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The future direction toward immunological issues of allo-and xeno-islet transplantation

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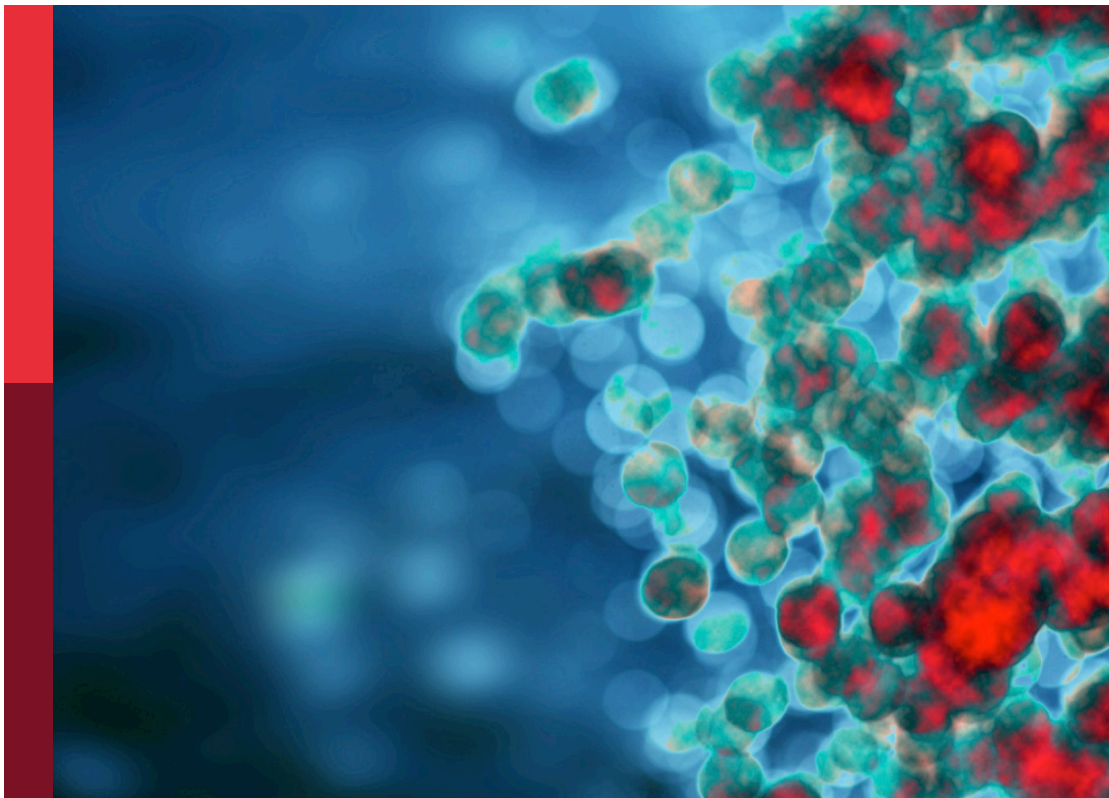

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The future direction toward immunological issues of allo-and xeno-islet transplantation

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Editorial: The future direction toward immunological issues of allo-and xeno-islet transplantation

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Editorial on the Research Topic
The future direction toward immunological issues of allo-and xeno-islet transplantation

The field of transplantation is advancing rapidly, particularly in addressing the immunological challenges associated with both allogeneic and xenogeneic transplants. Recent research highlights key developments in T-cell dynamics, innate immune regulation, and bioengineering innovations. This editorial summarizes these advancements and discusses strategies to overcome the barriers that continue to hinder successful graft outcomes.

T cell dynamics and molecular mechanisms in islet transplantation

The interaction of T cells within transplanted islets is critical for graft survival. Zhou et al. used single-cell RNA sequencing (scRNA-seq) to explore the molecular mechanisms behind T-cell dynamics in syngeneic and allogeneic islet transplantation. Their findings reveal significant heterogeneity among T-cell subpopulations, including CD4+ T cells, Tregs, and activated CD8+ T cells. The study highlights the differential activation of

pathways like interferon-alpha and TNF-alpha signaling, which are crucial for graft outcomes. These insights pave the way for more targeted immunosuppressive therapies tailored to specific immune responses.

Immunological challenges in allo-beta cell transplantation

Allo Beta Cell Transplantation faces significant immunological challenges, including immune rejection and autoimmunity recurrence. Caldara et al. focus on the interplay between glucose regulation, insulin, and immune activation. The study stresses the importance of standardized immunosuppression protocols, reliable methods for assessing graft rejection, and validated biomarkers for beta cell loss. Emerging strategies include alternative immunosuppressive regimens, targeted autoimmunity prevention, and innovative technologies like CAR-Tregs and genetically modified "stem stealth cells," which are vital for improving allo-beta cell transplantation outcomes.

Early microbiological identification in transplantation

The importance of early and accurate pathogen detection in transplant recipients cannot be overstated, particularly in lung transplantation where post-operative infections are a leading cause of morbidity and mortality. Zhang et al. demonstrated that metagenomic next-generation sequencing (mNGS) significantly improves pathogen detection rates compared to traditional microbial culture methods. This early detection capability is crucial for adjusting antimicrobial strategies promptly and effectively, thereby improving patient outcomes. The findings underscore the potential benefits of integrating mNGS into routine clinical practice for transplant recipients to better manage and prevent infections.

Innovations in bioengineering and stem cell approaches

To address the immunological challenges of islet transplantation, significant advancements have been made in bioengineering and stem cell technologies. Ho et al. discuss innovative strategies such as encapsulation technologies and the development of hypoimmune stem cells. These approaches aim to create an immunoprotective environment around transplanted islets, reducing the need for chronic immunosuppression. The review also explores the potential of human induced pluripotent stem cells (hiPSCs) as a renewable source for islet cells, highlighting the role of gene editing in enhancing their compatibility and function. These bioengineering advancements represent a critical step towards making islet transplantation a more viable and widely applicable treatment for type 1 diabetes (T1D).

Role of mesenchymal stem cells in enhancing islet transplantation

Mesenchymal stem cells (MSCs) have shown great potential in improving islet transplantation outcomes. Mou et al. highlight the immunomodulatory properties of MSCs, focusing on their ability to reduce immune rejection and support tissue repair. The potential of MSC-derived extracellular vesicles (EVs) to enhance graft survival is also discussed. Despite their promise, challenges like MSC heterogeneity and optimization in therapeutic applications remain. Advanced techniques, including AI and scRNA-seq, are proposed as solutions to these challenges, enabling more personalized treatment strategies.

Pig islet xenotransplantation: current status and challenges

Pig islet xenotransplantation offers a promising alternative to human donor pancreases, addressing the growing demand for islet transplants. Cooper et al. review the progress in this field, focusing on the development of gene-edited pigs that are more compatible with human recipients. The transplantation of neonatal pig islets (NICC) shows several advantages, including lower costs and simpler isolation processes. However, challenges such as the instant blood-mediated inflammatory reaction (IBMIR) and the need for effective immunosuppressive therapy persist. The review concludes that with continued advancements, pig islet xenotransplantation holds significant potential for clinical application.

Chronic rejection in lung transplantation: implications for islet transplantation

Chronic rejection remains a significant obstacle in lung transplantation and serves as a relevant model for understanding similar challenges in islet transplantation. Heigl et al. investigate the nature of chronic rejection in a murine orthotopic lung transplant model, revealing that rejection may begin as an arterial response rather than being airway-centered. These findings challenge traditional understandings and suggest that a broader perspective, including vascular and pleural involvement, is necessary for improving graft outcomes. This research has important implications for islet transplantation, where chronic rejection remains a critical challenge.

Advancements in innate immune regulation

The innate immune response, particularly by macrophages, poses a significant barrier to graft survival in islet transplantation. Duan et al. review strategies to regulate this response, including drug

therapies, optimization of islet preparation, and cotransplantation with MSCs. The study highlights the potential of blocking Toll-like receptor 4 (TLR4) signaling and inhibiting the NLRP3 inflammasome to reduce macrophage-induced inflammation, which is crucial for improving islet graft survival.

Optimal conditions for islet culture in xenotransplantation

Maintaining the viability and functionality of porcine islets during long-term culture is essential for successful xenotransplantation. Sakata et al. investigate the effects of different temperatures on the culture of adult porcine islets, concluding that 37°C is optimal for preserving islet morphology, promoting cell proliferation, and restoring endocrine function. This research provides valuable insights into the culture conditions necessary for maintaining the quality of porcine islets, which are a promising source for xenotransplantation.

Advancements in understanding T-cell dynamics, immunomodulatory therapies, and bioengineering are paving the way for improved outcomes in both allo- and xeno-islet transplantation. These developments not only enhance graft survival but also address broader immunological challenges in transplantation. Continued integration of these strategies will be key to overcoming barriers and advancing the field.

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Etiologic characteristics revealed by mNGS-mediated ultra-early and early microbiological identification in airway secretions from lung transplant recipients

Xiaoqin Zhang[1,2†], Xuemei Tang[2†], Xiaoli Yi[3†], Yu Lei[2], Sen Lu[2], Tianlong Li[2], Ruiming Yue[2], Lingai Pan[2], Gang Feng[4*], Xiaobo Huang[2*], Yiping Wang[2*] and Deyun Cheng[1*]

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Background: Post-operative etiological studies are critical for infection prevention in lung transplant recipients within the first year. In this study, mNGS combined with microbial culture was applied to reveal the etiological characteristics within one week (ultra-early) and one month (early) in lung transplant recipients, and the epidemiology of infection occurred within one month.

Methods: In 38 lung transplant recipients, deep airway secretions were collected through bronchofiberscope within two hours after the operation and were subjected to microbial identification by mNGS and microbial culture. The etiologic characteristics of lung transplant recipients were explored. Within one month, the infection status of recipients was monitored. The microbial species detected by mNGS were compared with the etiological agents causing infection within one month.

Results: The detection rate of mNGS in the 38 airway secretions specimens was significantly higher than that of the microbial culture (P<0.0001). MNGS identified 143 kinds of pathogenic microorganisms; bacterial pathogens account for more than half (72.73%), with gram-positive and -negative bacteria occupying large proportions. Fungi such as *Candida* are also frequently detected. 5 (50%) microbial species identified by microbial culture had multiple drug resistance (MDR). Within one month, 26 (68.42%) recipients got infected (with a median time of 9 days), among which 10 (38.46%) cases were infected within one week. In the infected recipients, causative agents were detected in advance by mNGS in 9 (34.62%) cases, and most of them (6, 66.67%) were infected within one week (ultra-early). In the infection that occurred after one week, the consistency between mNGS results and the etiological agents was decreased.

Conclusion: Based on the mNGS-reported pathogens in airway secretions samples collected within two hours, the initial empirical anti-infection regimes covering the bacteria and fungi are reasonable. The existence of bacteria with MDR forecasts the high risk of infection within 48 hours after transplant, reminding us of the necessity to adjust the antimicrobial strategy. The predictive role of mNGS performed within two hours in etiological agents is time-limited, suggesting continuous pathogenic identification is needed after lung transplant.

KEYWORDS

lung transplant, mNGS, airway secretions, early infection, etiology

Introduction

Although the first human lung transplant was performed in 1963, the operation became a clinical reality for treating end-stage lung diseases until the mid-1980s, after overcoming most surgical and pharmacologic challenges (1, 2). Nevertheless, the morbidity and mortality remain high, and the survival rate in lung transplant recipients is lower than of other solid organ transplant recipients, with a 5-year survival rate of 55.6% (3). Rejection- and infection-related complications are the main factors for overall morbidity and mortality in lung transplant recipients (4, 5). For lung transplant recipients, infection is a significant complication. It represents the most common cause of death within the first year, and pulmonary infection-related respiratory failure is the leading cause of death during post-operative admission (<30 days) (6, 7). So far, most of the post-operative etiological studies in lung transplants mainly focus on the episodes of infection that occurred within three months or one year following the operation. In a previous epidemiological study in which 51 lung transplant recipients were followed for a mean of 38.2 months, 42% of infectious episodes occurred within the first three months, and 75% developed within the first year after transplant (8). However, infections that occur within one week (ultra-early) and one month (early) after transplantation are rarely paid attention to, and the associated etiological study is insufficient.

Traditional etiological diagnosis methods of bronchoscopy specimens include airway secretions for microbial culture, smear microscopy, and histopathology (9). In lung transplant recipients, airway secretions microbial culture is the most frequently adopted for etiological examination to diagnose pulmonary infection (10, 11). However, the positive rate of microbial culture is low because of the limitation in microbial cultivating techniques and the impact of lesions surrounded by fibrous tissue and antibiotic application history (9). Consequently, molecular diagnostic technologies are emerging as complementary methodologies for pathogenic detection (12), including the polymerase chain reaction that focuses on a specific pathogen (13).

Metagenomic next-generation sequencing (mNGS), an unbiased and practical approach for pathogen identification with a shorter turn-around time, has been employed to diagnose infectious diseases (14). In liver transplant recipients, mNGS was adopted in the diagnosis and treatment guidance of post-operative infection, showing distinct advantages in detecting mixed, viral, and parasitic infections over the traditional culture method (15). Compared with urine culture, mNGS performed more remarkably in etiological diagnosis for kidney transplant recipients with urinary tract infections (16). In lung transplant recipients, mNGS is committed to pathogenic detection in airway secretions samples, with a shorter turn-around time, providing timely information for diagnosing pulmonary infections (17). These findings highlight the great potential of mNGS in detecting pathogenic microorganisms and identifying infection in lung transplant recipients. Herein, the secretions samples were absorbed through a bronchofiberscope from the deep airway within two hours after lung transplant. Airway secretions were subjected to mNGS test and microbial culture to reveal the ultra-early microbial characteristics and analyze the pulmonary infection within one month in recipients. Our data may offer a critical reference for antimicrobial regimens to prevent infections developed within one week or month, thereby reducing the related mortality.

Materials and methods

Lung transplant recipient enrollment

Patients undergoing lung transplantation at Sichuan Provincial People's Hospital from October 2018 to June 2022 were included in this study. The inclusive and exclusive criteria for donor lungs were described in our previous study (18), and listed as follows.

Donor lungs inclusion criteria: (a) Age < 60 years old, smoking history < 20 packs/year. (b) No chest injury. (c) Continuous mechanical ventilation < 1 week. (d) PaO_2 > 300 mmHg (FiO_2 = 100%, PEEP = 5cm H_2O). (e) X-ray or CT shows that the lung field is relatively clear. (f) No abscess secretion was found through bronchoscopy in the lung bronchus.

Donor lungs exclusion criteria: (a) Age > 60 years old, smoking history > 20 packs/year. (b) Chest trauma and lung contusion. (c)

Continuous mechanical ventilation > 1 week. (d) PaO_2 < 300 mmHg (FiO_2 = 100%, PEEP = 5cm H_2O). (e) X-ray or CT shows that the lung field is infected. (f) There are purulent secretions at bronchoscopy in the donor's lower airways. (g) The percentage of white blood cells, neutrophils, C-reactive protein, and procalcitonin increases gradually compared with the situation at the onset of the disease. (h) The donor's body temperature is higher than normal. (i) Blood culture is positive.

Study design and sample collection

Basic information about the enrolled recipients, including age, sex, primary indications for a lung transplant, types of lung transplantation (bilateral or unilateral), and infection status within one month following the operation, was recorded. Prognostic information on the enrolled patients' antimicrobial use, mechanical ventilation, and ICU hospitalization was recorded in detail.

In most lung transplant centers in China, timely bronchofiberscopy after surgery is a routine examination aiming to clean the airway secretions through a bronchofiberscope, which helps to avoid obstructing the small airway and reduce pathogens. Therefore, airway secretions were absorbed from the deep airway by bronchofiberscope two hours after the operation and sent for traditional microbial culture and mNGS for pathogen detection immediately. In the following days, within one month, airway secretions or BALFs were collected for microbial culture every few days, depending on the actual conditions in recipients. Microbial culture for the above samples was conducted in our hospital. The yielded pathogen spectrum was analyzed and compared between these two methods. The incidence of infection within one month and the occurrence time in these recipients were determined. The causative agents for infection were compared with the pathogenic microorganisms reported by mNGS in airway secretions collected within two hours to evaluate the role of mNGS in forewarning potential pathogens.

mNGS procedure

The whole process of mNGS was completed by Genoxor Medical Science and Technology Inc. (Shanghai, China). The airway secretions samples were stored at 4°C and sent for mNGS detection within 24h. These steps included pre-treatment, DNA extraction, library construction, sequencing, bioinformatic analysis, and interpretation of data (19). A 1.5ml microcentrifuge tube containing 0.6ml of sample, enzyme, and 1.0g of glass beads (0.5mm) was attached to a horizontal platform on a vortex mixer and agitated vigorously at 2,800–3,200 rpm for 30 min. Then DNA in airway secretions samples was extracted using the TIANamp Micro DNA Kit (DP316, Tiangen Biotech) according to the manufacturer's instructions. After DNA concentration and purity detection, the libraries were constructed undergoing DNA fragmentation, end-repair, adapter ligation, and PCR amplification. DNA library concentration was measured by Qubit 2.0. An Agilent 2100 test achieved quality control of the DNA libraries. After being pre-quantified by qRT-PCR, quality-qualified libraries were sequenced on the NextSeq™ 550DX platform in SE-75 sequencing type according to the manufacturer's instructions.

Data analysis and quality control

Bioinformatics analysis of the mNGS data was performed according to the procedure described in a previous study (20). Raw data (raw reads) were subjected to a quality control process for trimming adapter sequences and removing low-quality tails, reads, and connector sequences using Trimmomatic v0.36 (21). The obtained high-quality and adequate data are called clean reads. Reads mapping to the human genome GRCh37 were removed using the calibration software Bowtie v2.2.6 (22), and the remaining were called unmapped reads (microbial reads). All the microbial reads were deposited in the database under the Sequence Read Archive (SRA) accession number PRJNA932550. Unmapped% refers to the proportion of microbial reads in the clean reads. Duplicated reads introduced in the PCR step were deleted using FASTX-Toolkit, Fulcrum, FastUniq, and CD-HIT-DUP tools (23). Subsequently, Kraken v2.0.9-beta (24) was adopted for the taxonomic classification of microbial reads, with a microbial genome database in NCBI constructed using 51543 genomes of about 27000 species (ftp://ftp.ncbi.nlm.nih.gov/genomes/) (25). The number of reads in the Kraken classification report was further estimated by the Bayesian algorithm named Bracken to produce species-level abundance estimates (26). The estimates of the percentage relative abundance of each species were computed using the reads per kilobase of transcript per million mapped reads (RPKM), a normalization method for mNGS reads, and RPKM was calculated using the formula: gene reads/[the total mapped reads (millions) × genome length (KB)] (27).

Criteria for defining positive results of mNGS

The mNGS assay was employed for detecting microorganisms, including bacteria, viruses, fungi, and parasites, and a positive result will be judged if it satisfies any of the following criteria described previously (17). 1) The relative abundance of bacteria (excluding *M. tuberculosis* complex) and fungi was greater than 30% at the genera level; 2) Virus detection was considered when the stringent map read number (SMRN) was ≥3. 3) For *M. tuberculosis* complex, at least one number of reads should be aligned to the reference genome at the species or the genus level. However, a positive mNGS finding did not invariably indicate the presence of causative pathogens. Microorganisms detected with mNGS were categorized into colonized, putative, and pathogenic microorganisms. It would be the clinician's responsibility to determine the putative pathogens and pathogenic microorganisms through comprehensive clinical assessments. In the pathogenic spectrum analysis, the proportion of the pathogenic species, the detection frequency, was calculated with a formulation: the number

of samples in which a particular species was detected/the total number of samples.

Diagnosis of infection and judgment of pathogenic agents

Before and after the lung transplant, the infectious risk and status of the recipients were monitored. The suspicion and diagnosis of infection were based on several clinical symptoms, including body temperature, computed tomography, etiological examination, and immune indicators. In the infected recipients, the putative pathogens and pathogenic microorganisms were judged based on a comprehensive analysis of clinical data, including the number of reads for mNGS, the clinical presentations, radiologic manifestations, conventional detection findings, clinical epidemiology, and the treatment effect of the antibiotic therapy. The putative pathogens or pathogenic microorganisms could be ascertained if the two clinicians approved. Further discussion by senior clinicians is needed in case of a significant disagreement between the first two clinicians. Then, the targeted antibiotic therapy was formulated to fight against infection, and a favorable outcome further confirmed the causative agent. The consistency of mNGS with the causative agents in the infected recipients was evaluated at the species level.

Statistical analysis

Descriptive statistics were computed for the overall samples and stratified by the positive pathogen detected by mNGS on airway secretions samples. Mean ± standard deviation (SD) or median (interquartile range, IQR) was used for describing the continuous variables. Chi-squared or Fisher's Exact test was used to compare the two groups' differences. The significance level was set at 0.05. All statistical analyses were performed using the GraphPad software 8.0.

Results

General information of study participants

From October 2018 to June 2022, 40 patients received lung transplant surgery in our hospital, and 38 eligible patients were included for the final analysis. Two recipients were excluded because of death quickly without any microbial culture result. Basic information of these patients was provided in Supplementary Table 1. Of all 38 lung transplant recipients, the mean age was 58.13 years (ranges 33-70), including 33 (86.84%) males. The most common primary disease was COPD (19, 50%), followed by interstitial lung disease (18, 47.37%), with the addition of one patient with pneumosilicosis. In terms of the lung transplant types, 23 (60.53%) underwent bilateral transplantation and 15 (39.47%) unilateral transplantation. In the 38 recipients, new-onset infection within one month occurred in 26 (68.42%). These clinical characteristics were recorded and demonstrated in Table 1. After lung transplant, the initial antibiotic regimens frequently include Sulbactam/Cefopcrazone and Piperacillin Sodium/Tazobactam Sodium. Immunosuppressant regiments comprise cyclosporin A, tacrolimus, and methylprednisolone.

Pathogenic spectrum generated by mNGS and traditional microbial culture

38 airway secretions samples from 38 lung transplant recipients were collected within two hours after surgery and simultaneously sent for etiological examination by traditional microbial culture and mNGS. The study design is illustrated in Figure 1. The detecting results of the two methods in each patient were provided in Supplementary Table 1. This supplementary material also included detailed information concerning each sample's sequencing number of reads (raw reads, clean reads, clean reads/raw reads, unmapped reads, and unmapped %), as well as the putative pathogens in each patient and their relative abundance. It demonstrates that the raw reads range from 4M to 57M, with an average of 20M; most ratios of clean reads to raw reads are above 90%. Unmapped% refers to the proportion of microbial reads in the clean reads, ranging from 0.69% to 79.21%.

143 kinds of pathogenic microorganisms were found in 35 (92.11%, 35/38) airway secretions specimens using mNGS, while the detection rate by microbial culture was 26.31% (10/38) (P<0.0001) (Table 2). Statistically, mNGS identified pathogenic microorganisms at the level of species or genus, which were further classified into five types, including bacteria (72.73%), fungi (13.29%), virus (11.89%), mycoplasma (1.4%), and parasites (0.7%) (Figure 2A). When analyzed at the species level, *S. pneumoniae* (28.95%) and *H. parainfluenzae* (23.68%) were the

TABLE 1 Characteristics of the lung transplant recipients.

Characteristics	Values
Lung transplant recipients (n)	38
Median age, y (IQR)	60.5 (52.8-65.3)
Sex (male, %)	33 (86.84%)
Primary indications for lung transplantation, n (%)	
COPD	19 (50%)
Interstitial lung disease	18 (47.37%)
Pneumosilicosis	1 (2.63%)
Types of lung transplantation, n (%)	
Bilateral lung transplantation	23 (60.53%)
Unilateral lung transplantation	15 (39.47%)
Infection status within one month, n (%)	
Infected	26 (68.42%)
Uninfected	12 (31.58%)

COPD, chronic obstructive pulmonary disease.

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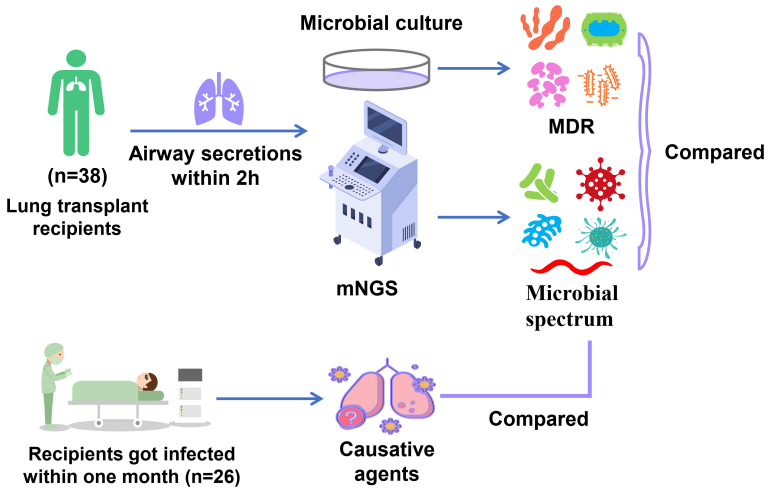

FIGURE 1
The flowchart of the study design.

TABLE 2 The number of pathogenic microorganisms detected by mNGS and microbial culture in the airway secretions samples collected within two hours from lung transplant recipients.

Samples	mNGS			Microbial culture		Infection status of patients
	Bacteria	Fungi	Viruses	Bacteria	Fungi	
S1	1	0	0	1	0	Infected
S2	13	0	0	1	0	Infected
S3	25	1	1	0	0	Infected
S4	21	1	1	0	0	Infected
S5	1	0	0	0	0	Infected
S6	3	0	2	0	0	Infected
S7	3	0	0	0	0	Infected
S8	13	1	0	1	0	Infected
S9	0	0	0	0	0	Infected
S10	4	0	0	0	0	Infected
S11	12	1	0	1	0	Infected
S12	11	0	0	1	0	Infected
S13	21	3	1	0	0	Infected
S14	16	0	2	0	0	Infected
S15	2	0	0	1	0	Infected
S16	29	1	2			Infected
S17	6	1	2	0	0	Infected
S18	0	1	0	0	0	Infected
S19	22	1	1	0	0	Infected
S20	2	0	0	0	0	Infected
S21	0	0	0	0	0	Infected

(Continued)

TABLE 2 Continued

Samples	mNGS			Microbial culture		Infection status of patients
	Bacteria	Fungi	Viruses	Bacteria	Fungi	
S22	22	0	0	0	0	Infected
S23	0	1	0	0	0	Infected
S24	18	0	1	0	0	Infected
S25	2	1	0	0	0	Infected
S26	5	1	2	1	0	Infected
S27	2	1	0	0	0	Uninfected
S28	1	0	0	1	0	Uninfected
S29	1	0	0	1	0	Uninfected
S30	1	1	1	0	1	Uninfected
S31	3	0	0	0	0	Uninfected
S32	0	0	0	0	0	Uninfected
S33	4	0	0	0	0	Uninfected
S34	1	0	1	0	0	Uninfected
S35	2	0	0	0	0	Uninfected
S36	16	0	0	0	0	Uninfected
S37	31	1	0	0	0	Uninfected
S38	16	2	1	0	0	Uninfected

top two bacteria, followed by *S. aureus* (21.05%), *S. pseudopneumoniae* (21.05%), *K. pneumoniae* (21.05%), and *A. baumannii complex* (21.05%) (Figure 2B). *C. albicans* (21.05%) was the most dominant fungi detected with mNGS. *Human betaherpesvirus 5* (18.42%) was the most prevalent virus. Seven pathogenic microorganisms were detected through the traditional culture method in 10 airway secretions samples. *K. pneumoniae* was detected in three cases (7.89%); *S. aureus* was detected in two samples (5.26%) (Figure 2C). The other bacteria include *A. baumannii* and *A. ursingii*, and fungi like *C. parapsilosis* were detected in one sample (2.63%).

Time distribution of infection within one month after transplant and the consistency between mNGS-reported pathogens and the causative agents

Figure 3 illustrates the results of etiological identification by mNGS and traditional culture and the information on causative agents in recipients infected within one month. Within one month, 26 (68.42%) of the 38 recipients got infected, and the median time of new-onset infection was 9 days, ranging from 3 to 25 days. Among the 26 infected recipients, 10 (38.46%) got infected within one week following the lung transplant operation, and infection in 7 (26.92%) cases occurred within one to two weeks. The remaining 7 (26.92%) and 2 (7.69%) got infected within two to three weeks and three to four weeks, respectively (Table 3). Consequently, infection onset within one week was the highest, and more than half (65.38%) of recipients developed an infection within two weeks. The drug sensitivity of the pathogens was also examined through microbial culture and demonstrated in Figure 3. Multiple drug resistance was observed in *S. aureus* (case 1), *A. baumannii* (case 8), *S. maltophilia* (case 11), and *B. multivorans* (case 15).

The consistency of two hours airway secretions-mNGS and microbial culture results with the causative agents in the infected recipients was determined at the species level and illustrated in Figure 3. In 9 (34.62%, 9/26) infected recipients (patient 1, 2, 3, 5, 7, 8, 12, 13, 26), their causative agents were detected by mNGS in advance (in the airway secretions collected within two hours), who got an infection at the 3rd, 3rd, 3rd, 5th, 5th, 5th, 8th, 8th, and 25th day, respectively, after lung transplant (Figure 3). Except for an infection caused by *A. fumigatus* on the 25th day, the median time of infection occurring in the rest 8 recipients was 5 days following the operation. Namely, most of them (6, 66.67%) were infected within one week, 2 (22.22%) cases suffered between one to two weeks, and 1 (11.11%) at three to four weeks (Table 4). A decreased trend was observed in consistency, along with the prolonged infection time.

Discussion

Our study retrospectively investigated the ultra-early and early etiological characteristic in lung transplant recipients, whose results may provide reference for early antimicrobial strategy in lung

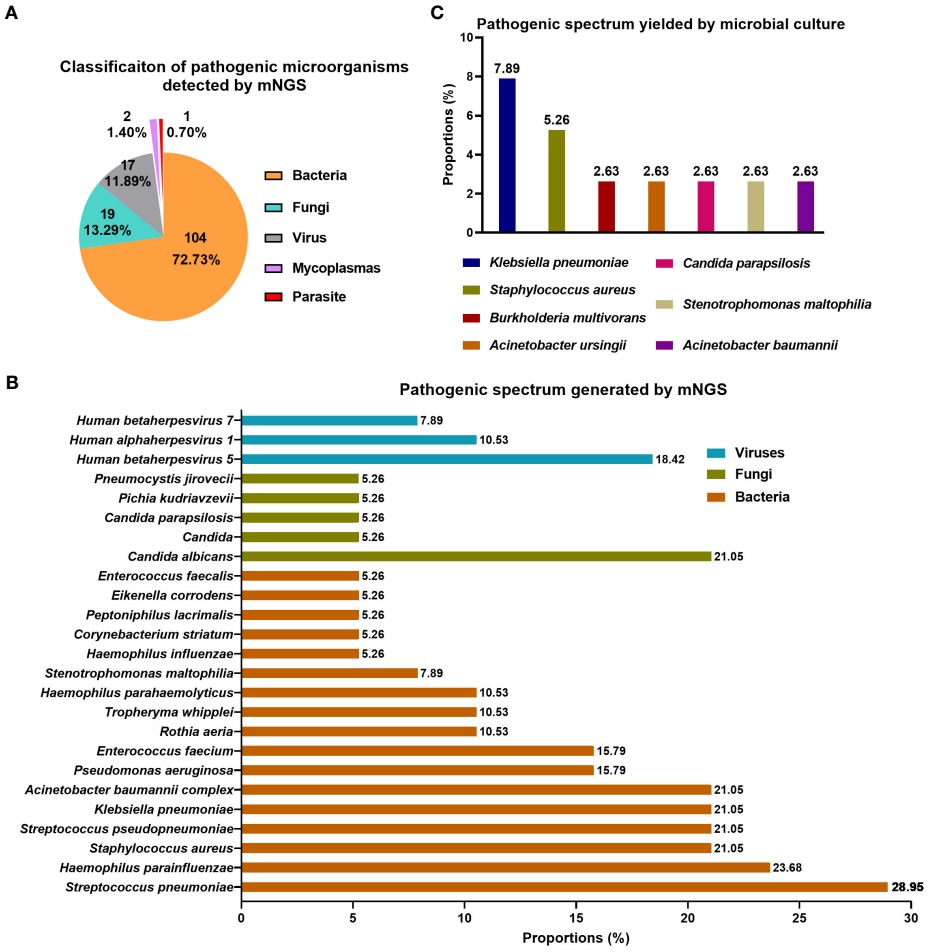

FIGURE 2
Pathogen spectrum detected by mNGS and traditional culture in airway secretions collected within two hours following lung transplant. **(A)** Classification of pathogenic microorganisms detected by mNGS; **(B)** Pathogenic spectrum detected by mNGS; **(C)** Pathogenic spectrum detected by conventional microbial culture.

transplant recipients. This study completed pathogen identification through the mNGS technology and microbial culture. In general, mNGS performed well in finding diverse microbial species and might serve as an effective supplementary means to traditional etiological detection methods.

In various infectious diseases, the diagnostic accuracy of mNGS is frequently compared with that of conventional detection methods (28). In this study, the traditional culture method served as the control group versus mNGS, whose positive rate for pathogen identification was shallow compared to that of mNGS (26.31% vs. 92.11%). Ju et al. also observed a significantly higher positive rate of mNGS than conventional detection methods (83.4% vs. 55.8%) in airway secretions specimens, with a higher diversity of pathogens simultaneously (17). In our 38 airway secretions samples, mNGS identified 143 kinds of microorganism, ranging from bacteria (72.73%), fungi (13.29%), virus (11.89%), mycoplasma (1.4%), to parasites (0.7%) (Figure 2A). The pathogen spectrum revealed that mNGS reported more total amount of pathogen than microbial culture (Figures 2B, C). Moreover, mNGS showed absolute superiority in the detection of virus and parasite. Viral infection after lung transplant is common and classified into diseases caused by cytomegalovirus or by other community-acquired respiratory viruses (4, 29). It has been reported that viral pathogens are involved in 25 of 71 infectious episodes in a cohort of lung transplant recipients, with cytomegalovirus-related diseases accounting for 68% of them (8). Without doubt, the conventional diagnosis of parasitic infections in lung transplant recipients is complicated, with clinical suspicion combined with molecular diagnostic methods such as PCR (30). Therefore, the application of mNGS benefits the etiological diagnosis of rare pathogens. To sum up, we claimed that mNGS is superior to the conventional culture in detection rate and in finding more pathogenic microorganisms with a higher diversity, contributing to a wider reference of pathogen screening and the later prophylactic treatment.

Bacterial infections are the most frequent infectious complications. In a Swiss transplant cohort study, 55% of all lung transplant recipients developed infections in the first year, and 63% were bacterial (31). More than half of the pathogens detected in the current study were bacterial microbes, and *S. pneumoniae* (28.95%) and *H. parainfluenzae* (23.68%) were the top two bacteria, followed by

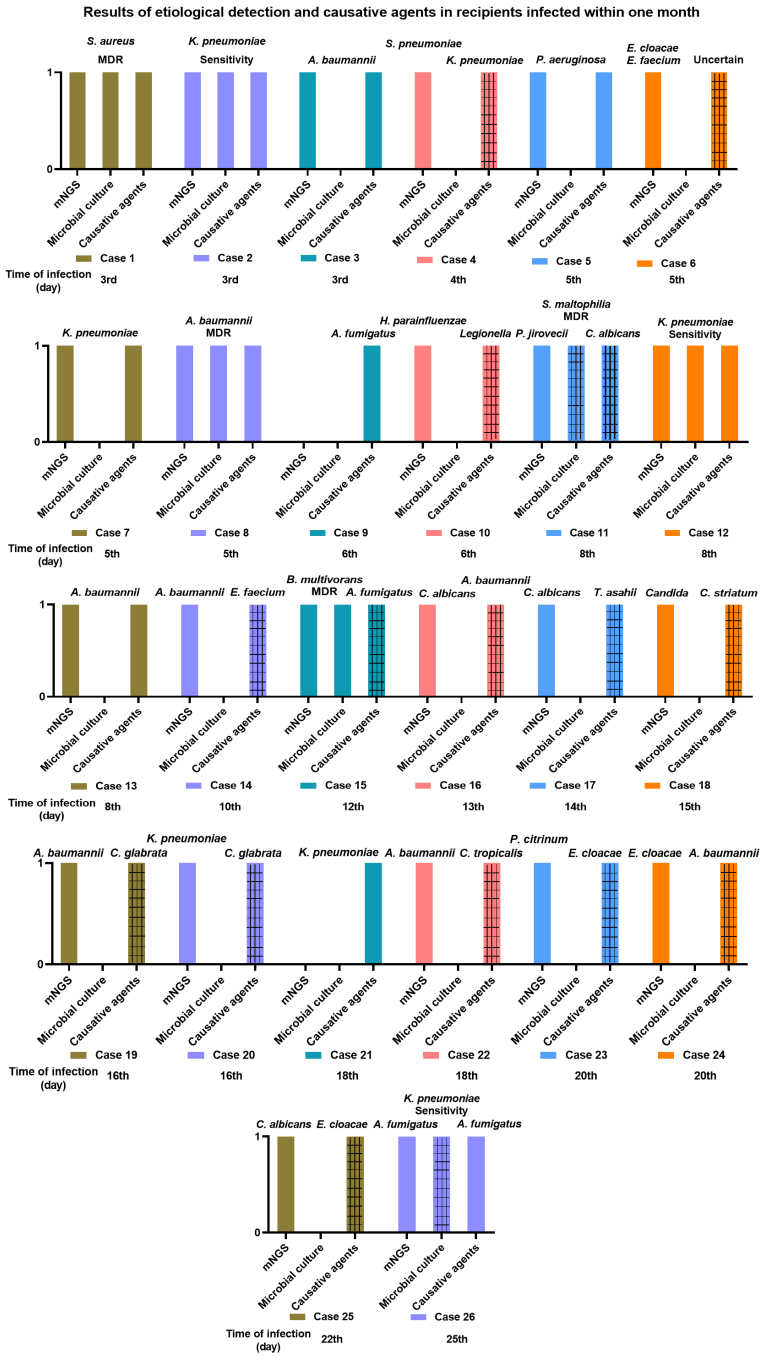

FIGURE 3
The information of two hours airway secretions mNGS and microbial culture results and the causative agents in recipients infected within one month. The gridlines stand for different pathogenic microorganism when comparing the results of mNGS and microbial culture with the causative agents.

S. aureus (21.05%), *S. pseudopneumoniae* (21.05%), *K. pneumoniae* (21.05%), and *A. baumannii complex* (21.05%) (Figure 2B). They are all the common opportunistic pathogen invading the respiratory tract, and are more likely to invoking infection following lung transplant under immunosuppression (32–34). Thereinto, *S. pneumoniae* and *H. influenzae* are among the main vaccine-preventable bacterial infections in immunocompromised individuals like recipients of solid organ transplants, resulting in a large proportion of hospitalization (34). It has been proven that *K. pneumoniae* is commonly isolated after lung transplantation, and carbapenem-resistant *K. pneumoniae* acquisition is associated with an increased risk of bronchial dehiscence and reduced survival among recipients (33, 35). As reported, fungi are frequently isolated before and after transplantation from respiratory samples, and fungal infections are more common in lung transplant recipients than in most other solid organs (11, 36, 37). In the fungi detected in our samples, *Candida* (34.21%) was the most frequently detected, with *C. albicans* (21.05%) as the predominant species. It led to one infection event in case 11 at

TABLE 3 The time distribution of infection within one month in lung recipients.

Onset time of infection	Number of infected cases	Proportions in the infected patients
Within one week	10	38.46%
One-two weeks	7	26.92%
Two-three weeks	7	26.92%
Three-four weeks	2	7.69%

8th day after the operation. *Candida* leads to most fungal extrapulmonary infections in lung transplant recipients, and frequently occurs one-month after the transplant (38). It has been reported that the average period of *Aspergillus*-related infection is 42 days after lung transplantation (12). Our data demonstrated that recipients 9, 15, and 26 were infected by *Aspergillus* on the 6th, 12th, and 25th days after transplant, respectively. In the pathogenic microorganisms identified by mNGS, bacterial pathogens account for more than half (72.73%), with gram-positive and -negative bacteria occupying large proportions. Fungi such as *Candida* are also frequently detected. Therefore, the initial empirical anti-infection regimes covering the bacteria and fungi are reasonable, and the broad spectrum antimicrobial drugs can be substituted by the narrows after the mNGS results produced.

Within one month, 68.42% (26/38) of recipients got infected, and more than half of the infections happened within two weeks. According to Table 4, mNGS could predict the causative agents in early infection, especially for the infection onset within one week. Notoriously, donor-derived infections generally manifest during the first few weeks after lung transplant (31). Many deceased donors were more likely to carry pathogens with multiple drug resistance (MDR) or suffered from hospital infections because they stay in the intensive care unit (39, 40). Our Figure 3 indicated that MDR bacteria were detected in airway secretions samples from 4 cases, and they were *S. aureus* (case 1), *A. baumannii* (case 8), *S. maltophilia* (case 11), and *B. multivorans* (case 15). Bunsow reported that MDR bacteria were isolated from 4.9% (12/243) of donors, including *Enterobacterales*, *S. maltophilia*, *P. aeruginosa*, and *S. aureus* (41). These MDR bacteria should be highly suspected in the cases of infection occurred within 48h or infection worsened after transplant.

TABLE 4 The consistency of mNGS results in two hours of airway secretions with the causative agents in infected recipients.

Onset time of infection	Number of cases in which mNGS was consistent with the causative agents	Proportions
Within one week	6	66.67%
One-two weeks	2	22.22%
Two-three weeks	0	0%
Three-four weeks	1	11.11%

In the present studies concerning post-operative infection after lung transplant, many researchers focus on a longer duration, such as three months, one year, even five years (8, 42–44), but early infection within one month has rarely been highlighted. Our study revealed that the median time of new-onset infection was nine days, 38.46% of recipients got infected within one week, and even 65.38% developed infection within two weeks. The high incidence of infection in lung transplant recipients may be associated with the destruction of the mucosal barrier, which was improved with the repair of the mucous membrane (45). Therefore, it is essential to repair the mucosal barrier by removing the tracheal catheters as soon as possible (46). In the infections that occurred shortly after the transplant, the consistency between mNGS results and the etiological agents was high but decreased with the prolonged time interval. That is, the predictive role of mNGS in etiological agents is time-limited, suggesting that continuous pathogenic screening is indispensable for infection prevention (47). With the deepening of research on pathogenic microorganisms affecting lung transplant recipients and advances in pathogen detection technologies, the infection risks are expected to be perceived earlier and specifically intervened to prevent infection and improve their survival rate.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: PRJNA932550 (SRA).

Ethics statement

The studies involving humans were approved by Medical Ethics Committee of Sichuan Provincial People's Hospital (No. 2021-399). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

XZ: Conceptualization, Data curation, Writing – original draft, Writing – review & editing. XT: Data curation, Formal Analysis, Investigation, Visualization, Writing – original draft. XY: Data curation, Software, Writing – review & editing. YL: Data curation, Methodology, Writing – review & editing. SL: Data curation, Methodology, Writing – review & editing. TL: Data curation, Methodology, Writing – review & editing. RY: Investigation, Methodology, Writing – review & editing. LP: Investigation, Methodology, Writing – review & editing. GF: Project administration, Software, Supervision, Visualization, Writing – review & editing. XH: Project administration, Software, Supervision, Visualization, Writing – review & editing. YW: Conceptualization, Formal Analysis, Methodology, Writing – review & editing. DC: Conceptualization, Formal Analysis, Methodology, Writing – original draft, Writing – review & editing.

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Conflict of interest

Author XY was employed by the company Genoxor Medical Science and Technology Inc.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2023.1271919/full#supplementary-material

References

1. Ivulich S, Westall G, Dooley M, Snell G. The evolution of lung transplant immunosuppression. *Drugs* (2018) 78(10):965–82. doi: 10.1007/s40265-018-0930-6

2. Young KA, Dilling DF. The future of lung transplantation. *Chest* (2019) 155 (3):465–73. doi: 10.1016/j.chest.2018.08.1036

3. Lehr CJ, Blackstone EH, McCurry KR, Thuita L, Tsuang WM, Valapour M. Extremes of age decrease survival in adults after lung transplant. *Chest* (2020) 157 (4):907–15. doi: 10.1016/j.chest.2019.06.042

4. Burguete SR, Maselli DJ, Fernandez JF, Levine SM. Lung transplant infection. *Respirology* (2013) 18(1):22–38. doi: 10.1111/j.1440-1843.2012.02196.x

5. Grewal HS, Highland KB, McCurry K, Akindipe O, Budev M, Mehta AC. Bacterial meningitis as a cause of death in lung transplant donors: early outcomes in recipients. *Clin Transplant* (2018) 32(8):e13307. doi: 10.1111/ctr.13307

6. Costa J, Benvenuto LJ, Sonett JR. Long-term outcomes and management of lung transplant recipients. *Best Pract Res Clin Anaesthesiol* (2017) 31(2):285–97. doi: 10.1016/j.bpa.2017.05.006

7. Hu CX, Chen WH, He JX, Jiang GN, Li XS, Wei D, et al. Lung transplantation in China between 2015 and 2018. *Chin Med J (Engl)* (2019) 132(23):2783–9. doi: 10.1097/CM9.0000000000000543

8. Parada MT, Alba A, Sepulveda C. Early and late infections in lung transplantation patients. *Transplant Proc* (2010) 42(1):333–5. doi: 10.1016/j.transproceed.2009.12.002

9. Huang J, Jiang E, Yang D, Wei J, Zhao M, Feng J, et al. Metagenomic next-generation sequencing versus traditional pathogen detection in the diagnosis of peripheral pulmonary infectious lesions. *Infect Drug Resist* (2020) 13:567–76. doi: 10.2147/IDR.S235182

10. Stjarne Aspelund A, Hammarstrom H, Inghammar M, Larsson H, Hansson L, Christensson B, et al. Heparin-binding protein, lysozyme, and inflammatory cytokines in bronchoalveolar lavage fluid as diagnostic tools for pulmonary infection in lung transplanted patients. *Am J Transplant* (2018) 18(2):444–52. doi: 10.1111/ajt.14458

11. Qiao W, Zou J, Ping F, Han Z, Li L, Wang X. Fungal infection in lung transplant recipients in perioperative period from one lung transplant center. *J Thorac Dis* (2019) 11(4):1554–61. doi: 10.21037/jtd.2019.03.18

12. Kennedy CC, Pennington KM, Beam E, Razonable RR. Fungal infection in lung transplantation. *Semin Respir Crit Care Med* (2021) 42(3):471–82. doi: 10.1055/s-0041-1729173

13. Jacobs F, Knoop C, Brancart F, Gilot P, Melot C, Byl B, et al. Human herpesvirus-6 infection after lung and heart-lung transplantation: a prospective longitudinal study. *Transplantation* (2003) 75(12):1996–2001. doi: 10.1097/01.TP.0000058809.42027.66

14. Han D, Li Z, Li R, Tan P, Zhang R, Li J. mNGS in clinical microbiology laboratories: on the road to maturity. *Crit Rev Microbiol* (2019) 45(5-6):668–85. doi: 10.1080/1040841X.2019.1681933

15. Zhao D, Guo L, Lian D, Gu Y, Yan X, Hu H, et al. Diagnostic value and clinical application of mNGS for post-liver transplantation infection: A cross-sectional study with case reports. *Front Microbiol* (2022) 13:919363. doi: 10.3389/fmicb.2022.919363

16. Duan W, Yang Y, Zhao J, Yan T, Tian X. Application of metagenomic next-generation sequencing in the diagnosis and treatment of recurrent urinary tract infection in kidney transplant recipients. *Front Public Health* (2022) 10:901549. doi: 10.3389/fpubh.2022.901549

17. Ju CR, Lian QY, Guan WJ, Chen A, Zhang JH, Xu X, et al. Metagenomic next-generation sequencing for diagnosing infections in lung transplant recipients: a retrospective study. *Transpl Int* (2022) 35:10265. doi: 10.3389/ti.2022.10265

18. Zhang XQ, Lei Y, Tan XL, Guo L, Huang XB, Yang FX, et al. Optimization of early antimicrobial strategies for lung transplant recipients based on metagenomic next-generation sequencing. *Front Microbiol* (2022) 13:839698. doi: 10.3389/fmicb.2022.839698

19. Pan L, Wu F, Cai Q, Xu Z, Hu H, Tang T, et al. Whole genome profiling of lung microbiome in solid organ transplant recipients reveals virus involved microecology may worsen prognosis. *Front Cell Infect Microbiol* (2022) 12:863399. doi: 10.3389/fcimb.2022.863399

20. Chen J, Sun L, Liu X, Yu Q, Qin K, Cao X, et al. Metagenomic assessment of the pathogenic risk of microorganisms in sputum of post-operative patients with pulmonary infection. *Front Cell Infect Microbiol* (2022) 12:855839. doi: 10.3389/fcimb.2022.855839

21. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* (2014) 30(15):2114–20. doi: 10.1093/bioinformatics/btu170

22. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods* (2012) 9(4):357–9. doi: 10.1038/nmeth.1923

23. Martin TC, Visconti A, Spector TD, Falchi M. Conducting metagenomic studies in microbiology and clinical research. *Appl Microbiol Biotechnol* (2018) 102(20):8629–46. doi: 10.1007/s00253-018-9209-9

24. Wood DE, Lu J, Langmead B. Improved metagenomic analysis with Kraken 2. *Genome Biol* (2019) 20(1):257. doi: 10.1186/s13059-019-1891-0

25. Kitts PA, Church DM, Thibaud-Nissen F, Choi J, Hem V, Sapojnikov V, et al. Assembly: a resource for assembled genomes at NCBI. *Nucleic Acids Res* (2016) 44(D1): D73–80. doi: 10.1093/nar/gkv1226

26. Lu J, Breitwieser FP, Thielen P, Salzberg SL. Bracken: estimating species abundance in metagenomics data. *Peerj Comput Sci* (2017) 3(1):e104. doi: 10.7717/peerj-cs.104

27. Wang C, You Z, Fu J, Chen S, Bai D, Zhao H, et al. Application of metagenomic next-generation sequencing in the diagnosis of pulmonary invasive fungal disease. *Front Cell Infect Microbiol* (2022) 12:949505. doi: 10.3389/fcimb.2022.949505

28. Chen J, Zhao Y, Shang Y, Lin Z, Xu G, Bai B, et al. The clinical significance of simultaneous detection of pathogens from bronchoalveolar lavage fluid and blood samples by metagenomic next-generation sequencing in patients with severe pneumonia. *J Med Microbiol* (2021) 70(1):001259. doi: 10.1099/jmm.0.001259

29. Almaghrabi RS, Omrani AS, Memish ZA. Cytomegalovirus infection in lung transplant recipients. *Expert Rev Respir Med* (2017) 11(5):377–83. doi: 10.1080/17476348.2017.1317596

30. van den Bogaart L, Cipriano A, Koutsokera A, Manuel O. Understanding rare infections post-lung transplantation. *Expert Rev Respir Med* (2021) 15(3):325–38. doi: 10.1080/17476348.2021.1843428

31. McCort M, MacKenzie E, Pursell K, Pitrak D. Bacterial infections in lung transplantation. *J Thorac Dis* (2021) 13(11):6654–72. doi: 10.21037/jtd-2021-12

32. Oh DH, Kim YC, Kim EJ, Jung IY, Jeong SJ, Kim SY, et al. Multidrug-resistant Acinetobacter baumannii infection in lung transplant recipients: risk factors and prognosis. *Infect Dis (Lond)* (2019) 51(7):493–501. doi: 10.1080/23744235.2018.1556400

33. Suh JW, Lee JG, Jeong SJ, Park MS, Kim SY, Paik HC. Risk of bronchial dehiscence in lung transplant recipients with Carbapenemase-producing Klebsiella. *Ann Thorac Surg* (2020) 110(1):265–71. doi: 10.1016/j.athoracsur.2020.01.076

34. Rezahosseini O, Moller DL, Sorensen SS, Perch M, Gustafsson F, Gelpi M, et al. An observational prospective cohort study of incidence and outcome of streptococcus pneumoniae and hemophilus influenzae infections in adult solid organ transplant recipients. *Microorganisms* (2021) 9(7):1371. doi: 10.3390/microorganisms9071371

35. Raviv Y, Shitrit D, Amital A, Fox B, Bakal I, Tauber R, et al. Multidrug-resistant Klebsiella pneumoniae acquisition in lung transplant recipients. *Clin Transplant* (2012) 26(4):E388–394. doi: 10.1111/j.1399-0012.2012.01671.x

36. Pappas PG, Alexander BD, Andes DR, Hadley S, Kauffman CA, Freifeld A, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). *Clin Infect Dis* (2010) 50 (8):1101–11. doi: 10.1086/651262

37. Adegunsoye A, Strek ME, Garrity E, Guzy R, Bag R. Comprehensive care of the lung transplant patient. *Chest* (2017) 152(1):150–64. doi: 10.1016/j.chest.2016.10.001

38. Villalobos AP, Husain S. Infection prophylaxis and management of fungal infections in lung transplant. *Ann Transl Med* (2020) 8(6):414. doi: 10.21037/atm.2020.03.102

39. Lee KH, Jeong SJ, Kim SY, Han SH, Park MS, Lee JG, et al. Effects of multidrug-resistant bacteria in donor lower respiratory tract on early posttransplant pneumonia in lung transplant recipients without pretransplant infection. *Transplantation* (2020) 104 (4):e98–e106. doi: 10.1097/TP.0000000000003102

40. Paglicci L, Borgo V, Lanzarone N, Fabbiani M, Cassol C, Cusi MG, et al. Incidence and risk factors for respiratory tract bacterial colonization and infection in lung transplant recipients. *Eur J Clin Microbiol Infect Dis* (2021) 40(6):1271–82. doi: 10.1007/s10096-021-04153-1

41. Bunsow E, Los-Arcos I, Martin-Gomez MT, Bello I, Pont T, Berastegui C, et al. Donor-derived bacterial infections in lung transplant recipients in the era of multidrug resistance. *J Infect* (2020) 80(2):190–6. doi: 10.1016/j.jinf.2019.12.006

42. Kesten S, Chaparro C. Mycobacterial infections in lung transplant recipients. *Chest* (1999) 115(3):741–5. doi: 10.1378/chest.115.3.741

43. Moffatt SD, Demers P, Robbins RC, Doyle R, Wienacker A, Henig N, et al. Lung transplantation: a decade of experience. *J Heart Lung Transplant* (2005) 24(2):145–51. doi: 10.1016/j.healun.2003.10.020

44. Monforte V, Ussetti P, Castejon R, Sintes H, Perez VL, Laporta R, et al. Predictive value of immune cell functional assay for non-cytomegalovirus infection in lung transplant recipients: a multicenter prospective observational study. *Arch Bronconeumol* (2021) 57(11):690–6. doi: 10.1016/j.arbr.2020.12.012

45. Biderman P, Bugaevsky Y, Ben-Zvi H, Bishara J, Goldberg E. Multidrug-resistant Acinetobacter baumannii infections in lung transplant patients in the cardiothoracic intensive care unit. *Clin Transplant* (2015) 29(9):756–62. doi: 10.1111/ctr.12575

46. Fishman JA. Infection in solid-organ transplant recipients. *N Engl J Med* (2007) 357(25):2601–14. doi: 10.1056/NEJMra064928

47. Henkle E, Winthrop KL. Nontuberculous mycobacteria infections in immunosuppressed hosts. *Clin Chest Med* (2015) 36(1):91–9. doi: 10.1016/j.ccm.2014.11.002

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Optimal temperature for the long-term culture of adult porcine islets for xenotransplantation

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Porcine islet xenotransplantation represents a promising therapy for severe diabetes mellitus. Long-term culture of porcine islets is a crucial challenge to permit the on-demand provision of islets. We aimed to identify the optimal temperature for the long-term culture of adult porcine islets for xenotransplantation. We evaluated the factors potentially influencing successful 28-day culture of islets at 24°C and 37°C, and found that culture at 37°C contributed to the stability of the morphology of the islets, the proliferation of islet cells, and the recovery of endocrine function, indicated by the expression of genes involved in pancreatic development, hormone production, and glucose-stimulated insulin secretion. These advantages may be provided by islet-derived CD146-positive stellate cells. The efficacy of xenotransplantation using islets cultured for a long time at 37°C was similar to that of overnight-cultured islets. In conclusion, 37°C might be a suitable temperature for the long-term culture of porcine islets, but further modifications will be required for successful xenotransplantation in a clinical setting.

KEYWORDS

islet transplantation, porcine, xenotransplantation, long-term culture, pancreatic stellate cell

1 Introduction

Pancreatic islet transplantation is a promising therapy for patients with severe diabetes mellitus (DM) and a lack of glucose control. However, this approach is limited by the size of the donor pool (1); therefore, alternative sources of islets are being evaluated, and the adult pig is considered to be an ideal donor. The adult porcine pancreas is similar to the human pancreas in size and contains a large enough number of islets to treat patients with diabetes. In addition, pigs can be readily bred to produce animals of an appropriate size and number.

Porcine-specific carbohydrate antigen (2–6) and the possibility of zoonosis (7, 8) are regarded as major challenges to the use of porcine islet xenotransplantation in the clinic. However, recent progress with gene-editing technology may permit the creation of porcine-specific antigen and porcine-derived pathogen-free pigs (9, 10). Such technological progress increases the feasibility of porcine islet xenotransplantation.

For the success of adult porcine islet xenotransplantation, large numbers of high-quality islets must be obtained. However, porcine islet isolation is technically difficult, because of the vulnerability of islets, compared with islet isolation for other species (11, 12). Previous studies have shown that the expression of collagen is low in the peripheral regions of porcine islets (13), and the basement membranes of porcine islets are easily damaged during routine islet isolation (14). This fragility contributes to the difficulty of obtaining sufficient high-quality islets and culturing and maintaining them.

The establishment of a porcine islet bank, in which a large number of porcine high-quality islets can be stockpiled and accessed on demand, is a pivotal challenge in the establishment of this therapy. For this purpose, the development of a suitable method for the long-term culture of porcine islets that can maintain their viability and function is essential. Long-term culture harbors some merits in increase of the purity of islets and reduction of immunogenicity, which might contribute to the engraftment of porcine islets (15). Previous studies have attempted to characterize the effects of long-term culture on porcine islets and to determine the most appropriate conditions (16, 17). However, detailed knowledge of the effects of long-term culture on porcine islets and the mechanisms involved is still lacking. Therefore, in the present study, we aimed to characterize the effects of long-term culture on porcine islets and identify the optimal temperature for these subcellular structures, to help establish porcine islet xenotransplantation as a viable therapy.

2 Materials and methods

2.1 Study approval

The care of the animals and the experimental procedures complied with the principles of laboratory animal care (Guide for the Care and Use of Laboratory Animals, 8th edition (National Research Council, 2011)), and the experimental protocol was approved by the Animal Care and Use Committee of Fukuoka University (approval number: 2114119).

2.2 Study design

The scheme of this study design is shown in Figure 1. In brief, porcine islets isolated from each microminipig (P112, P114, P116, P117, P118, P119, P120; Supplemental Table 1) were cultured for 28 days by different temperature conditions, 24 or 37°C. On Day 1, 7, 14, 21, and 28, some islets were sampled and used for assessment of morphology and viability of islets. Islet counts for assessing islet equivalents (IEQs)/islet number and residual rate were also performed on the days. Sampling for assessment of glucose-stimulated insulin/glucagon secretion, assessment of insulin/glucagon content, qPCR, flow cytometry, and RNA sequence was performed on Day 1 and 28. Islet xenotransplantation using diabetic nude mice also done on the same days. Medium was changed per 2 – 3 days.

2.3 Animals

Microminipigs (https://fujimicra.co.jp/eng/product.html#whats; Fuji Micra Inc., Fujinomiya, Japan) weighing approximately 25–30

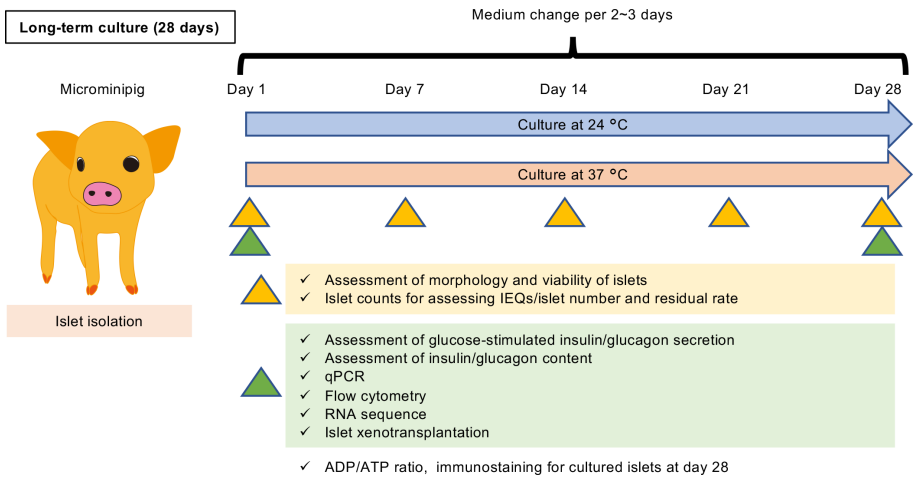

FIGURE 1
Scheme of this study design. Porcine islets isolated from each microminipig (P112, P114, P116, P117, P118, P119, P120; Supplemental Table 1) were cultured for 28 days by different temperature conditions, 24 or 37°C. On Day 1, 7, 14, 21, and 28, some islets were sampled and used for assessment of morphology and viability of islets. Islet counts for assessing islet equivalents (IEQs)/islet number and residual rate were also performed on the days. Sampling for assessment of glucose-stimulated insulin/glucagon secretion, assessment of insulin/glucagon content, qPCR, flow cytometry, and RNA sequence was performed on Day 1 and 28. Islet xenotransplantation using diabetic nude mice also done on the same days. Medium was changed per 2 – 3 days.

kg were used as donor animals. The age of these pigs was 2 – 3 years at islet isolations. Male BALB/c-nu mice (https://www.clea-japan.com/products/immunodeficiency/item_a0010; CLEA Japan Inc., Tokyo, Japan) aged 8–12 weeks were the diabetic recipients. The information of microminipigs is shown in Supplemental Table 1. These animals were housed under specific pathogen-free conditions and had free access to food and water.

2.4 Procurement of pancreata

Total pancreatectomy for organ procurement was performed under general anesthesia using isoflurane (Cat#095-06573; Fujifilm Wako Pure Chemical Co., Osaka, Japan). After laparotomy, an Argyle Salem Sump tube (Covidien Japan Inc., Tokyo, Japan) was inserted into the aorta, ligated in place, and used for heparinization by the intravenous injection of heparin sodium (400 IU/kg, Cat#873334; AY Pharmaceuticals Co., Tokyo, Japan). Subsequently, the pigs were exsanguinated by incising the vena cava in the thoracic cavity, and Belzer UW® Cold Storage Solution (https://amn.astellas.jp/content/dam/jp/amn/jp/ja/di/pdf/blz/Belzer_UW_Cold_Storage_Solution.pdf; Preservation Solutions, Inc. Elkhorn, WI) was infused via the tube while the abdominal organs were cooled using crushed ice. After the flushing of the circulation was completed, total pancreatomy was performed. An 18–24-gauge intravenous catheter (size according to the diameter of the pancreatic duct) was inserted into the pancreatic duct, and cold preservation solution (Cat#035-13121-2; ET-Kyoto solution; Otsuka Pharmaceutical Factory, Inc., Naruto, Japan and ulinastatin; Cat#3999405A2077; Mochida Pharmaceutical Co., Tokyo, Japan) was infused at 1 mL/g pancreas mass.

2.5 Porcine islet isolation and purification

A collagenase solution containing liberase MTF (0.5 g per 1 vial) and thermolysin (15 mg per 1 vial) (Cat#05339880001; Roche CustomBiotech, Penzberg, Germany) was instilled into the disinfected pancreas via the catheter placed in the pancreatic duct. The distended pancreas was cut into several pieces and then placed into a Ricordi Chamber. The digestion was started by commencing the gentle shaking of the Ricordi chamber, while warmed collagenase solution was circulated. When the digestion was stopped, the digested tissue was diluted in RPMI 1640 solution (Cat#11875085; Gibco™) containing 10% inactivated plasma (Fetal Bovine Serum, qualified, United States, Cat#26140079; Gibco™) and ulinastatin and then collected in Belzer UW® Cold Storage Solution. The purification process was performed using IBM 2991 (COBE 2991; Terumo BCT, Tokyo, Japan) by centrifugation with a continuous density gradient between 1.077 g/cm^3 and 1.100 g/cm^3 created using Optiprep (Cat#ST-07820; Veritas Co., Tokyo, Japan). After centrifugation, the gradient density solutions containing highly-purified islets (≥ 70%) were collected. The purity was determined using the percentage of the total number of cell clusters staining positive for dithizone (Cat#D5130; Sigma-Aldrich, St. Louis, MO, USA).

2.6 Islet culture conditions

Islets were cultured in CMRL1066 solution (Cat# 99-603-CV; Corning, Corning, NY, USA) containing 10% fetal bovine serum (FBS), 1% antibiotics, and 200 units/L rapid insulin agent and a 5% CO_2 atmosphere. Isolated islets were cultured at 24°C overnight and then at 24 or 37°C with 5% CO_2 atmosphere for a further 27 days (Figure 1A). Information regarding the donor microminipigs and isolated islets is provided in Supplemental Table 1.

2.7 Assessment of islet number and viability

Islet number was assessed using two methods: counts and IEQs. Islet number was defined as the number of cultured islets, and IEQ was defined as the number of 150 μm-diameter islets for the normalization of islet volume (18).

To assess the viability of islets, isolated islets were stained with Hoechst® 33342 (Cat#H1399; Invitrogen™) and propidium iodide (PI) (Cat#P1304MP; Invitrogen™) and the percentages of viable cells in each islet were calculated using the following formula: ([Hoechst® 33342 stained cells] – [PI stained cells])/[Hoechst® 33342 stained cells] × 100 (%).

Islet number, IEQ, and IEQ/islet number ratio, indicative of the mean size of the islets, and viability were measured 1, 7, 14, 21, and 28 days after islet isolation. Furthermore, morphology, assessed using islet quality score, and the residual percentage of the cells in the cultured islets were also assessed at these time points. The sums of the scores for islet shape (flat at 0 point, moderate at 1 point, oval or round at 2 point); surface (rough at 0 point, moderate at 1 point, smooth at 2 point); damage (fragmented at 0 point, moderate at 1 point, free at 2 point); the number of single cells in the culture medium (numerous at 0 point, moderate at 1 point, a few at 2 point); and the diameters of the islets (all islets <100 μm at 0 point, a few islets >200 μm at 1 point, over 10% of islets >200 μm at 2 point) were used to assess morphology (Supplemental Table 1). The percentage of residual islets was calculated as the percentage of cultured IEQs per IEQ 1 day following islet isolation.

2.8 Glucose-stimulated insulin and glucagon secretion

Glucose-stimulated insulin and glucagon secretion (GSIS and GSGS) were measured using 300 IEQs. Islets were preincubated with 3.3 mM glucose for 60 minutes, after which they were stimulated with glucose at concentrations of 3.3 mM (low glucose) or 16.5 mM (high glucose) for 60 minutes using cell culture inserts (Millicell Hanging Cell Culture Insert, PET 8 μm, 24-well; Cat#PTEP24H48; Merck Millipore, Tokyo, Japan). The porcine insulin and glucagon concentrations in the culture media were measured using an ELISA (LBIS Porcine Insulin ELISA Kit; Cat#AKRIN-013T and Glucagon ELISA Kit; Cat# 29280001; Fujifilm Wako Shibayagi Co., Shibukawa, Japan). The absorbance at 450 nm (optical density, OD450) was determined using an

iMark™ Microplate Absorbance Reader with Microplate Manager® Software 6 (Bio-Rad, Hercules, CA, USA).

2.9 Measurement of insulin and glucagon concentrations

Insulin and glucagon were extracted from 300 IEQs using 1 mL RIPA buffer (Cat#16488-34; Nacalai Tesque, Kyoto, Japan) containing ×100 protease and phosphatase inhibitor cocktails (Cat#07575-51 and Cat#07574-61; Nacalai Tesque). The insulin and glucagon concentrations were measured using an LBIS Porcine Insulin ELISA Kit and a Glucagon ELISA Kit (Wako), respectively.

2.10 Differentiation assay for attached cells

The cells that attached during culture at 37°C were detached by incubation with TrypLE™ Express (Cat#12605010; Gibco™) for 25 minutes, collected, and seeded into wells of a 24-well plate (5×10^4 cells/well). They were incubated in medium containing β-glycerophosphate, dexamethasone, and ascorbate for osteoblasts; or insulin, indomethacin, isobutylmethylxanthine, and dexamethasone for adipocytes (Cat#BMK-R006, Cat#BMK-R007, Cat#BMK-R008, Cat#BMK-R008; Bio future Technology, Tokyo, Japan) for over a week. The extent of differentiation into osteoblasts or adipocytes was assessed using Alizarin Red S and Oil Red O staining, respectively.

2.11 ELISA for transforming growth factor β1

The concentrations of TGF-β1 secreted into the medium by attached cells were measured using Human/Mouse/Rat/Porcine/Canine TGF-β1 Quantikine ELISA Kits (Cat#DB100B; R&D Systems, Inc., Minneapolis, MN, USA), in accordance with the manufacturer's instructions.

2.12 ADP/ATP ratio

The ADP and ATP contents of cultured islets 28 days after isolation were measured using an ADP/ATP Ratio Assay Kit-Luminescence (Cat#346-09911; Dojindo Laboratories, Mashiki, Japan), and the ADP/ATP ratio was calculated. The absorbance at 450 nm for both ADP and ATP was measured using Spark™ 10M multimode microplate reader (Tecan Ltd., Männedorf, Switzerland).

2.13 Real-time reverse transcription polymerase chain reaction analysis

RNA was extracted from porcine islet samples using TRIzol Reagent (Cat#15596026; Invitrogen) and purified using a PureLink® RNA Mini Kit (Cat#12183018A; Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturer's instructions. The mRNA concentrations were equalized using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc.). Reverse transcription was performed using a QuantiTect Reverse Transcription Kit (Cat#205311; Qiagen K.K., Tokyo, Japan). qRT-PCR analysis was performed using a CFX Connect Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and a Thunderbird SYBR qPCR Mix (Cat#QPS-101: Toyobo Co., Ltd., Osaka, Japan). The primers used for real-time RT-PCR are shown in Supplemental Table 2. These were designed by Fasmac Co., Ltd. (Atsugi, Japan). Relative quantitation was performed using LightCycler Software Version 4.1 and the results were normalized to the expression of a reference gene (*Actb*). The data are presented as a fold difference, calculated using the $2^{-\Delta\Delta Ct}$ method.

2.14 RNA sequencing

The extraction, purification, and standardization of RNA extracted from cultured islets (on Days 1 and 28, after culture at 24°C or 37°C) were performed as described above. RNA sequencing libraries were prepared from 1 μg of RNA using a TruSeq Stranded mRNA LT Sample Prep Kit (Cat#20020595; Illumina), as per the manufacturer's instructions. Cluster amplification and 151-bp paired-end sequencing were performed in accordance with the manufacturer's protocol for NovaSeq (Illumina).

RNA sequencing was performed in Cell Innovator (Fukuoka, Japan). Read quality analysis was performed on the raw data using FastQC v0.11.7 (http://bioinformatics.babraham.ac.uk/projects/fastqc/). Quality trimming and adapter clipping were performed using Trimmomatic version 0.38 (19): trailing bases were trimmed if below the mean quality of 15, to a minimum length of 36 bases, and to remove the Illumina adapters. Trimmed reads were mapped to transcripts in the reference data for the Sus scrofa (pig) genome Sscrofa11.1 using the Bowtie2 aligner within RNA-Seq by Expectation-Maximization (RSEM) (20). The abundance of both genes and isoforms was estimated using RSEM in transcripts per million (TPM) counts.

Differentially expressed genes (DEGs) were identified using the edgeR program (21). Normalized counts per million (CPM) values, log fold-changes (logFC), and p-values were obtained from the gene-level TPM counts. The criteria for DEGs were $p \leq 0.05$ and ratios ≥2 fold for upregulated genes.

2.15 Flow cytometry analysis

Porcine islets were dispersed to generate single islet cells using accutase (Cat#12679-54: Nacalai Tesque). These were washed in Hanks' buffer solution containing 10% bovine serum albumin, incubated with a blocking solution, and then incubated with a primary antibody (rabbit anti-insulin (Cat#ab46716, RRID: AB_881326; 1:50; Abcam), mouse anti α-Gal Epitope (Gal alpha1–3 Gal beta1–4 GlcNAc-R) (Cat#ALX-801-090-1, RRID :

AB_2111596; 1:5; Enzo Life Sciences, Inc., Lausen, Switzerland), mouse anti-pig SLA Class I (Cat#MCA2261GA, RRID : AB_324753; 1:10; Bio-Rad Laboratories, Inc.), mouse anti-pig SLA Class II DQ (Cat#MCA1335GA, RRID : AB_322326; 1:10; Bio-Rad Laboratories, Inc.), rabbit anti-CD146 antibody (Cat#ab75769, RRID : AB_2143375; 1:50; Abcam), mouse anti-smooth muscle actin (Cat#ATGA0358; 1:50; NKMAX, Seongnam, Gyeonggi, Republic of Korea), mouse anti-Ki-67 (Cat#F078801, RRID : AB_578672; 1:50; Dako (Agilent), Santa Clara, CA, USA), purified mouse IgM, κ Isotype Ctrl (Cat#401601; RRID : AB_2935847; 1:50; BioLegend, San Diego, CA, USA), purified mouse IgG1, κ Isotype Ctrl (Cat#401402, RRID : AB_2801451; 1:5; BioLegend), or purified rabbit polyclonal Isotype Ctrl (Cat#910801, RRID : AB_2722735; 1:43; BioLegend)). Donkey anti-mouse IgG A647 (Cat#ab150107, RRID : AB_2890037; 1:1,000; Abcam) and donkey anti-rabbit IgG A488 (Cat#ab98488, RRID : AB_10676096; 1:1,000; Abcam) secondary antibodies were used. Fixation/Permeabilization Solution Kit (Cat#554714; BD, Franklin Lakes, NJ, USA) was used for intracellular flow cytometry. Flow cytometry was performed using a BD Accuri™ C6 Plus flow cytometer (BD).

2.16 Induction of diabetes in recipient mice

Diabetes was induced in recipient mice by the intravenous injection of streptozotocin (220 mg/kg body mass; Cat#S0130; Sigma-Aldrich). Mice with blood glucose concentrations exceeding 400 mg/dL were used as diabetic recipients.

2.17 Islet transplantation

Recipient mice were anesthetized using isoflurane, then a dorsal incision was made through the muscle and peritoneum and the left kidney was mobilized outside the abdomen. The renal capsule was peeled off from the parenchyma to prepare the renal subcapsular space for the transplantation of islets. Overnight or 28-day-cultured porcine islets were placed into the space using Gastight Syringes 1002 RN (Hamilton Company Inc., Reno, NV, USA) and Intramedic polyethylene tubing 0.58 mm (Cat#BD427410; Becton Dickinson, Franklin Lakes, NJ, USA). After transplantation, the kidney was replaced into the abdomen and the incision was sutured.

2.18 Assessment of the function of transplanted islets

The function of the transplanted islets was assessed by monitoring the blood glucose and plasma porcine C-peptide concentrations. Normoglycemia was defined as a blood glucose concentration of <200 mg/dL. The plasma porcine C-peptide concentrations were measured using a Porcine C-peptide ELISA (Cat#10-1256-01; Mercodia, Winston Salem, NC, USA).

2.19 Histological assessment

The left kidneys of the recipient mice were dissected following euthanasia and the transplanted islets were evaluated. Cultured islets were embedded in agarose gel for the evaluation of any changes that had occurred during long-term culture. Three-micrometer-thick sections were either stained with hematoxylin and eosin (HE) or subjected to immunohistochemistry (for insulin to identify islets, for von Willebrand factor (vWF) to identify vessels, for porcine C-peptide to identify porcine islets, for mouse C-peptide to identify mouse islets, for Ki67 to evaluate cellular proliferation, for collagen I or fibronectin to identify ECM in the cultured islets, for integrin β1 or E-cadherin (adhesion factors), for CD146 or α smooth muscle actin (SMA) to identify PSCs, for PDX-1 (a marker of β cells or pancreatic progenitors), or α-Gal (a carbohydrate antigen)). The primary antibodies used were guinea pig anti-insulin (Cat#A056401-2, RRID : AB_2617169; 1:100; Agilent, Dako, Tokyo, Japan), rabbit anti-insulin (Cat#ab181547, RRID : AB_2716761; 1:1,000; Abcam, Cambridge, UK), sheep anti-glucagon (Cat#ab36232, RRID : AB_732575; 1:100; Abcam), rabbit anti-somatostatin (Cat#ab103790, RRID : AB_10711731; 1:500; Abcam), mouse anti-pig C-peptide (Cat#MAA447Po21; 1:200; Cloud-Clone Corp. MAA447Po21, Katy, TX, USA), mouse anti-mouse C-peptide (Cat#NBP1-05433, RRID : AB_1556271; 1:500; Novus Biologicals NBP1-05433, Centennial, CO, USA), rabbit anti-vWF antibody (Cat#ab179451, RRID : AB_2890242; 1:100; Abcam), rabbit anti-Ki67 antibody (Cat#ab66155, RRID : AB_1140752; 1:200; Abcam), rabbit anti-collagen I antibody (Cat#ab138492, RRID : AB_2861258; 1:500; Abcam), rabbit anti-fibronectin (Cat#ab2413, RRID : AB_2262874; 1:100; Abcam), rabbit anti-integrin β1 (Cat#ab179471, RRID : AB_2773020; 1:1,000; Abcam), rabbit anti-E cadherin (Cat#ab40772, RRID : AB_731493; 1:500; Abcam), rabbit anti-CD146 (1:200; Abcam), rabbit anti-αSMA (Cat#ab15734, RRID : AB_443242; 1:200; Abcam), mouse anti-PDX1 (Cat#sc-390792, RRID : AB_2938928; 1:100; SantaCruz), and mouse anti-α-Gal (1:5; Enzo Life Sciences, Farmingdale, NY, USA). After incubation with a primary antibody, donkey anti-mouse IgG (H+L) Alexa488 (Cat#715-547-003, RRID : AB_2340851; 1:100; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA), Alexa 488-conjugated donkey anti-guinea pig (Cat#715-547-003, RRID : AB_2340472; 1:100; Jackson ImmunoResearch Laboratories, Inc.), Alexa Fluor® 488 AffiniPure goat anti-rat IgG (H+L) (Cat#112-545-003, RRID : AB_2338351; 1:100; Jackson ImmunoResearch Laboratories, Inc.), Alexa Fluor® 647 AffiniPure goat anti-rabbit IgG (H+L) (Cat#111-605-144, RRID : AB_2338078; 1:100; Jackson ImmunoResearch Laboratories, Inc.), Cy3-conjugated goat anti-rabbit (Cat#111-165-144, AB_2338006; 1:100; Jackson ImmunoResearch Laboratories, Inc.), Alexa 488 anti-goat, Alexa 647 anti-goat, Alexa 647 anti-mouse, and Cy3 anti-goat were used as secondary antibodies. Nuclear staining was performed using 4′,6-diamidino-2-phenylindole (DAPI: Cat#340-07971; Dojindo). Histological images were obtained using a BZ-X700 microscope (Keyence, Itasca, IL, USA) and immunostaining was quantified using ImageJ® software (https://imagej.nih.gov/ij/index.html; National Institutes of Health, Bethesda, MD, USA).

2.20 TUNEL assay

TdT-mediated dUTP nick end labeling (TUNEL) assay was performed to long-term cultured islets using TACS2 TdT *in situ* Apoptosis Detection Kit-Fluorescein (Cat# 4812-30-K; R&D Systems), to detect apoptotic islet cells. Double staining for insulin, glucagon or somatostatin was done to the same specimen for detecting apoptotic cells in β, α, δ cells, respectively.

2.21 Statistical analysis

The blood glucose and plasma C-peptide concentrations and the changes in blood glucose concentration during glucose tolerance testing were compared using two-way repeated measures analysis of variance, followed by Dunnett's test, as appropriate. Data are presented as the mean ± standard error of the mean. $p < 0.05$ was used to define statistical significance. All tests were two-sided. Statistical analyses were conducted using JMP®12.0.0 (SAS Institute Inc., Cary, NC, USA).

3 Results

3.1 Culture at 37°C stabilizes the morphology and promotes the cellular proliferation of long-term cultured porcine islets

First, we aimed to characterize long-term (28-day) cultured islets. Figure 2 shows the morphological changes of the islets during the long-term culture. On Day 0 (i.e. at preculture), the porcine islets in this assay accompanied with round shape (islet shape: 2 point) and smooth surface (surface: 2 point). There were no damaged islets (damage: 2 point) with few dispersed single cells (the number of single cells: 2 point). On the other hand, a few over 200 μm-sized islets were seen (the diameters of the islets: 1 point). The islet quality score of these islets was 9 points (Figures 2A, B). On Day 1, the islet quality score was declined to 7 points because the shape of islets became flat and fragmented islets were moderately shown (Figures 2A, B, C, E). After that, the surface of the cultured islets became smooth over time, especially those cultured at 37°C. Most of the islets cultured at 37°C became solid, with a smooth surface, between days 7 and 14 (Figure 2A). The islet quality score was 10 points during the span (Figure 2B). On the other hand, most islets cultured at 24°C had a rough and frayed surface at these time points (Figure 2A). The islet quality score was lower comparing with islets cultured at 37°C during the observation span (Figure 2B). Final islet quality score on Day 28 was 9 points at 37°C and 8 point at 24°C. Score in islet shape, surface, damage and the diameter of the islets was exceeded in 37°C, while there were no change in the number of single cells (Figures 2C–G).

In this morphological assessment, aggregation of the islets was noticeable after 21 days of culture at 37°C (Figure 2A). We considered that these morphological changes might be the result of higher expression of extracellular matrix (ECM) proteins and adhesion factors that strengthen cell-to-cell junctions. As representative ECM proteins, the expression of collagen I and fibronectin in long-cultured islets was evaluated by qPCR and immunofluorescence staining. The expressions of *Col1A1*, which encodes collagen I, tended to be high in long-term cultured islets, and especially in those cultured at 37°C, while there were no significant differences among the three culture conditions (Figure 3A; Supplemental Figure 1A). Collagen I was mainly expressed on the surfaces of the endocrine cells, but the level of expression was very weak (Figure 3B; Supplemental Figure 1B). The collagen I-positive area of the long-term cultured islets was significantly larger in those cultured at 37°C than in those cultured at 24°C ($p = 0.005$; Figure 3C). In contrast, the expression of *Fn1*, which encodes fibronectin, was significantly lower, as was the area of the islets that was immunopositive for fibronectin (Supplemental Figures 2A–C).

Integrin β1 and E-cadherin are the major adhesion factors that contribute to cell-to-cell junctions. The former is a receptor for various ECMs, including collagen and fibronectin, and forms focal adhesions, multi-protein complexes that mediate contact between cells and the ECM. Integrin β1 is expressed on the surfaces of endocrine cells in mouse islets (22), and E-cadherin is expressed on cell membranes, where it interacts with similar molecules on other cells, to form cell-cell adherens junctions (23). In the present study, we found that the expression of *Intgb1*, which encodes integrin β1, in islets cultured at 37°C for 28 days was significantly higher than in islets cultured overnight and at 24°C for 28 days, in each isolation (#1 - #3 islet isolation) (37°C Day 28 vs. Day 1: $p = 0.013$, $p = 0.001$, $p = 0.049$; 37°C Day 28 vs. 24°C Day 28: $p = 0.016$, $p = 0.040$, $p > 0.05$; Supplemental Figure 1C; Figure 3D). Furthermore, the expression of integrin β1 was high in cell-to-cell junctions after culture at 37°C, and lower after culture at 24°C ($p < 0.001$; Figures 3E, F). The expression of *Cdh1*, which encodes E-cadherin, was higher after long-term culture, especially at 24°C (Supplemental Figure 2D), but the expression of E-cadherin protein was relatively low at both 24°C and 37°C (Supplemental Figures 2E, F). Thus, the expression of collagen I and integrin β1 following long-term culture at 37°C is consistent with an enhancement of cell-ECM junctions, which might contribute to the stability of the islets.

We also found that long-term culture was associated with an increase in islet size. The IEQ/islet number ratio, an index of the mean size of an islet, increased during long-term culture, and was higher after culture at 37°C than at 24°C (Figures 2A, 4A). We considered that the larger islet size after culture at 37°C might reflect greater cellular proliferation. To elucidate the mechanism underlying this difference, we assessed the expression of Ki67, a marker of cellular proliferation, in the islets, and found few Ki67-positive cells, especially at 37°C (ratio of Ki67-positive cells: $p = 0.02$; Figures 4B, C). We next quantified the population of insulin/Ki67 double-positive cells in the long-term cultured islets using flow cytometry, and found that long-term culture increased the numbers of Ki67-positive cells in islets cultured at 24°C, and especially at 37°C, indicating a promotion of cellular proliferation ($Insulin^-/Ki67^+$: 0.00503% on Day 1, 0.11% at 24°C on Day 28, 1.06% at 37°C on Day 28; $Insulin^+/Ki67^+$: 0.00902% on Day 1, 0% at 24°C on Day 28, 0.87% at 37°C on Day 28 for #1 islet isolation; $Insulin^+/Ki67^+$: 0.22% at 24°C on Day 28, 4.57% at 37°C on Day 28 for #2 islet isolation;

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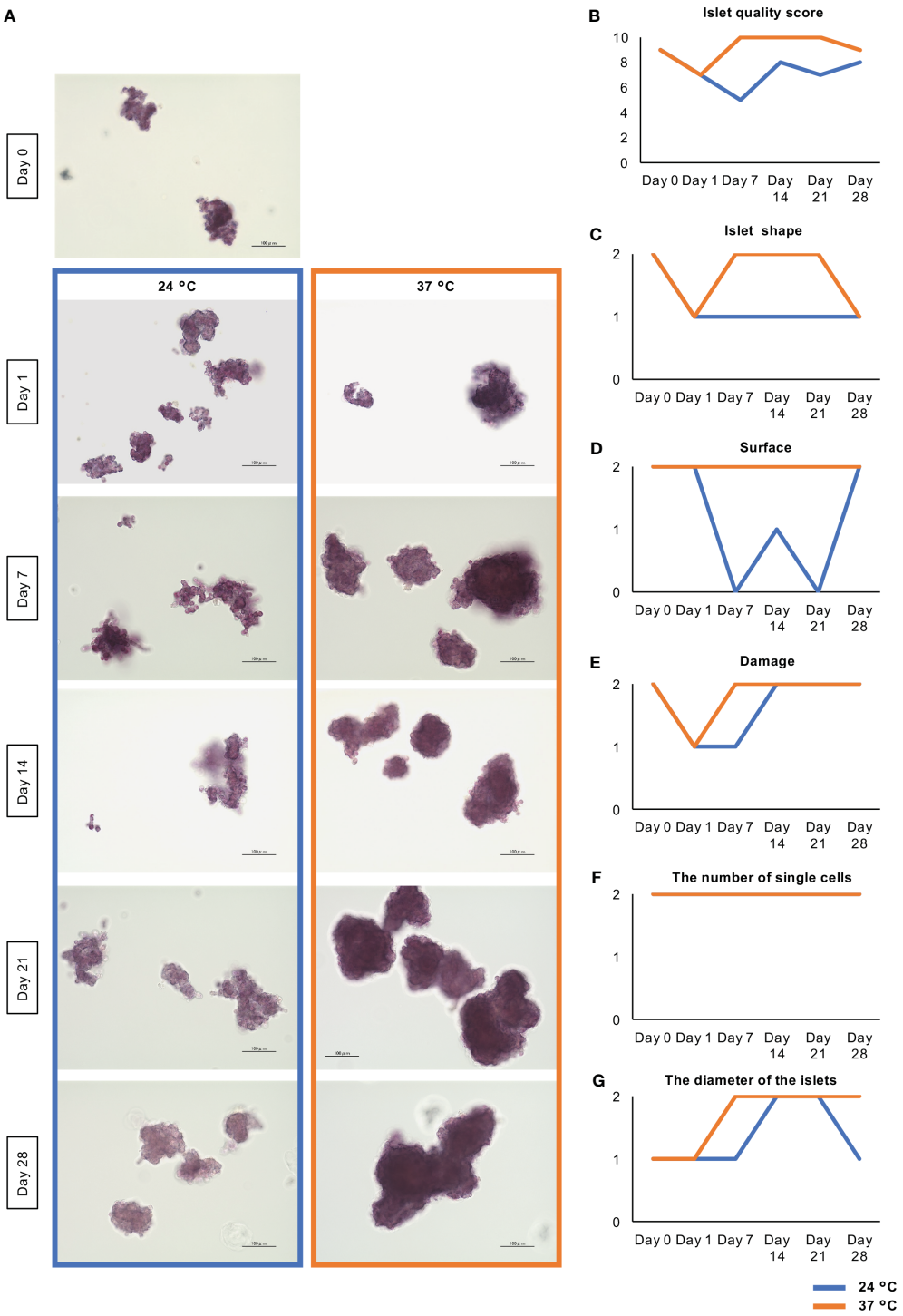

FIGURE 2
Morphological change of long-term cultured porcine islets. **(A)** Dithizone-stained islets isolated from one pig cultured at 24°C (blue) and 37°C (orange) on Days 0, 1, 7, 14, 21, and 28. Most of the islets cultured at 37°C became solid with a smooth surface throughout the observation span. On the other hand, most islets cultured at 24°C had a rough and frayed surface. Scale bar: 100 µm. **(B)** Change of the islet quality score. This score is composed of he sums of the scores for islet shape (flat at 0 point, moderate at 1 point, oval or round at 2 point); surface (rough at 0 point, moderate at 1 point, smooth at 2 point); damage (fragmented at 0 point, moderate at 1 point, free at 2 point); the number of single cells (numerous at 0 point, moderate at 1 point, a few at 2 point); and the diameters of the islets (all islets <100 µm at 0 point, a few islets >200 µm at 1 point, over 10% of islets >200 µm at 2 point). **(C-G)**. Change of the parameters for calculating islet quality scores including islet shape **(C)**, surface **(D)**, damage **(E)**, the number of single cells **(F)**, and the diameter of the islets **(G)**.

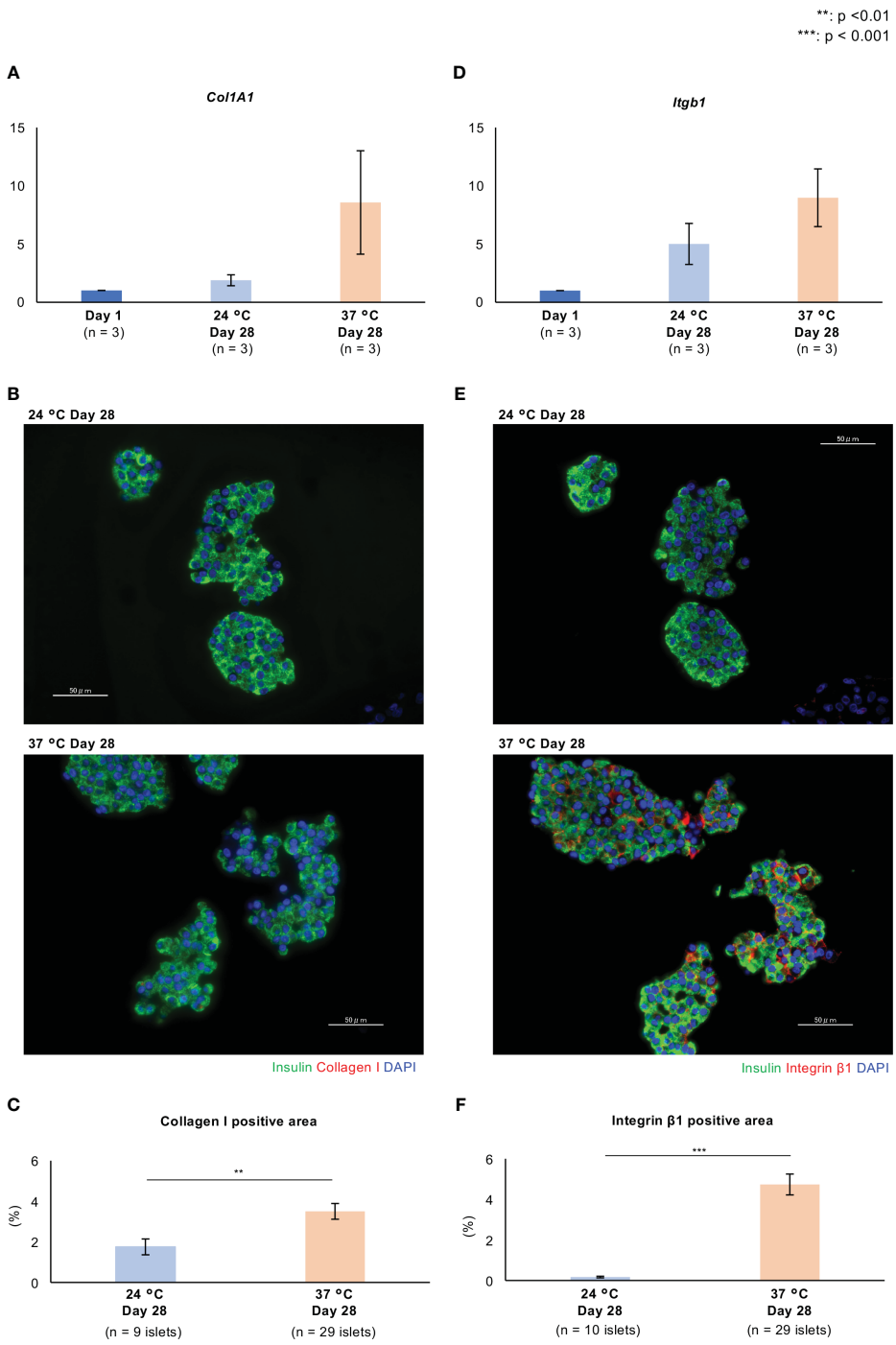

FIGURE 3
Expression of extracellular matrix proteins and adhesion factors in long-term cultured porcine islets. **(A, D)**. Expression of *Col1a1* **(A)** and *Itgb1* **(D)** in cultured porcine islets. *Col1a1* and *Itgb1* encode collagen I and integrin β1, respectively. The ratios of the expression between Day 1 and other culture conditions (24°C Day 28 and 37°C Day 28), quantified using the $2^{-\Delta\Delta Ct}$ method. n = 3 islet isolations. **(B, E)**. Histology of isolated islets which were cultured at 24°C (upper) and 37°C (lower). Sections are immunostained with anti-insulin (green), anti-collagen I red in **(B)**, and anti-integrin β1 red in **(E)** antibodies. **(C, F)**. Collagen I **(C)** and integrin β1 **(F)**-positive areas per islet area. DAPI (blue) counterstaining for nuclei was used. ** $p < 0.01$, *** $p < 0.001$. Scale bar: 50 μm.

Figure 4D). These data indicate that the proliferation of islet cells, and especially β cells, is more highly upregulated after long-term culture at 37°C than at 24°C.

Immunofluorescence staining for Ki67 and PDX1, which is a marker of endocrine differentiation that is expressed by pancreatic progenitors and adult β cells, showed Ki67-positive and PDX-negative cells in long-term cultured islets, especially islets at 37°C (Figure 4E). We speculate that these cells might be not only non-β cells, but also extra-endocrine cellular components that contribute to the increase in islet size.

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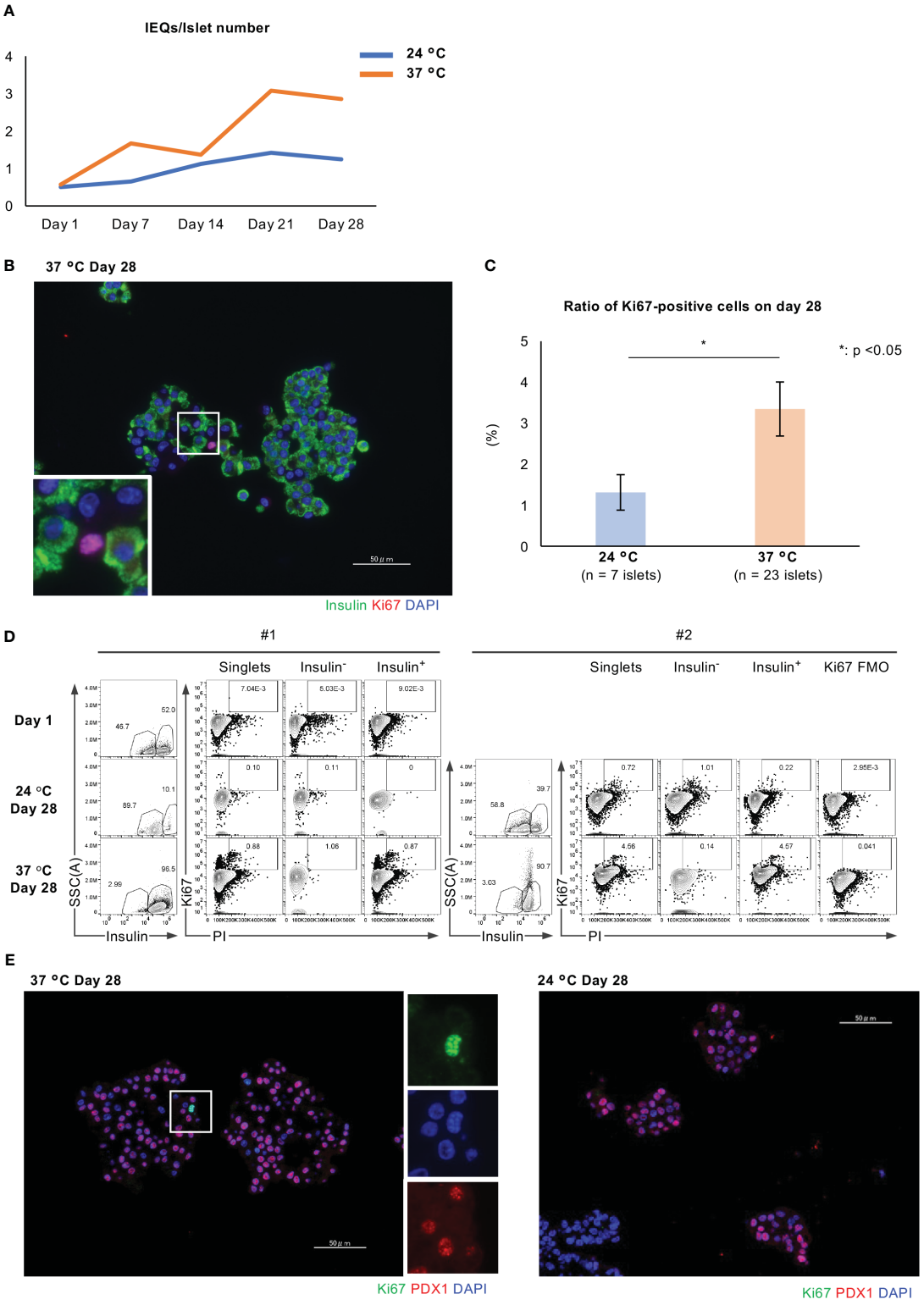

FIGURE 4
Cellular proliferation of long-term cultured porcine islets. **(A)** Change in the IEQ/islet number of long-term cultured porcine islets isolated from a pig at 24°C (blue) and 37°C (orange). IEQ/islet number indicates the average size of islets. **(B)** Histology of isolated islets which were cultured at 37°C, immunostained for insulin (green) and Ki67 (red). **(C)** Ratio of Ki67-positive islet cells per total islet cells in the islets cultured at 24°C (pale blue) or 37°C (pale orange) for 28 days. **(D)** Results of the flow cytometry analysis of Ki67-positive cells, Ki67-positive and insulin-negative cells, and insulin/Ki67 double-positive cells in islets on Day 1, after culture at 24°C on Day 28, and after culture at 37°C on Day 28. #1 and #2 means the number of islet isolations, respectively (i.e. islet isolation number 1 and 2). We had two islet isolations for these flow cytometry analyses. **(E)** Histology of long-term cultured islets at 37°C (left) and 24°C (right) on Day 28. They were immunostained for Ki67 (green) and PDX1 (red), and counterstained using DAPI (blue). * $p < 0.05$. Scale bar: 50 μm.

3.2 Long-term culture does not reduce the viability of porcine islets

Figure 5 shows the viability of long-term cultured porcine islets. Regarding the percentage of residual islets (residual islet equivalents, compared to Day 1), the percentage of residual islets similarly decreased after culture at either 24°C or 37°C (Figure 5A). On the other hand, over 95% of the viability of the islet cells was retained during long-term culture at either temperature, with no significant difference between the two (Figures 5B, C). Furthermore, there was no significant difference in the ADP/ATP ratio, indicative of damage to mitochondria, between the two conditions after 28 days of culture (Figure 5D). We also assessed the apoptosis of long-term cultured islets, and could not detect any apoptotic cells in β, α and δ cells of the islets at both 24°C and 37°C (Figures 5E-G).

3.3 Long-term culture of porcine islets at 37°C is associated with partial recovery of endocrine function, associated with increases in the expression of genes involved in pancreatic differentiation

Figures 6A–E and Supplemental Figures 3A-E show the expression of genes involved in pancreatic differentiation (*Pdx1* and *Neurog3*) and encoding hormones (*Ins*, *Gcg*, *Sst*). Long-term culture at 37°C increased the expression of all of these genes. GSIS and the insulin content of the islets were markedly reduced by 28 days of culture at either 24°C or 37°C (Figure 6F); however, the secretion of insulin in response to a high glucose concentration and the insulin content tended to be higher in islets cultured at 37°C than in those cultured at 24°C (Figures 6F, G; Supplemental Figures 3F, G). These data imply that long-term culture at 37°C is associated with the partial recovery of the endocrine function of porcine islets. The GSGS and glucagon content of the islets was attenuated by long-term culture (Supplemental Figures 4A, B).

3.4 Porcine islets contain multipotent stem cells that might mediate cellular proliferation and the recovery of endocrine function when cultured long-term at 37°C

As shown in Figure 5A, the percentage of residual islets similarly decreased after culture at either 24°C or 37°C. Cells derived from islets attached to and proliferated on the bottom of culture flasks at 37°C, but not at 24°C. This phenomenon trapped many islets and contributed to the reduction in percentage of residual islets (Figure 7A).

Although the attachment of cells caused a loss of long-term cultured islets, we hypothesized that this might provide some benefits to the islets with respect to cellular proliferation and endocrine function. Therefore, the characteristics of the islet cells that attached to the culture vessel during long-term culture at 37°C were assessed. We first evaluated the multipotency of these attached cells using a Mesenchymal Stem Cell (MSC) Identification Kit. Some of the cells were capable of differentiating into osteoblasts and inducing calcification (Figure 7B), or into adipocytes containing lipid droplets (Figure 7C). Furthermore, the attached cells produced transforming growth factor beta 1 (TGF-β1) (Figure 7D). Thus, the attached cells include MSCs that are multipotent and secrete substances with paracrine effects. We speculate that these cells might be pancreatic stellate cells (PSCs), which are pancreas-resident stem cells.

PSCs play a critical role in pancreatic fibrosis in chronic pancreatitis and pancreatic cancer (24) and act as multipotent stem cells, generating pancreatic β cells (25). There are several specific markers of PSCs, and we used two in the present study: CD146 and α-smooth muscle actin (SMA). CD146 is also known as melanoma cell adhesion molecule (26) and is a marker of early MSCs (27–29). CD146-positive MSCs have a high level of multipotency, and can give rise to endothelial cells, osteoblasts, chondrocytes, and adipocytes (30, 31). αSMA is also a marker of activated PSCs (32). Flow cytometry analysis of the attached cells derived from porcine islets revealed that they included CD146-positive and αSMA-positive cells, and the numbers of each were higher comparing with those in islets cultured at either 24°C or 37°C (Figure 7E). The immunofluorescence staining of sections of porcine pancreas revealed a few CD146-positive cells in both intra- and extra-islet tissues (Figure 7F), and also in long-term cultured islets, especially if they were cultured at 37°C ($p = 0.04$; Figure 7G, H). Some of the CD146-positive cells were also immunopositive for αSMA (data not shown). We also assessed the characteristics of the CD146-positive cells in the islets as the potential progenitors of pancreatic cells. Immunofluorescence staining of long-term cultured islets for CD146/PDX1 revealed the presence of double-positive cells, which might represent pancreatic progenitors that can differentiate into endocrine cells (Figures 7I, J).

3.5 The expression of α-Gal is not high in porcine islets

We next aimed to determine whether long-term culture reduces the immunogenicity of cultured islets because this would increase the chances of successful xenotransplantation (33). Supplemental Figure 5A, B shows the expression of *Ggta1p* and *Cmah*. Long-term culture reduced the expression of *Cmah* in islets cultured at 24°C ($p = 0.0008$; Supplemental Figure 5B). The expression of *Ggta1p* was reduced by long-term culture at 37°C ($p = 0.0016$), but the expression level was significantly higher after culture at 24°C than after culture at 37°C ($p = 0.0028$; Supplemental Figure 5A).

Next, we performed a flow cytometry analysis of long-term cultured porcine islets isolated from three pigs (#1 - #3 islet isolation) to further characterize the expression of α-Gal. Interestingly, no α-Gal-positive islet cells were obtained from #1 or #3 islet isolation (Supplemental Figure 5C), but they were identified in the islets obtained from #2 islet isolation, and there were more in the islets cultured at 37°C than at 24°C (Supplemental Figure 5C). Immunofluorescence staining for α-Gal did not show

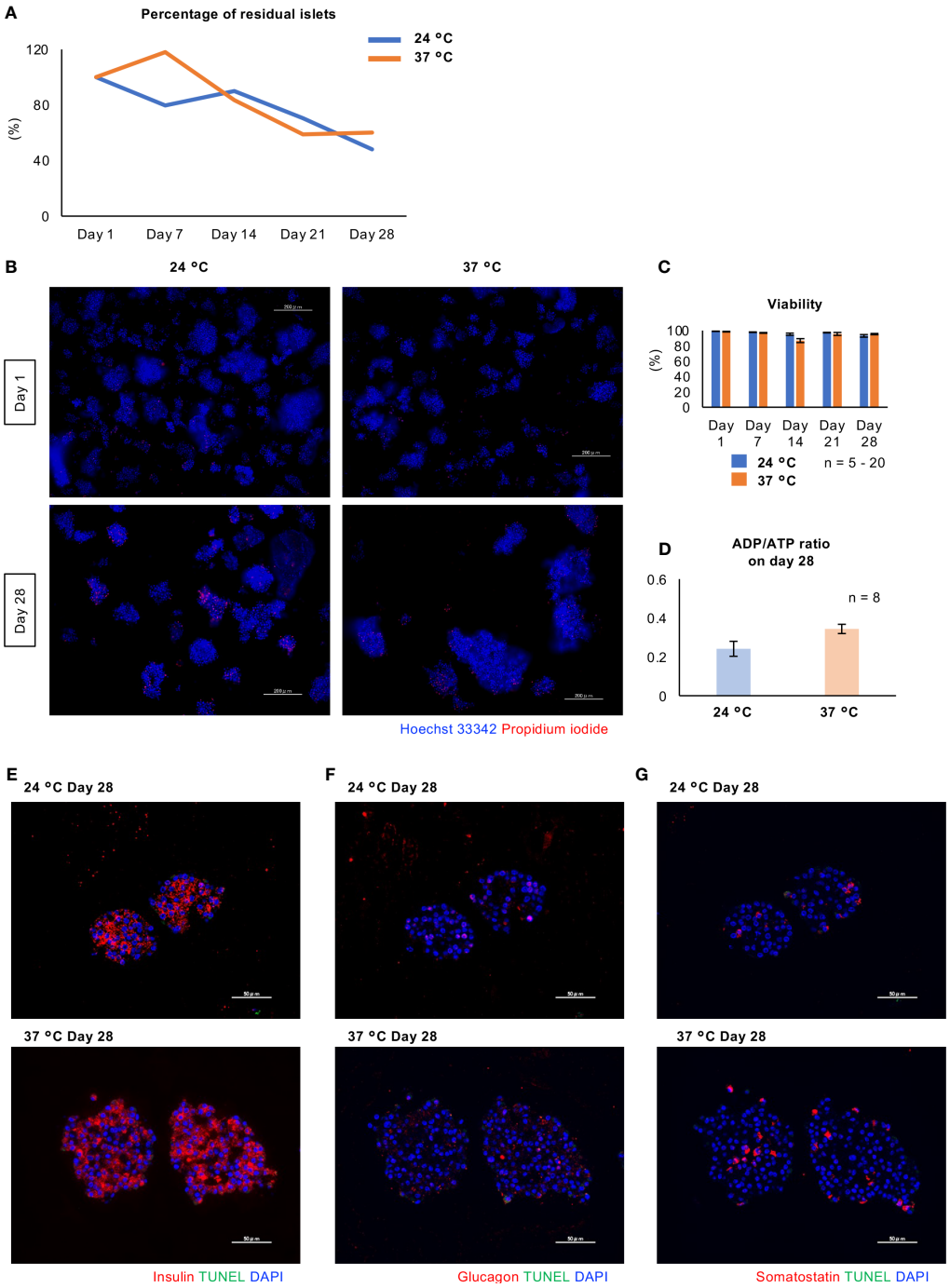

FIGURE 5
Viability of long-term cultured porcine islets. **(A)** Change of percentage of residual islets isolated from a pig during long-term culture (24°C: blue, 37°C: orange). **(B)** Cultured islets at 24°C (left) and 37°C (right), stained with Hoechst 33342 (blue) and propidium iodide (red) on Days 1 and 28. **(C)** Viability of the porcine islets during the culture. Viability was assessed by the formula ([Hoechst® 33342 stained cells] – [PI stained cells])/[Hoechst® 33342 stained cells] × 100 (%). **(D)** ADP/ATP concentration ratio, indicating mitochondrial damage, for the cultured islets on Day 28.(Prior to **(D)**) Scale bar: 100 μm. **(E - G)** TUNEL staining (green) for long-term cultured islets. Double staining with insulin (**E**; red), glucagon (**F**; red) and somatostatin (**G**; red). DAPI (blue) was used for counter staining. Scale bar: 50 μm.

any α-Gal positive cells in islets cultured at either 24 or 37°C (Supplemental Figure 5D), but the endothelium of the abdominal artery showed strong immunostaining (Supplemental Figure 5E). Thus, we could not find evidence of α-Gal expression in porcine islets. The α-Gal-positivity obtained using flow cytometry might have been a pseudo-positive finding because of the high level of background staining (Supplemental Figure 5C).

As a further assessment of immunogenicity, we performed flow cytometry to identify cultured islet cells that expressed SLA class I or SLA class II DQ. Swine leukocyte antigens (SLAs) are porcine major histocompatibility (MHC) antigens, which are mediators of humoral rejection following transplantation (34). Both SLA classes I and II can cause the rejection of xenotransplants because they can be targeted by human

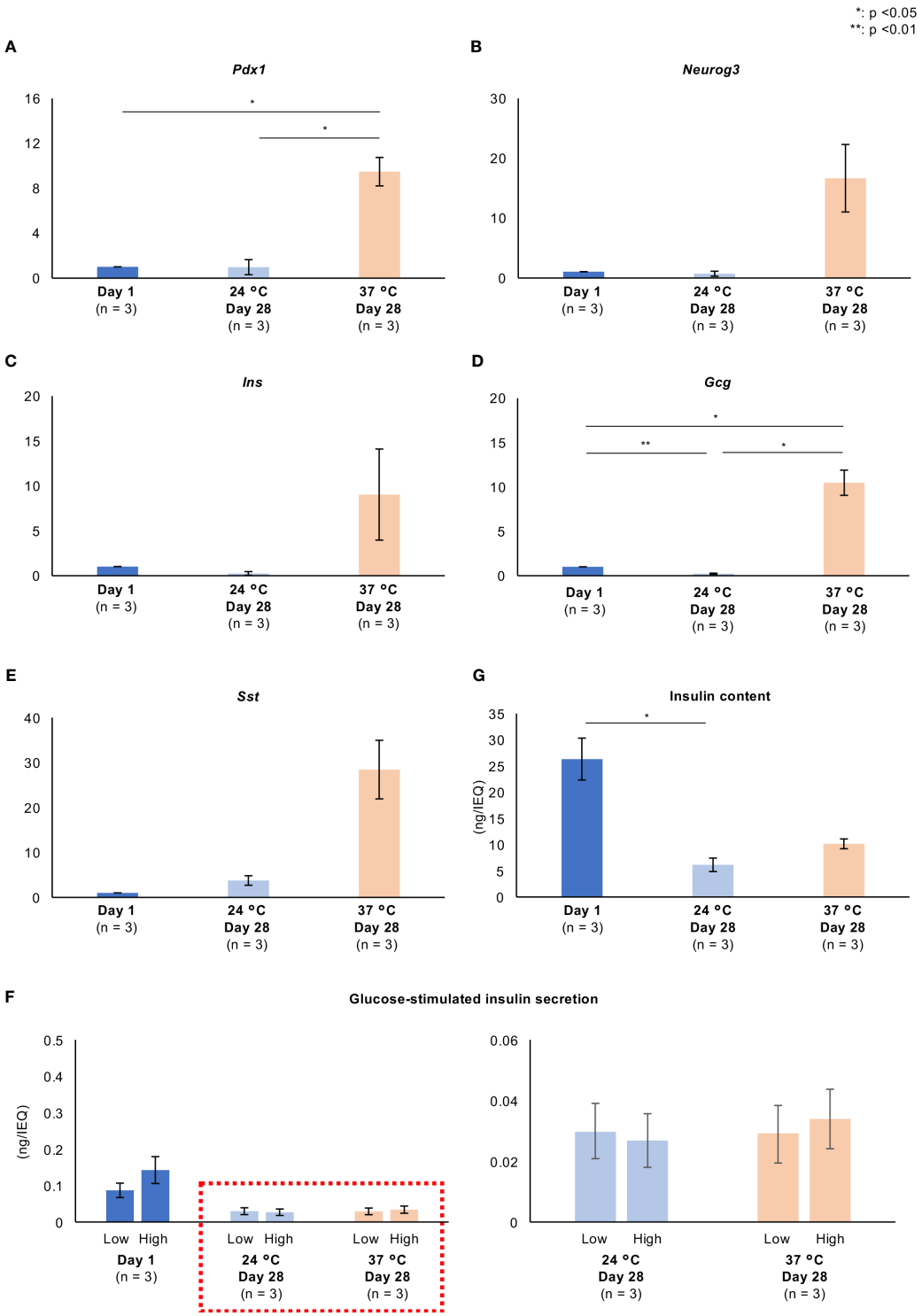

FIGURE 6
Pancreatic differentiation and endocrine function of long-term cultured porcine islets. **(A–E)**. Expression of genes involved in pancreatic differentiation **(A)**: *Pdx1*, **(B)**: *Neurog3*) and encoding hormones **(C)**: *Ins*, D: *Gcg*, E: *Sst*) in cultured porcine islets. Day 1: blue, 24°C Day 28: pale blue, and 37°C Day 28: pale orange. The ratios of the expression after 1 day and 28 days (both temperatures) are shown as $2^{-\Delta\Delta Ct}$ values. **(F)** Glucose-stimulated insulin secretion by cultured islets in response to low and high glucose stimulations. **(G)** Insulin content per islet. n = 3 islet isolations. * $p < 0.05$, ** $p < 0.01$.

leukocyte antigen-specific antibodies (35). We found that SLA class I was expressed by overnight-cultured porcine islets (Day 1) (Supplemental Figure 5F), and SLA class II DQ was expressed in islets from #1 islet isolation, but not in those from #3 islet isolation (Supplemental Figure 5F). Although the number of SLA class I-positive cells did not change, the number of SLA class II DQ-positive cells increased during long-term culture, contrary to our expectation that the expression would decrease.

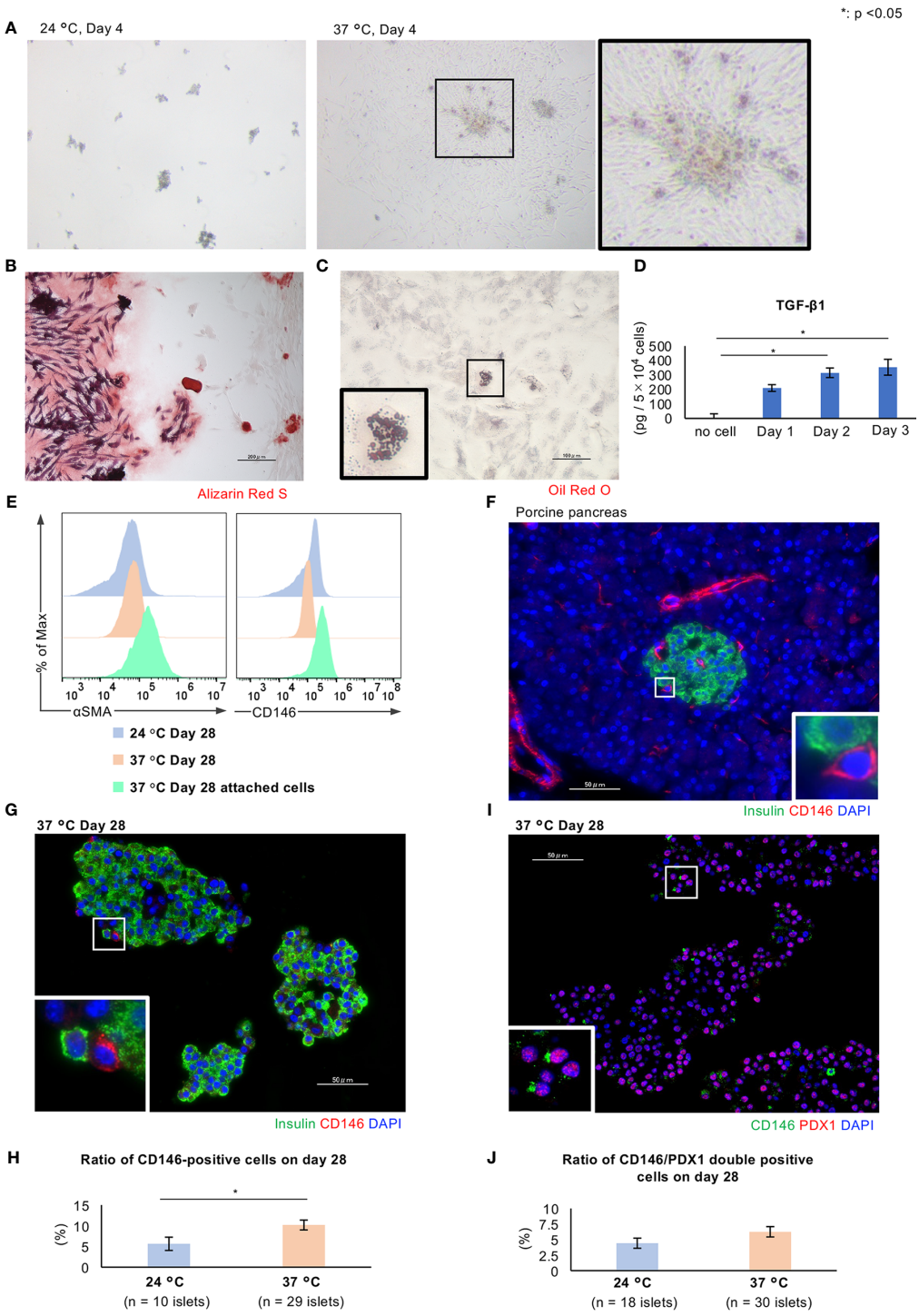

FIGURE 7
Characteristics of CD146-positive cells in long-term cultured porcine islets. **(A)** Porcine islets cultured at 24°C (left) or 37°C (center) for 4 days. Spindle cells derived from the islets attached to the bottom of the culture flask and proliferated (center and right). **(B)** Differentiation of the attached cells into osteoblasts. Alizarin Red S staining to demonstrate differentiated osteoblasts. **(C)** Differentiation of the attached cells into adipocytes. Oil red O staining to demonstrate differentiated adipocytes. **(D)** Concentration of TGF-β1 secreted by attached cells. **(E)** Results of flow cytometry analysis of the attached cells (pale green) and islets cultured at 24°C (pale blue) or 37°C (pale orange) for the detection of CD146 (right) or αSMA (left)-positive cells. **(F)** CD146-positive cells in the porcine pancreas immunostained for insulin (green) and CD146 (red). **(G)** Histology of islets cultured at 37°C, immunostained for insulin (green) and CD146 (red). **(H)** Ratio of CD146-positive cells in islets cultured at 24°C (pale blue) or 37°C (pale orange) for 28 days. **(I)** Histology of islets cultured at 37°C, immunostained for CD146 (green) and PDX1 (red). DAPI (blue) was used for counterstaining. **(J)** Ratio of CD146/PDX1-positive cells in islets cultured at 24°C (pale blue) or 37°C (pale orange) for 28 days. * $p < 0.05$. Scale bar: 50 μm.

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3.6 RNA sequencing revealed that islets cultured long-term at 37°C demonstrate higher levels of insulin secretion, focal adhesion, and cellular proliferation

Sequencing of RNA extracted from porcine islets and cultured overnight, or at 24°C or 37°C for 28 days, followed by data analysis using the Database for Annotation, Visualization and Integrated Discovery (DAVID: https://david.ncifcrf.gov) was next performed to further characterize the effects of long-term culture. We found that the expression of genes involved in ion transport (*Kcnip1*, *Calb1*), cell differentiation (*Ush2a*, *Th*, *Fev*), and the downregulation of apoptosis (*Scg2*) was higher in islets cultured for 28 days at 37°C (Table 1) than in those cultured at 24°C. We then used the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway classification to interrogate the functions of the upregulated genes. A comparison with 24°C-cultured cells on Day 28 showed that "Metabolic pathway", "Protein processing in endoplasmic reticulum", and "Insulin secretion" were upregulated in the 37°C-cultured cells (Table 2). Specifically, genes involved in insulin secretion, ion transport, including the calcium signaling pathway, and the cAMP signaling pathway were upregulated at 37°C. We hypothesized that these differences might have been induced by a thermosensitive system that included transient receptor potential (TRP) channels, and therefore measured the expression of genes encoding these channels. Of these, *Trpm5* (log-fold difference 5.09, p = 1.45E–44) was found to be upregulated in cells cultured for 28 days at 37°C.

Figure 8 shows a heat map (Figure 8A) and volcano plot (Figures 8B–D), which display the up- and downregulated genes for comparisons of the Day 1, 24°C Day 28, and 37°C Day 28 groups. Islets cultured for 28 days at 37°C showed differences in the expression of genes involved in the repression of immunity (*Tcim*), temperature-dependent insulin secretion (*Trpv4*), cell cycle promotion (*Gadd45b*, *Gadd45g*, *Ccnb2*, and *Ccnb3*), adhesion and the ECM (*Tnr*, *Itgb8*, *Itgb6*, *Itga4*, and *Lama1*), and endocrine function and pancreatic differentiation (*Glp1r*, *Rapgef4*, *Gpr119*, *Pdx1*, and *Gcg*).

3.7 The effects of the transplantation of islets cultured long-term at 37°C were not inferior to those of overnight-cultured islets

Finally, we performed islet xenotransplantation into diabetic nude mice using long-term cultured porcine islets. The number of transplanted islets was 2,000 and 4,000 IEQs (purity: over 90%, tissue volume: less than 100 μL per 2,000 IEQs). Two thousand IEQs is considered as the minimum number which enables to improve blood glucose. The blood glucose concentrations of the mice were not normalized when they were transplanted with islets

TABLE 1 The 20 most upregulated genes in islets cultured for a long period at 37°C, compared with 24°C.

log fold-changes, p-value	Gene symbol and name	Accession number
7.16 4.76E-30	SLIT1 (Slit guidance ligand 1)	XM_021072814
7.12 1.92E-29	CAPS (Calcyphosine)	NM_001244975
6.89 5.46E-144	SCG2 (Secretogranin II)	NM_001012299
6.76 1.98E-24	ST8SIA2 (ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 2)	NM_001315676
6.44 1.14E-20	USH2A (Usherin)	XM_021064292
6.33 2.10E-73	MOXD1 (Monooxygenase DBH like 1)	XM_001926931
6.29 9.83E-121	HEPACAM2 (HEPACAM family member 2)	XM_003357438
6.05 1.40E-71	KCNIP1 (Potassium voltage-gated channel interacting protein 1)	NM_001031777
5.86 4.81E-23	PRODH2 (Proline dehydrogenase 2)	XM_021097109
5.84 2.32E-114	TH (Tyrosine hydroxylase)	XM_021085452
5.84 2.77E-48	DPEP2 (Dipeptidase 2)	XM_003355779
5.59 3.69E-60	GRIK1 (Glutamate ionotropic receptor kainate type subunit 1)	XM_003358905
5.59 1.17E-12	FEV (FEV transcription factor, ETS family member)	XM_021075453
5.55 2.33E-72	DGKB (Diacylglycerol kinase beta)	XM_021102437
5.54 3.66E-82	CALB1 (Calbindin 1)	NM_001130226
5.45 1.92E-36	GALNTL6 (Polypeptide N-acetylgalactosaminyltransferase like 6)	XM_021072439
5.37 5.91E-44	LDHD (Lactate dehydrogenase D)	XM_021093784
5.20 1.96E-20	CLVS2 (Clavesin 2)	XM_013992600
5.10 2.27E-72	SSTR3 (Somatostatin receptor 3)	NM_001167628
5.09 1.45E-44	TRPM5 (Transient receptor potential cation channel subfamily M member 5)	XM_021082616

A total of 9,511 genes were identified.

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TABLE 2 KEGG pathway functional classification of the upregulated genes in islets cultured for a long period of time at 37°C, compared to 24°C Top 20 in terms of *p*-valueTop 20 in terms of gene expression.

Pathway	Count	p-value
Metabolic pathway	231	2.10E-16
Protein processing in endoplasmic reticulum	45	5.20E-11
Insulin secretion	25	3.30E-07
Retrograde endocannabinoid signaling	33	3.30E-06
Cardiac muscle contraction	23	7.50E-06
Dopaminergic synapse	29	1.30E-05
Adrenergic signaling in cardiomyocytes	31	1.50E-05
GABAergic synapse	23	1.60E-05
Maturity onset diabetes of the young	11	5.20E-05
Morphine addiction	22	6.30E-05
Dilated cardiomyopathy	22	1.00E-04
Arrhythmogenic right ventricular cardiomyopathy	19	1.30E-04
Serotonergic synapse	25	1.40E-04
Various types of N-glycan biosynthesis	13	1.60E-04
Glutamatergic synapse	24	1.90E-04
Amphetamine addiction	17	2.70E-04
Hypertrophic cardiomyopathy	20	4.10E-04
N-Glycan biosynthesis	14	4.10E-04
Cholinergic synapse	23	4.90E-04
Circadian entrainment	21	5.30E-04
Pathway	**Count**	**p-value**
Metabolic pathway	231	2.10E-16
Pathways of neurodegeneration - multiple diseases	64	1.80E-02
Neuroactive ligand-receptor interaction	49	5.40E-03
Alzheimer disease	48	2.20E-02
Protein processing in endoplasmic reticulum	45	5.20E-11
Parkinson disease	37	1.20E-02
Thermogenesis	35	3.70E-07
cAMP signaling pathway	34	3.20E-03
Retrograde endocannabinoid signaling	33	3.30E-06
Diabetic cardiomyopathy	33	1.80E-03
Calcium signaling pathway	33	2.30E-02
Adrenergic signaling in cardiomyocytes	31	1.50E-05
Dopaminergic synapse	29	1.30E-05
Oxytocin signaling pathway	27	8.20E-04
Cell adhesion molecules	27	1.50E-03
Axon guidance	26	2.50E-02
Insulin secretion	25	3.30E-07
Serotonergic synapse	25	1.40E-04
cGMP-PKG signaling pathway	25	1.70E-02
Glutamatergic synapse	24	1.90E-04

(Continued)

TABLE 2 Continued

Top 20 in terms of gene expression.
A total of 9,511 genes were identified. The genes in bold were in the top 20 for both p-value and abundance.

cultured for 1 day or 28 days at 24°C (Supplemental Figures 6A, B), but their plasma porcine C-peptide concentrations gradually increased with time and the transplanted islets were found to have successfully engrafted in both groups (Supplemental Figures 6C–F). The plasma concentrations of porcine C-peptide in the 24°C Day 28 group were significantly lower than those in the Day 1 group (Supplemental Figures 6C, D). However, the transplantation efficacy of islets cultured long-term at 37°C was similar to that of overnight-cultured islets. The blood glucose concentrations were similar in the Day 1 and 37°C Day 28 groups (Figure 9A) and their plasma porcine C-peptide concentrations increased after transplantation to similar levels (9.44 ± 1.28 pmol/L at transplantation vs. 55.50 ± 21.13 pmol/L 84 days later, $p = 0.04$; Figure 9B). Nevertheless, four of the five mice in the 37°C Day 28 group failed to return to normoglycemia (Figure 9C). The one mouse that became normoglycemic did not show an increase in blood glucose concentration following graftectomy (Figure 9E). In contrast, an increase in plasma porcine C-peptide concentration occurred in all the mice (Figure 9D), and this decreased in four out of five of the mice in the 37°C Day 28 group following graftectomy (55.50 ± 21.13 pmol/L before graftectomy vs. 7.79 ± 1.41 pmol/L after graftectomy, $p = 0.04$; Figure 9E). The transplanted islets cultured for 28 days at 37°C remained engrafted 3 months after xenotransplantation (Figure 9F). Thus, 37°C is a more effective culture temperature than 24°C for the long-term culture of porcine islets to be used in xenotransplantation. Thus, the transplantation efficacy of long-term cultured islets at 37°C was not inferior to that of overnight-cultured islets.

4 Discussion

The xenotransplantation of porcine organs, including porcine islets, may be a feasible therapeutic approach in the future. For the promotion of porcine islet xenotransplantation, further innovation is needed to prolong the lifetime of grafts. The identification of the optimal long-term culture temperature for porcine islets represents a substantial challenge to successful porcine islet xenotransplantation because high-quality porcine islets are required. Extensive research has been carried out on the long-term culture of porcine islets, but the characteristics and function of the islets and the optimal temperature for such culture remain a subject for discussion. For example, Brandhorst and colleagues found that 37°C was the optimal temperature for the maintenance of insulin secretion, but it was not suitable for the preservation of cell number (16). Krickhahn and colleagues showed that porcine islets were significantly attenuated in

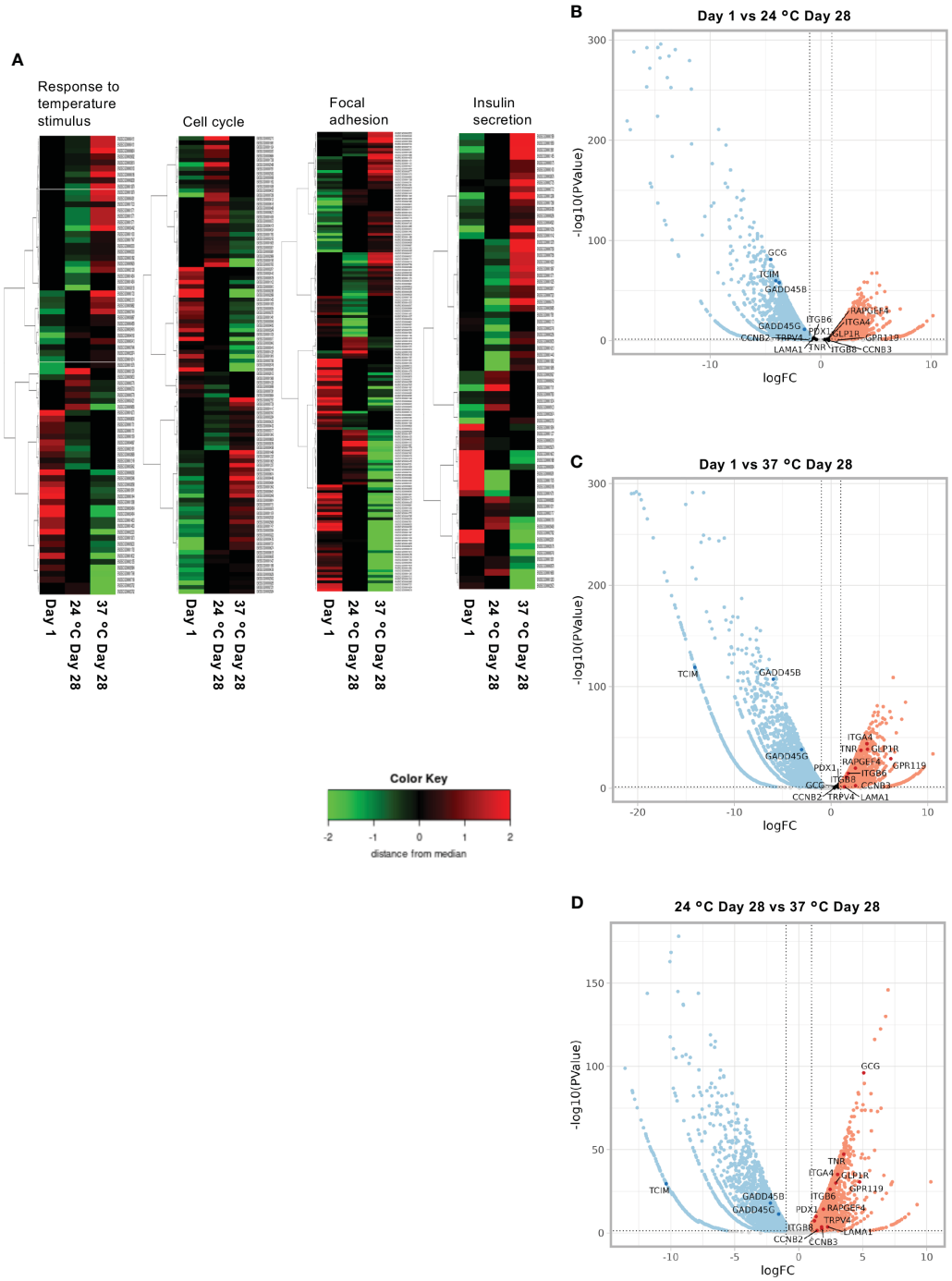

FIGURE 8
Results of the RNA sequencing of long-term cultured islets. **(A)** Heat map showing the upregulated and downregulated genes involved in the response to temperature stimulus, cell cycle, focal adhesion, and insulin secretion for Day 1, 24°C Day 28, and 37°C Day 28 islets. **(B–D)**. Volcano plots of the gene expression of porcine islets to compare Day 1 and 24°C Day 28 **(B)**, Day 1 and 37°C Day 28 **(C)**, and 24°C Day 28 and 37°C Day 28 **(D)**.

function after 11 days of culture at 24°C (36). However, Rijkelijkhuizen and colleagues achieved a 5-month period of survival of grafts in diabetic rats that were derived from porcine islets cultured at 37°C for 1.5–3 weeks (37).

In the present study, we have assessed the effects of long-term (28-day) culture on porcine islets at either 24°C or 37°C. The long-term culture did not affect the viability of the islets. Furthermore, we made three findings relevant to the establishment of the optimal long-term culture conditions. Firstly, long-term culture at 37°C promoted the morphological stability of the islets. In general, porcine islets are fragile and are considered not to be suitable for long-term culture. We found that most of the islets cultured at 37°C became solid and compact, with a smooth surface, between days 7 and 14, whereas most of them cultured at 24°C had rough, frayed

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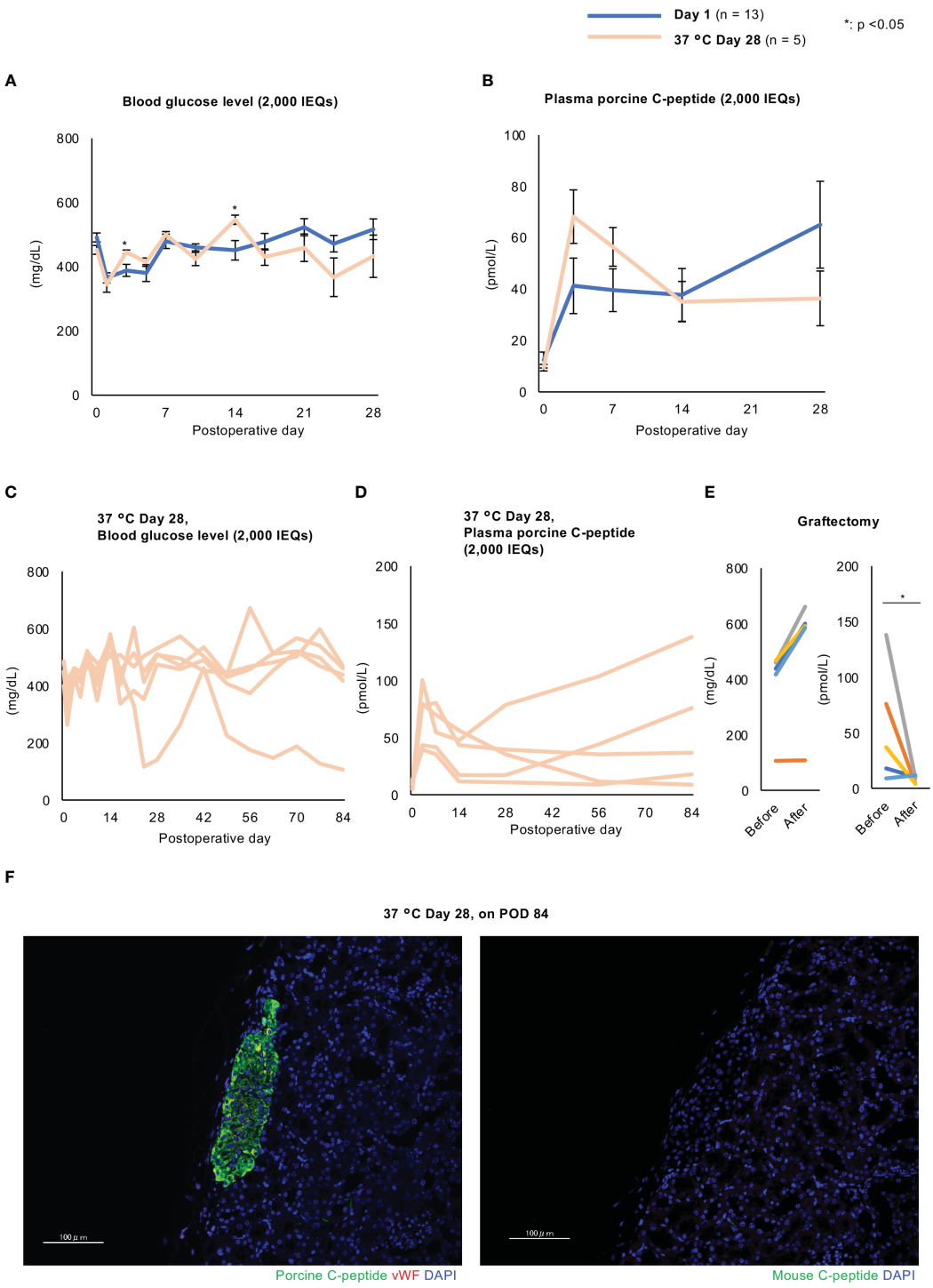

FIGURE 9
Effects of the xenotransplantation of long-term cultured islets into diabetic nude mice. **(A, B)** Blood glucose **(A)** and plasma porcine C-peptide **(B)** concentrations in diabetic nude mice after the xenotransplantation of porcine islets cultured for 1 day (Day 1 group, blue) or 28 days (37°C Day 28 group, pale orange) (2,000 IEQs) at 37°C. C and **(D)** Individual data for mice transplanted with 37°C Day 28 islets. Blood glucose **(C)** and plasma porcine C-peptide **(D)** concentrations. **(E)** Blood glucose (left) and plasma porcine C-peptide (right) concentrations before and after graftectomy. **(F)** Transplanted porcine islets cultured for 28 days at 37°C and examined 84 days after transplantation, immunostained for porcine C-peptide (green, left) or mouse C-peptide (green, right) and von Willebrand factor (red), and counterstained with DAPI (blue). Scale bar: 100 μm. * $p < 0.05$.

surfaces. We hypothesized that this stabilization was provided by a strengthening of cell-to-cell junctions, secondary to the proliferation of ECM and adhesion factors, and we found that the expression of collagen I and integrin β1 in the cell membranes of porcine islets is significantly increased by long-term culture at 37°C. RNA sequencing of islets cultured long-term at 37°C also showed the upregulation of genes involved in ECM and adhesion. These increases in expression might be responsible for the strengthened

cell-ECM junctions, and therefore contribute to the stability of the islets. This strengthening might also be responsible for the improvement in insulin production and secretion in long-term cultured porcine islets (38).

Second, long-term culture at 37°C promoted the proliferation of cells within the cultured porcine islets, which caused an increase in islet size. Ki67-positive islet cells, including both β and non-β cells, were significantly more numerous in the islets following long-term culture at 37°C. Furthermore, RNA sequencing revealed that this culture temperature promoted cell cycle progression (downregulation of *Gadd45b* and *Gadd45g*, upregulation of *Ccnb2* and *Ccnb3*) (39). These data indicate that long-term culture at 37°C promotes the proliferation of endocrine cells by activating the cell cycle. We also consider that the increase in islet size was the result not only of cellular proliferation but also of endocrine differentiation from pancreatic progenitors. The long-term cultured islets were found to contain CD146-positive cells, which are considered to be PSCs, and some of the CD146-positive cells were also found to express PDX-1.

Third, long-term culture at 37°C led to a recovery of the endocrine function of long-term cultured islets. Although long-term culture attenuated the endocrine function of porcine islets, assessed using GSIS and insulin content, this change was mitigated by culture at 37°C. Furthermore, the expression of genes involved in pancreatic regeneration and encoding hormones was higher when long-term culture was performed at 37°C. RNA sequencing analysis also revealed that the expression of the genes involved in insulin secretion, positive regulation of the cAMP signaling pathway, and calcium homeostasis was upregulated when the islets were cultured at 37°C, which may increase insulin secretion. This might be explained by activation of the thermosensitive system controlled by TRP channels, and the expression of TRPM5 was found to be upregulated after culture at 37°C. This encodes a Ca^{2+}-activated cation channel that is activated at 15–35°C (40). Previous studies have shown that TRPM5 is expressed in islets, where it regulates the frequency of Ca^{2+} oscillations and contributes to insulin secretion (41–43).

We consider that PSCs, which are CD146-positive cells, were the principal contributors to the morphological change, cellular proliferation, and recovery of endocrine function during long-term culture at 37°C. PSCs are minor cellular components of the periacinar, perivascular, and periductal spaces in the pancreas (44, 45), and previous studies have revealed the roles of PSCs in the synthesis of various ECM proteins, such as procollagen III, collagen I, laminin, and fibronectin (46), which maintain the periinsular basement membrane (47). PSCs are also a key player in the fibrosis that occurs in chronic pancreatitis and pancreatic cancer (48, 49). PSCs also contribute to cellular proliferation through paracrine effects (50, 51). Furthermore, PSCs are multipotent cells that can differentiate into insulin-producing cells (25). Recently, Paul and colleagues assessed whether co-culture with PSCs improves the viability and function of porcine islets. They found that islets co-cultured with PSCs at 37°C showed less fragmentation and disaggregation, higher viability, greater insulin and glucagon production, higher PDX-1 expression, and superior GSIS (52). In the present study, we found that long-term culture at 37°C promoted the proliferation of spindle-like cells derived from islets that attached to the culture vessels. Although this caused the trapping of the islets, the attached cells included multipotent stem cells, which are considered to be CD146-positive PSCs. CD146-positive cells were significantly more abundant in islets cultured at 37°C and might contribute to the superior stability, cellular proliferation, and endocrine function of the islets via paracrine effects and pancreatic differentiation.

Regulation of immunity is also essential for success of xenotransplantation, as same as allogeneic transplantation. However, further knowledges about mechanism of rejection against xenograft are necessary for the success (53). Three predominant carbohydrate antigens, α-Gal, New5Gc and SDa, are the targets for rejection of xenograft. Human naturally harbor antibodies against the antigens in serum. In hyperacute rejection, the antibodies induce complement activation via classical pathway at porcine xenotransplantation. The activated complements injure xenogeneic endothelial cells (54). Binding of the antibodies also induce activation of xenogeneic endothelial cells, which might cause intravascular thrombosis (55). In antibody-mediated rejection, activated B cells and $CD4^+$ T cells via presentation of xenoantigens by antigen-presenting cells (APCs) attack xenograft by production of xenoantigen-specific antibodies (56, 57). And in cellular rejection, the rejection is induced by activated $CD4^+$T cells and $CD8^+$T cells through xenoantigen presented by donor APCs via SLA and recipient APCs via human leukocyte antigen (9, 58, 59). In this study, we attempted to elucidate the immunogenicity of porcine islet under long-term culturing. Interestingly, immunofluorescence and flow cytometry provided no evidence of α-Gal expression in the porcine islets. The α-Gal epitope is a porcine-specific carbohydrate that is a mediator of hyperacute rejection in pig-to-human xenotransplantation (60). Indeed, 70%–90% of human antibodies target the α-Gal epitope (61). Therefore, the regulation of α-Gal is essential for successful heart and kidney xenotransplantation (62, 63). Bottino and colleagues revealed that over 8 months of graft survival could be achieved using islets from pigs with disruptions to their α1,3-galactosyltransferase genes (GTKO pig) with anti-CD154 antibody (64). However, we consider that manipulating α-Gal might not be necessary for successful islet xenotransplantation, in contrast to the requirement for successful organ transplantation. Other molecules might have a larger role in the success of the islet xenotransplantation, but further studies are recommended.

We selected 2 – 3 years-old adult pigs as donors for islets in this study. On the other hand, some groups showed superiorities of neonatal porcine islets in easiness of islet isolation and reverse of diabetes in transplantation (65, 66). Indeed, age is an important factor in deciding suitable donor. Regarding procedure of islet isolation, the procedure for neonatal pigs is easy and inexpensive similar to rodent islet isolation. In a study by Korbutt et al., pancreas acquired from 1 - 3 day-old neonatal pigs with 1.5 ~ 2.0 kg body weight are minced to 1 ~ 2 mm^3 size. They were transferred to a collagenase solution and gently shaken in a water bath at 37°C for 16 ~ 18 minutes. After filtrating in a 500 um pore-size mesh filter and washing with a buffer solution, the digestion was cultured in Petri dishes. A purification process before a culture is not necessary. Approximately 50,000 islet equivalents (IEQs) can be obtained by

this method (67). Unfortunately, neonatal islets harbor some disadvantages in islet yield and immaturity. Published islet yield from fetal and neonatal pancreases were ~ 8,000 IEQs and 20,000 ~ 50,000 IEQs, respectively (68–71). It might be difficult to improve patients with diabetes with that number. Regarding the immaturity of the islets, the therapeutic effect of transplantation to diabetic nude mice was delayed in neonatal islets (6 ~ 10 weeks) (72). Furthermore, the expression of α-Gal in fetal and neonatal islets is stronger than in adult islets (73). In contrast, adult pigs have a larger number of mature islets (68, 69, 71, 74). The isolation procedure is difficult and expensive, although the concept is the same as neonatal islet isolation. The adult porcine pancreas is larger than a neonatal porcine pancreas. More expensive materials are needed, including collagenase, washing buffer, density gradient solution, cold preservation solution for procurement and culture medium, and perfusion, digestion, and purification equipment are required for adult porcine islet isolation. And as previously mentioned, the fragility of adult porcine islets adds to the difficulty (75). Particularly, young adult porcine islets are more fragile than older porcine islets (75). According to Buhler and colleagues, islet capsules could not be found in young porcine islets (76). Meyer and colleagues found that the expressions of collagen I, III, and IV in peri-islet are higher in older pigs than in younger pigs (77).

In this study, we focused on the influence of culture temperature for long-term culture of porcine islets. However, many issues should be considered for establishment of the optimal condition of long-term culture, including oxygenation, composition of culture medium, 2D or 3D culture. Among them, size of islets might be critical for the success of long-term culture. It is obvious for the success of islet transplantation to transplant high volume of islets, i.e. high IEQs. IEQs are influenced by the number of larger islets. However, there are no correlations between size and function of islets. For example, large islets are sensitive to hypoxia. Necrosis is frequently seen at the center of large islets by hypoxia

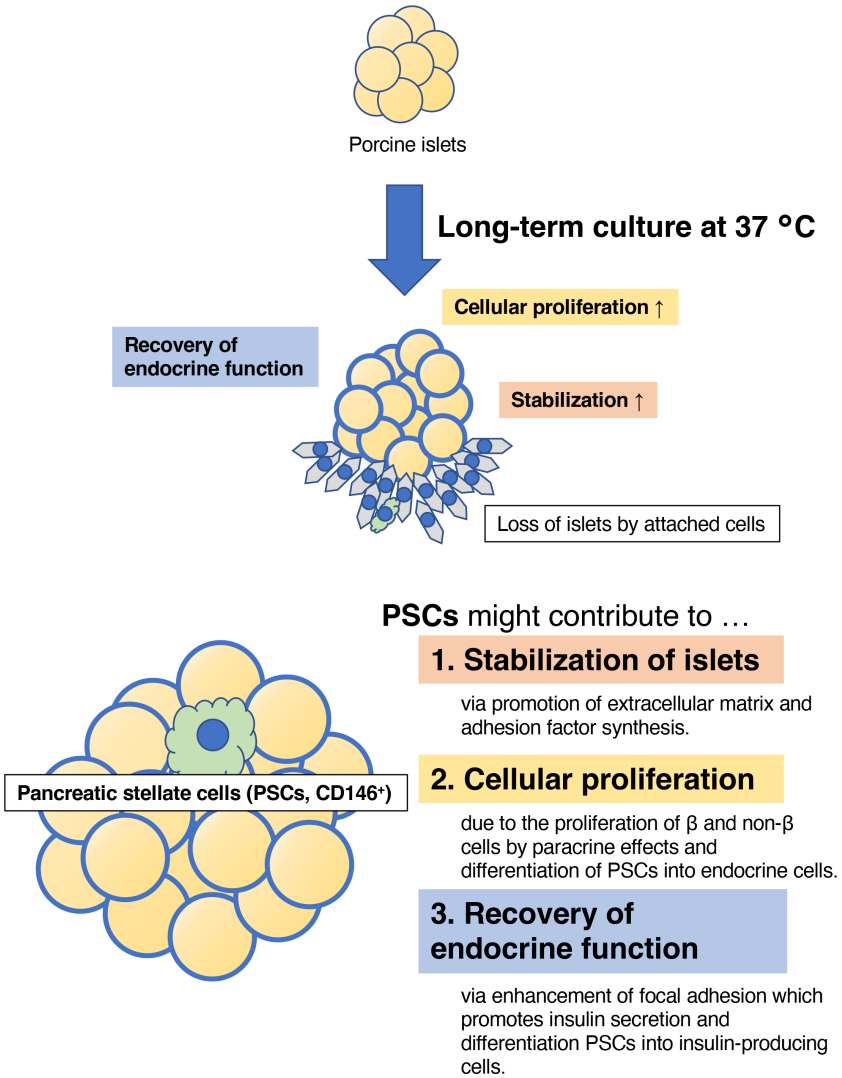

FIGURE 10
The estimated therapeutic effects of long-term culture on porcine islets. Culture at 37°C contributed to the stability of the morphology of the islets, the proliferation of islet cells, and the recovery of endocrine function, indicated by the expression of genes involved in pancreatic development, hormone production, and glucose-stimulated insulin secretion. These advantages may be provided by islet-derived CD146-positive stellate cells.

during the culture (78). Furthermore, there are differences in endocrine cell composition among the size of islets. While small islets harbor a lot of β cells, the population of α and δ cells is increased in large islets, especially human islets (79). Therefore, reconstruction of islets in uniform size might be considerable issue for long-term culture of porcine islets.

The present study had two principal limitations. The first was the sample size. The main reason for the small sample size was the difficulty of guaranteeing the quality of the porcine islets, owing to the technical difficulty of islet isolation. The second was a failure of the recipient mice to achieve normoglycemia following transplantation. Porcine islets are fragile, and therefore it might be difficult to prepare recipient animals appropriately for transplantation. In addition, the difference in pig and mouse insulin might have influenced the transplantation efficacy. However, we have shown that 37°C is superior for the successful long-term culture of porcine islets and the mechanism involved. The loss of islets and functional impairment vs. fresh islets are the key challenges to the successful use of long-term culture. Alternative culture methods, including perfusion culture and large-scale three-dimensional culture, should be evaluated in the future for clinical use.

In conclusion, we have assessed the optimal temperature for the long-term culture of porcine islets, and found that a temperature of 37°C provides some benefits in better stability, cellular proliferation, and the recovery of insulin secretion in culture (Figure 10). Therefore, 37°C might be a suitable temperature for the long-term culture of porcine islets, but further modifications will be required for successful xenotransplantation in a clinical setting.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: DOI: 10.6084/m9.figshare.24203670.

Ethics statement

The animal study was approved by the Animal Care and Use Committee of Fukuoka University. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

NS: conceptualization, data curation, formal analysis, funding acquisition, investigation, writing – original draft. GY: investigation, methodology, writing – review & editing. RK: investigation, methodology, writing – review & editing. CA: investigation, methodology, writing – review & editing. SK: investigation, methodology, supervision, writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2023.1280668/full#supplementary-material

References

1. Cayabyab F, Nih LR, Yoshihara E. Advances in pancreatic islet transplantation sites for the treatment of diabetes. *Front Endocrinol (Lausanne)* (2021) 12:732431. doi: 10.3389/fendo.2021.732431

2. Nanno Y, Sterner E, Gildersleeve JC, Hering BJ, Burlak C. Carbohydrate antigen microarray analysis of serum IgG and IgM antibodies before and after adult porcine islet xenotransplantation in cynomolgus macaques. *PloS One* (2021) 16:e0253029. doi: 10.1371/journal.pone.0253029

3. Kirchhof N, Shibata S, Wijkstrom M, Kulick DM, Salerno CT, Clemmings SM, et al. Reversal of diabetes in non-immunosuppressed rhesus macaques by intraportal porcine islet xenografts precedes acute cellular rejection. *Xenotransplantation* (2004) 11:396–407. doi: 10.1111/j.1399-3089.2004.00157.x

4. Byrne GW, Azimzadeh AM, Ezzelarab M, Tazelaar HD, Ekser B, Pierson RN, et al. Histopathologic insights into the mechanism of anti-non-Gal antibody-mediated pig cardiac xenograft rejection. *Xenotransplantation* (2013) 20:292–307. doi: 10.1111/xen.12050

5. Oriol R, Ye Y, Koren E, Cooper DK. Carbohydrate antigens of pig tissues reacting with human natural antibodies as potential targets for hyperacute vascular rejection in pig-to-man organ xenotransplantation. *Transplantation* (1993) 56:1433–42. doi: 10.1097/00007890-199312000-00031

6. Galili U. Natural anti-carbohydrate antibodies contributing to evolutionary survival of primates in viral epidemics? *Glycobiology* (2016) 26:1140–50. doi: 10.1093/glycob/cww088

7. Patience C, Takeuchi Y, Weiss RA. Infection of human cells by an endogenous retrovirus of pigs. *Nat Med* (1997) 3:282–6. doi: 10.1038/nm0397-282

8. Specke V, Rubant S, Denner J. Productive infection of human primary cells and cell lines with porcine endogenous retroviruses. *Virology* (2001) 285:177–80. doi: 10.1006/viro.2001.0934

9. Estrada JL, Martens G, Li P, Adams A, Newell KA, Ford ML, et al. Evaluation of human and non-human primate antibody binding to pig cells lacking GGTA1/CMAH/beta4GalNT2 genes. *Xenotransplantation* (2015) 22:194–202. doi: 10.1111/xen.12161

10. Niu D, Wei HJ, Lin L, George H, Wang T, Lee IH, et al. Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9. *Science* (2017) 357:1303–7. doi: 10.1126/science.aan4187

11. Toso C, Brandhorst D, Oberholzer J, Triponez F, Buhler L, Morel P. Isolation of adult porcine islets of Langerhans. *Cell Transplant* (2000) 9:297–305. doi: 10.1177/096368970000900301

12. Sakata N, Yoshimatsu G, Kawakami R, Chinen K, Aoyagi C, Kodama S. Procedure of adult porcine islet isolation. *Tissue Eng Part C Methods* (2023) 29:144–53. doi: 10.1089/ten.tec.2023.0020

13. Van Deijnen JH, Van Suylichem PT, Wolters GH, Van Schilfgaarde R. Distribution of collagens and type V in the pancreas of rat, dog, pig and man. *Cell Tissue Res* (1994) 277:115–21. doi: 10.1007/BF00303087

14. Wang RN, Paraskevas S, Rosenberg L. Characterization of integrin expression in islets isolated from hamster, canine, porcine, and human pancreas. *J Histochem Cytochem* (1999) 47:499–506. doi: 10.1177/002215549904700408

15. Kneteman NM, Halloran PF, Sanden WD, Wang T, Seelis RE. Major histocompatibility complex antigens and murine islet allograft survival. *Transplantation* (1991) 51:247–51. doi: 10.1097/00007890-199101000-00041

16. Brandhorst D, Brandhorst H, Hering BJ, Bretzel RG. Long-term survival, morphology and in *vitro* function of isolated pig islets under different culture conditions. *Transplantation* (1999) 67:1533–41. doi: 10.1097/00007890-199906270-00006

17. Mueller KR, Martins KV, Murtaugh MP, Schuurman HJ, Papas KK. Manufacturing porcine islets: culture at 22 degrees C has no advantage above culture at 37 degrees C: a gene expression evaluation. *Xenotransplantation* (2013) 20:418–28. doi: 10.1111/xen.12048

18. Ricordi C, Gray DW, Hering BJ, Kaufman DB, Warnock GL, Kneteman NM, et al. Islet isolation assessment in man and large animals. *Acta Diabetol Lat* (1990) 27:185–95. doi: 10.1007/BF02581331

19. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* (2014) 30:2114–20. doi: 10.1093/bioinformatics/btu170

20. Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinf* (2011) 12:323. doi: 10.1186/1471-2105-12-323

21. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* (2010) 26:139–40. doi: 10.1093/bioinformatics/btp616

22. Sakata N, Yoshimatsu G, Tanaka T, Yamada T, Kawakami R, Kodama S. Mechanism of transplanted islet engraftment in visceral white adipose tissue. *Transplantation* (2020) 104:2516–27. doi: 10.1097/TP.0000000000003400

23. Canel M, Serrels A, Frame MC, Brunton VG. E-cadherin-integrin crosstalk in cancer invasion and metastasis. *J Cell Sci* (2013) 126:393–401. doi: 10.1242/jcs.100115

24. Nielsen MFB, Mortensen MB, Detlefsen S. Identification of markers for quiescent pancreatic stellate cells in the normal human pancreas. *Histochem Cell Biol* (2017) 148:359–80. doi: 10.1007/s00418-017-1581-5

25. Mato E, Lucas M, Petriz J, Gomis R, Novials A. Identification of a pancreatic stellate cell population with properties of progenitor cells: new role for stellate cells in the pancreas. *Biochem J* (2009) 421:181–91. doi: 10.1042/BJ20081466

26. Wang Z, Xu Q, Zhang N, Du X, Xu G, Yan X. CD146, from a melanoma cell adhesion molecule to a signaling receptor. *Signal Transduct Target Ther* (2020) 5:148. doi: 10.1038/s41392-020-00259-8

27. Shi S, Gronthos S. Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. *J Bone Miner Res* (2003) 18:696–704. doi: 10.1359/jbmr.2003.18.4.696

28. Dmitrieva RI, Minullina IR, Bilibina AA, Tarasova OV, Anisimov SV, Zaritskey AY. Bone marrow- and subcutaneous adipose tissue-derived mesenchymal stem cells: differences and similarities. *Cell Cycle* (2012) 11:377–83. doi: 10.4161/cc.11.2.18858

29. Martin-Rendon E, Sweeney D, Lu F, Girdlestone J, Navarrete C, Watt SM. 5-Azacytidine-treated human mesenchymal stem/progenitor cells derived from umbilical cord, cord blood and bone marrow do not generate cardiomyocytes in *vitro* at high frequencies. *Vox Sang* (2008) 95:137–48. doi: 10.1111/j.1423-0410.2008.01076.x

30. Blocki A, Wang Y, Koch M, Peh P, Beyer S, Law P, et al. Not all MSCs can act as pericytes: functional in *vitro* assays to distinguish pericytes from other mesenchymal stem cells in angiogenesis. *Stem Cells Dev* (2013) 22:2347–55. doi: 10.1089/scd.2012.0415

31. Tsang WP, Shu Y, Kwok PL, Zhang F, Lee KK, Tang MK, et al. CD146+ human umbilical cord perivascular cells maintain stemness under hypoxia and as a cell source for skeletal regeneration. *PloS One* (2013) 8:e76153. doi: 10.1371/journal.pone.0076153

32. Hamada S, Matsumoto R, Masamune A. Pancreatic stellate cells and metabolic alteration: physiology and pathophysiology. *Front Physiol* (2022) 13:865105. doi: 10.3389/fphys.2022.865105

33. Csete ME, Afra R, Mullen Y, Drazan KE, Benhamou PY, Shaked A. Adenoviral-mediated gene transfer to pancreatic islets does not alter islet function. *Transplant Proc* (1994) 26:756–7.

34. Zachary AA, Leffell MS. Desensitization for solid organ and hematopoietic stem cell transplantation. *Immunol Rev* (2014) 258:183–207. doi: 10.1111/imr.12150

35. Diaz Varela I, Sanchez Mozo P, Centeno Cortes A, Alonso Blanco C, Valdes Canedo F. Cross-reactivity between swine leukocyte antigen and human anti-HLA-specific antibodies in sensitized patients awaiting renal transplantation. *J Am Soc Nephrol* (2003) 14:2677–83. doi: 10.1097/01.ASN.0000088723.07259.CF

36. Krickhahn M, Meyer T, Buhler C, Thiede A, Ulrichs K. Highly efficient isolation of porcine islets of Langerhans for xenotransplantation: numbers, purity, yield and in *vitro* function. *Ann Transplant* (2001) 6:48–54.

37. Rijkelijkhuizen JK, Tons A, Terpstra OT, Bouwman E. Transplantation of long-term cultured porcine islets in the rat: prolonged graft survival and recipient growth on reduced immunosuppression. *Cell Transplant* (2010) 19:387–98. doi: 10.3727/096368909X484257

38. Laidmae I, Aints A, Uibo R. Growth of MIN-6 cells on salmon fibrinogen scaffold improves insulin secretion. *Pharmaceutics* (2022) 14:941. doi: 10.3390/pharmaceutics14050941

39. Vairapandi M, Balliet AG, Hoffman B, Liebermann DA. GADD45b and GADD45g are cdc2/cyclinB1 kinase inhibitors with a role in S and G2/M cell cycle checkpoints induced by genotoxic stress. *J Cell Physiol* (2002) 192:327–38. doi: 10.1002/jcp.10140

40. Perez CA, Huang L, Rong M, Kozak JA, Preuss AK, Zhang H, et al. A transient receptor potential channel expressed in taste receptor cells. *Nat Neurosci* (2002) 5:1169–76. doi: 10.1038/nn952

41. Brixel LR, Monteilh-Zoller MK, Ingenbrandt CS, Fleig A, Penner R, Enklaar T, et al. TRPM5 regulates glucose-stimulated insulin secretion. *Pflugers Arch* (2010) 460:69–76. doi: 10.1007/s00424-010-0835-z

42. Colsoul B, Schraenen A, Lemaire K, Quintens R, Van Lommel L, Segal A, et al. Loss of high-frequency glucose-induced Ca2+ oscillations in pancreatic islets correlates with impaired glucose tolerance in Trpm5-/- mice. *Proc Natl Acad Sci U.S.A.* (2010) 107:5208–13. doi: 10.1073/pnas.0913107107

43. Liman ER. A TRP channel contributes to insulin secretion by pancreatic beta-cells. *Islets* (2010) 2:331–3. doi: 10.4161/isl.2.5.12973

44. Apte MV, Haber PS, Applegate TL, Norton ID, McCaughan GW, Korsten MA, et al. Periacinar stellate shaped cells in rat pancreas: identification, isolation, and culture. *Gut* (1998) 43:128–33. doi: 10.1136/gut.43.1.128

45. Bachem MG, Schneider E, Gross H, Weidenbach H, Schmid RM, Menke A, et al. Identification, culture, and characterization of pancreatic stellate cells in rats and humans. *Gastroenterology* (1998) 115:421–32. doi: 10.1016/S0016-5085(98)70209-4

46. Apte MV, Haber PS, Darby SJ, Rodgers SC, McCaughan GW, Korsten MA, et al. Pancreatic stellate cells are activated by proinflammatory cytokines: implications for pancreatic fibrogenesis. *Gut* (1999) 44:534–41. doi: 10.1136/gut.44.4.534

47. Means AL. Pancreatic stellate cells: small cells with a big role in tissue homeostasis. *Lab Invest* (2013) 93:4–7. doi: 10.1038/labinvest.2012.161

48. Hwang RF, Moore T, Arumugam T, Ramachandran V, Amos KD, Rivera A, et al. Cancer-associated stromal fibroblasts promote pancreatic tumor progression. *Cancer Res* (2008) 68:918–26. doi: 10.1158/0008-5472.CAN-07-5714

49. Masamune A, Watanabe T, Kikuta K, Shimosegawa T. Roles of pancreatic stellate cells in pancreatic inflammation and fibrosis. *Clin Gastroenterol Hepatol* (2009) 7:S48–54. doi: 10.1016/j.cgh.2009.07.038

50. Masamune A, Kikuta K, Watanabe T, Satoh K, Hirota M, Shimosegawa T. Hypoxia stimulates pancreatic stellate cells to induce fibrosis and angiogenesis in pancreatic cancer. *Am J Physiol Gastrointest Liver Physiol* (2008) 295:G709–17. doi: 10.1152/ajpgi.90356.2008

51. Jiang J, Bai J, Qin T, Wang Z, Han L. NGF from pancreatic stellate cells induces pancreatic cancer proliferation and invasion by PI3K/AKT/GSK signal pathway. *J Cell Mol Med* (2020) 24:5901–10. doi: 10.1111/jcmm.15265

52. Paul PK, Das R, Drow TJ, de Souza AH, Balamurugan AN, Belt Davis D, et al. Pancreatic stellate cells prolong ex vivo islet viability and function and improve engraftment. *Stem Cells Transl Med* (2022) 11:630–43. doi: 10.1093/stcltm/szac018

53. Goerlich CE, Singh AK, Griffith BP, Mohiuddin MM. The immunobiology and clinical use of genetically engineered porcine hearts for cardiac xenotransplantation. *Nat Cardiovasc Res* (2022) 1:715–26. doi: 10.1038/s44161-022-00112-x

54. Dalmasso AP, Platt JL, Bach FH. Reaction of complement with endothelial cells in a model of xenotransplantation. *Clin Exp Immunol* (1991) 86 Suppl 1:31–5.

55. Rose AG, Cooper DK, Human PA, Reichenspurner H, Reichart B. Histopathology of hyperacute rejection of the heart: experimental and clinical observations in allografts and xenografts. *J Heart Lung Transplant* (1991) 10:223–34.

56. Ezzelarab MB, Ekser B, Isse K, Iwase H, Morelli AE, Ayares D, et al. Increased soluble CD154 (CD40 ligand) levels in xenograft recipients correlate with the development of *de novo* anti-pig IgG antibodies. *Transplantation* (2014) 97:502–8. doi: 10.1097/TP.0000000000000042

57. Elgueta R, Benson MJ, de Vries VC, Wasiuk A, Guo Y, Noelle RJ. Molecular mechanism and function of CD40/CD40L engagement in the immune system. *Immunol Rev* (2009) 229:152–72. doi: 10.1111/j.1600-065X.2009.00782.x

58. Martens GR, Reyes LM, Li P, Butler JR, Ladowski JM, Estrada JL, et al. Humoral reactivity of renal transplant-waitlisted patients to cells from GGTA1/CMAH/B4GalNT2, and SLA class I knockout pigs. *Transplantation* (2017) 101:e86–92. doi: 10.1097/TP.0000000000001646

59. Dorling A, Lombardi G, Binns R, Lechler RI. Detection of primary direct and indirect human anti-porcine T cell responses using a porcine dendritic cell population. *Eur J Immunol* (1996) 26:1378–87. doi: 10.1002/eji.1830260630

60. Galili U, Swanson K. Gene sequences suggest inactivation of alpha-1,3-galactosyltransferase in catarrhines after the divergence of apes from monkeys. *Proc Natl Acad Sci U.S.A.* (1991) 88:7401–4. doi: 10.1073/pnas.88.16.7401

61. Tector AJ, Mosser M, Tector M, Bach JM. The possible role of anti-neu5Gc as an obstacle in xenotransplantation. *Front Immunol* (2020) 11:622. doi: 10.3389/fimmu.2020.00622

62. Kuwaki K, Tseng YL, Dor FJ, Shimizu A, Houser SL, Sanderson TM, et al. Heart transplantation in baboons using alpha1,3-galactosyltransferase gene-knockout pigs as donors: initial experience. *Nat Med* (2005) 11:29–31. doi: 10.1038/nm1171

63. Knosalla C, Yazawa K, Behdad A, Bodyak N, Shang H, Buhler L, et al. Renal and cardiac endothelial heterogeneity impact acute vascular rejection in pig-to-baboon xenotransplantation. *Am J Transplant* (2009) 9:1006–16. doi: 10.1111/j.1600-6143.2009.02602.x

64. Bottino R, Wijkstrom M, van der Windt DJ, Hara H, Ezzelarab M, Murase N, et al. Pig-to-monkey islet xenotransplantation using multi-transgenic pigs. *Am J Transplant* (2014) 14:2275–87. doi: 10.1111/ajt.12868

65. Gao Q, Davis R, Fitch Z, Mulvihill M, Ezekian B, Schroder P, et al. Anti-thymoglobulin induction improves neonatal porcine xenoislet engraftment and survival. *Xenotransplantation* (2021) 28:e12713. doi: 10.1111/xen.12713

66. Hawthorne WJ, Salvaris EJ, Chew YV, Burns H, Hawkes J, Barlow H, et al. Xenotransplantation of genetically modified neonatal pig islets cures diabetes in baboons. *Front Immunol* (2022) 13:898948. doi: 10.3389/fimmu.2022.898948

67. Korbutt GS, Elliott JF, Ao Z, Smith DK, Warnock GL, Rajotte RV. Large scale isolation, growth, and function of porcine neonatal islet cells. *J Clin Invest* (1996) 97:2119–29. doi: 10.1172/JCI118649

68. Park CG, Bottino R, Hawthorne WJ. Current status of islet xenotransplantation. *Int J Surg* (2015) 23:261–6. doi: 10.1016/j.ijsu.2015.07.703

69. Zhu HT, Yu L, Lyu Y, Wang B. Optimal pig donor selection in islet xenotransplantation: current status and future perspectives. *J Zhejiang Univ Sci B* (2014) 15:681–91. doi: 10.1631/jzus.B1400120

70. Liu Z, Hu W, He T, Dai Y, Hara H, Bottino R, et al. Pig-to-primate islet xenotransplantation: past, present, and future. *Cell Transplant* (2017) 26:925–47. doi: 10.3727/096368917X694859

71. Nagaraju S, Bottino R, Wijkstrom M, Trucco M, Cooper DK. Islet xenotransplantation: what is the optimal age of the islet-source pig? *Xenotransplantation* (2015) 22:7–19. doi: 10.1111/xen.12130

72. Vizzardelli C, Molano RD, Pileggi A, Berney T, Cattan P, Fenjves ES, et al. Neonatal porcine pancreatic cell clusters as a potential source for transplantation in humans: characterization of proliferation, apoptosis, xenoantigen expression and gene delivery with recombinant AAV. *Xenotransplantation* (2002) 9:14–24. doi: 10.1034/j.1399-3089.2002.0o128.x

73. Rayat GR, Rajotte RV, Hering BJ, Binette TM, Korbutt GS. *In vitro* and in *vivo* expression of Galalpha-(1,3)Gal on porcine islet cells is age dependent. *J Endocrinol* (2003) 177:127–35. doi: 10.1677/joe.0.1770127

74. Bottino R, Balamurugan AN, Smetanka C, Bertera S, He J, Rood PP, et al. Isolation outcome and functional characteristics of young and adult pig pancreatic islets for transplantation studies. *Xenotransplantation* (2007) 14:74–82. doi: 10.1111/j.1399-3089.2006.00374.x

75. Rood PP, Buhler LH, Bottino R, Trucco M, Cooper DK. Pig-to-nonhuman primate islet xenotransplantation: a review of current problems. *Cell Transplant* (2006) 15:89–104. doi: 10.3727/000000006783982052

76. Buhler L, Deng S, Mage R, Bubloz C, Rohner A, Morel P. Pig islet isolation: new aspects. *Transplant Proc* (1994) 26:628–9.

77. Meyer T, Czub S, Chodnewska I, Beutner U, Hamelmann W, Klock G, et al. Expression pattern of extracellular matrix proteins in the pancreas of various domestic pig breeds, the Goettingen Minipig and the Wild Boar. *Ann Transplant* (1997) 2:17–26.

78. Komatsu H, Cook C, Wang CH, Medrano L, Lin H, Kandeel F, et al. Oxygen environment and islet size are the primary limiting factors of isolated pancreatic islet survival. *PloS One* (2017) 12:e0183780. doi: 10.1371/journal.pone.0183780

79. Kilimnik G, Jo J, Periwal V, Zielinski MC, Hara M. Quantification of islet size and architecture. *Islets* (2012) 4:167–72. doi: 10.4161/isl.19256

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Allo Beta Cell transplantation: specific features, unanswered questions, and immunological challenge

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Type 1 diabetes (T1D) presents a persistent medical challenge, demanding innovative strategies for sustained glycemic control and enhanced patient well-being. Beta cells are specialized cells in the pancreas that produce insulin, a hormone that regulates blood sugar levels. When beta cells are damaged or destroyed, insulin production decreases, which leads to T1D. Allo Beta Cell Transplantation has emerged as a promising therapeutic avenue, with the goal of reinstating glucose regulation and insulin production in T1D patients. However, the path to success in this approach is fraught with complex immunological hurdles that demand rigorous exploration and resolution for enduring therapeutic efficacy. This exploration focuses on the distinct immunological characteristics inherent to Allo Beta Cell Transplantation. An understanding of these unique challenges is pivotal for the development of effective therapeutic interventions. The critical role of glucose regulation and insulin in immune activation is emphasized, with an emphasis on the intricate interplay between beta cells and immune cells. The transplantation site, particularly the liver, is examined in depth, highlighting its relevance in the context of complex immunological issues. Scrutiny extends to recipient and donor matching, including the utilization of multiple islet donors, while also considering the potential risk of autoimmune recurrence. Moreover, unanswered questions and persistent gaps in knowledge within the field are identified. These include the absence of robust evidence supporting immunosuppression treatments, the need for reliable methods to assess rejection and treatment protocols, the lack of validated biomarkers for monitoring beta cell loss, and the imperative need for improved beta cell imaging techniques. In addition, attention is drawn to emerging directions and transformative strategies in the field. This encompasses alternative immunosuppressive regimens and calcineurin-free immunoprotocols, as well as a reevaluation of induction therapy and recipient preconditioning methods. Innovative approaches targeting autoimmune recurrence, such as CAR Tregs and TCR Tregs, are explored, along with the potential of stem stealth cells, tissue engineering, and encapsulation to

overcome the risk of graft rejection. In summary, this review provides a comprehensive overview of the inherent immunological obstacles associated with Allo Beta Cell Transplantation. It offers valuable insights into emerging strategies and directions that hold great promise for advancing the field and ultimately improving outcomes for individuals living with diabetes.

KEYWORDS

islet transplant, immunosuppression, type 1 diabetes, autoimmunity, beta cell replacement, immunomodulation

1 Introduction: "Beta is better."

1.1 Despite the availability of insulin therapy, T1D patients face challenges in achieving optimal blood sugar control, chronic complications, and mental health burden

In 2022, around 8.75 million people with T1D were living with the condition, with 1.52 million under 20 (IDF Diabetes Atlas 10th edition, https://diabetesatlas.org/). In the early 20th century, T1D was often fatal, with children dying within a short time after diagnosis (1). The discovery of insulin by Banting, Best, Collip, and Macleod in 1921 revolutionized diabetes care, offering hope to countless individuals (2). Leonard Thompson became the first T1D patient to receive insulin, marking the beginning of a century of innovations in diabetes treatment. Over time, the treatment goal for T1D has shifted from merely keeping patients alive to achieving nearly normal blood sugar levels. While insulin was once seen as a highly effective treatment, it is now recognized as insufficient, as it transforms a fatal condition into a chronic and degenerative disease (3). Healthy individuals maintain blood glucose levels close to 99 mg/dL on average, with minimal variability (4). Even with advanced technologies like closed-loop systems (5) and adjunctive therapies (i.e., SGLT-2 inhibitor, low-carb diet), T1D patients struggle to achieve these levels (6–8). Current consensus guidelines define a target range of 70-180 mg/dL (9, 10), which still falls far from healthy norms (11). Maintaining blood sugar levels as close to normal as possible is essential (12–19). Lowering blood sugar levels is associated with reduced risks of complications and mortality in T1D (20–22). A 1% reduction in HbA1c has been linked to decreased risks of myocardial infarction, stroke, microvascular complications, and more (23). Despite the availability of advanced treatments, a substantial proportion of T1D patients fail to meet glycemic targets (24). Registries and clinics report that many children, adolescents, and adults do not achieve HbA1c goals (25–28). Even with the use of technology (5), blood sugar control remains elusive (6–8). Patients with T1D face acute complications related to insulin therapy, including hypoglycemia (29–33). Hypoglycemia rates remain significant, impacting patients' cognitive function (34–38), cardiovascular health (39–42), and quality (43, 44) and quantity of life. Chronic complications continue to develop, despite advances in insulin and devices, affecting kidney function, retinopathy, and more. Insulin therapy can also have a significant negative impact on mental health, contributing to diabetes distress (45). Approximately 20-30% of T1D individuals experience this burden, which persists even with new technologies (46, 47). While there has been a decline in T1D-related mortality (48, 49), it still presents a significant risk. Patients with T1D face a relative risk of mortality 3.1 to 5.8 times higher than those without diabetes (North America (50, 51), Europe (52–54) and Australia (55)). This results in an estimated loss of 10 to 13 years of life (54–57). In conclusion, insulin therapy has undeniably been a life-saving treatment for individuals with T1D. However, it falls short of providing a normal and healthy life. Achieving optimal blood sugar control remains a significant challenge, and chronic complications continue to be a concern. Moreover, the psychological toll of managing T1D cannot be overlooked.

1.2 Pancreas and islet transplantation are effective treatments for T1D, improving glycemic control and life expectancy

As we commemorate a century of insulin discovery two years ago, it is imperative to renew our commitment to finding more effective treatments and ultimately striving for a world where individuals with T1D can live without the constraints of insulin therapy (3). Over the past three decades, clinical trials have demonstrated that restoring beta-cell function through islet or pancreas transplantation can lead to more physiologic regulation of blood sugar levels compared to exogenous insulin in diabetes patients (58). Clinical trials are essential for evaluating the safety and efficacy of new treatments, and they have played a vital role in the development of islet transplantation for T1D. Four successful large-scale Phase 3 clinical trials in islet transplantation have been published recently: CIT-07 (multicenter, single-arm) (59), CIT06 (pivotal trial) (60), TRIMECO (multicenter, open-label, randomized) (61) and REP0211 (multicenter, Double blind, randomized) (62). All these studies have provided compelling evidence that the transplantation of human islets into patients with T1D who experience impaired awareness of hypoglycemia

and severe hypoglycemic events is not only safe but also highly effective in maintaining optimal glycemic control (3). Attaining freedom from the need for insulin can be realized by transplanting a sufficient quantity of islets (63). Following islet transplantation, the likelihood of sustaining insulin independence for up to five years may reach as high as 50%. Furthermore, a substantial proportion of patients, approximately one in four, may continue to be insulin independent, maintaining HbA1c levels at or below 6.5%, for a period spanning at least a decade. This favorable outcome can be achieved through either islet transplantation as a standalone procedure or in conjunction with a kidney transplant (64, 65). The glucose control achieved with excellent islet graft function closely resembles glucose values observed in healthy adults, with median glucose levels at 103 mg/dl, a standard deviation around the mean value of 14, and no time spent above 180 mg/dl or below 54 mg/dl. HbA1c levels typically fall within the range of 5.6 to 5.8 (66). Moreover, standardized psychometric instruments and psychologist-conducted interviews have confirmed a significant improvement in the quality of life following islet transplantation (67–75). Additionally, there is evidence of positive effects on the microvascular complications of T1D, including the stabilization or slower progression of retinopathy (76–79) and neuropathy (77, 80–82), as well as improvements in micro- and macroangiopathy (74, 76, 83–91). Pancreatic transplantation, in conjunction with islet transplantation, stands as the other effective treatment option for reinstating normal glycemic control in individuals with T1D (92). Simultaneous pancreas-kidney (SPK) transplantation is the most commonly performed type of pancreas transplantation (93), primarily T1D patients with end-stage renal failure. After more than five decades of worldwide experience and over 80,000 reported cases to the International Pancreas Transplant Registry, there is substantial evidence demonstrating that SPK transplantation enhances life expectancy (94–96) and mitigates the progression of diabetic complications (97–99). Similarly, sequential pancreas after kidney (PAK) transplantation, whether following a living or deceased donor kidney transplant, has shown improvements in long-term patient and kidney graft survival rates (100). Pancreas transplantation alone (PTA) is also considered a rational therapy for appropriately selected T1D patients experiencing life-threatening metabolic complications (101–104).

1.3 Clinical trials using stem cell-derived islet cells for the treatment of T1D are ongoing, with promising preliminary results

The field of cellular therapies for the treatment of T1D is rapidly evolving and a new exciting era has already begun. Human pluripotent stem cells, including both embryonic stem (ES) and induced pluripotent stem (iPS) cells, are considered the most promising candidates for generating β cells due to their capacity for unlimited growth and differentiation. Multiple laboratories have developed effective protocols for differentiating these pluripotent cells into β cells, focusing on producing cellular products that are consistently potent and safe for clinical use (105–113). Currently, there are nine clinical trials registered in ClinicalTrial.gov utilizing human pluripotent stem cells for the treatment of T1D (NCT04678557, NCT02939118, NCT03162926 NCT02239354, NCT03163511 NCT05210530, NCT05565248, NCT04786262, NCT05791201). Three of these trials are active and recruiting patients, two have been completed, one was terminated, and three are active but not recruiting. Seven trial are using pancreatic precursor cells (PEC-01) derived from pluripotent stem cells (genetically modified in two trials, PEC211) in combination with durable, removable, close or perforated devices (114). These cells are a mixed population of pancreatic precursor cells (73%–80% $NKX6.1^{+}/PDX1^{+}$ pancreatic precursor) fully committed to further differentiating into mature endocrine pancreatic cells (115) once implanted within an encapsulation device in a subcutaneous space. Interim results from some of these clinical trials, reported in December 2021, were promising but not yet clinically meaningful. Over a follow-up period of up to 1 year, patients experienced a 20% reduction in insulin requirements, spent 13% more time within the target blood glucose range, maintained stable average HbA1c levels below 7.0%, and improved hypoglycemic awareness. Additionally, C-peptide levels, a marker of insulin production, were detected at approximately 1/100th of normal levels within explanted grafts, which included various types of pancreatic cells, including cells with a mature β cell phenotype. The immunosuppressive treatment appeared effective in preventing graft rejection, and the cell product demonstrated safety and tolerability, with no teratoma formation observed (116, 117). In 2021, VX-880, an investigational cell therapy for T1D, was approved as a second cell product. VX-880 comprises fully differentiated insulin-producing pancreatic islet cells derived from pluripotent stem cells. A Phase 1/2 clinical trial was approved for patients with T1D who have impaired hypoglycemic awareness and severe hypoglycemia. VX-880 is administered through infusion into the portal vein, and concomitant immunosuppressive therapy is necessary to protect the islet cells from immune rejection. Preliminary results suggest that β cells derived from stem cells and transplanted into the liver can engraft and begin secreting insulin shortly after infusion and provide insulin independence in patients with T1D (118). In addition to the ongoing clinical efforts, several commercial and academic organizations have announced their plans to conduct clinical trials using functional stem cell-derived islets.

1.4 Allo Beta Cell transplantation offers hope for a cure for T1D, but further research is needed to address the challenges of long-term immunosuppression and graft rejection

Allo Beta Cell transplantation is a promising cure for T1D, but it is not yet a widely available option because it requires patients to take lifelong immunosuppressive drugs. These drugs have serious side effects, including an increased risk of infection and cancer. Therefore, it is important to carefully weigh the risks and benefits of Allo Beta Cell transplantation for each individual patient. Factors to consider include the severity of T1D, the risk of complications from chronic immunosuppression, the patient's willingness to comply

with treatment, and their life expectancy. Allo Beta Cell transplantation may be a good option for people with severe T1D or a high risk of complications, or for people who have tried other treatments without success. Researchers are working on ways to protect transplanted beta cells from immune rejection without the need for chronic immunosuppression. This would make Allo Beta Cell transplantation a more viable option for a wider range of people with T1D. One promising approach is to use encapsulation devices. Encapsulation devices protect transplanted beta cells from the immune system by enclosing them in a semipermeable membrane. This allows the beta cells to secrete insulin into the bloodstream, but it prevents the immune system from attacking them. Another promising approach is to use gene editing to modify the beta cells before transplantation. This could make them less susceptible to attack by the immune system. Researchers are also working to develop new immunosuppressive drugs that are more effective and have fewer side effects. These advances could make Allo Beta Cell transplantation a safe and effective cure for T1D in the near future.

2 Immunological specific hallmark in Allo Beta Cell transplantation

Allo Beta Cell Transplantation presents distinct immunological hurdles when compared to the transplantation of other organs or tissues. These challenges are primarily associated with the unique functions and biology of beta cells, the site of infusion, and the individual characteristics of the recipient.

2.1 The importance of glucose regulation and insulin in immune activation

The regulation of glucose levels and the presence of insulin are pivotal factors in immune activation (119). A significant association between post-transplant glycemic control and the development of subsequent rejection was reported for solid organ transplantation (120–122). In contrast to other transplanted organs, beta cells are responsible for producing insulin and maintaining glucose equilibrium. Consequently, in Allo Beta Cell Transplantation, the effectiveness of the graft is also crucial for the immunological response.

2.1.1 Insulin and immunity

Insulin, a key hormone in glucose metabolism, also has immunomodulatory effects, promoting both pro- and anti-inflammatory responses in a variety of immune cells (122, 123). In macrophages and neutrophils, insulin activates insulin receptors (InsR) and insulin-like growth factor 1 receptors (IGF1R), which triggers signaling pathways that lead to the production of pro-inflammatory cytokines, chemokines, and adhesion molecules (123, 124). Insulin increases the production of reactive oxygen species (ROS), which can activate pro-inflammatory signaling pathways and produce pro-inflammatory mediators (125, 126). Insulin also promotes the activation and survival of eosinophils (127), the maturation and scavenger receptor expression of dendritic cells (128), and the activation, cytokine production, and differentiation of natural killer (NK) cells and innate lymphoid cells (ILCs) (129). In adaptive immunity, insulin predominantly assumes a pro-inflammatory role by optimizing T cell activation, enhancing their responsiveness to key cytokines, and facilitating migration to sites of infection or inflammation (130–134). T cells without InsR have metabolic and functional problems, resulting in less production of important immune molecules, such as IFNγ, and impaired expansion in response to specific antigens (135). In addition, InsR signaling seems to affect the balance of regulatory T cells (Tregs) in the immune system, which could have implications for conditions where insulin signaling weakens the suppressive function of Tregs (136). T cells also express the IGF1R, which plays a role in regulating the differentiation of T helper 17 (Th17) cells and Tregs (137). The precise influence of IGF1R signaling on these processes depends on contextual factors, such as the differentiation stage of the T cells and the presence of specific ligands. In B cells, while the exact role of InsR signaling remains less clear, it is known that B cells express InsR (138). Elevated local and systemic insulin levels are common in patients who have received islet transplants, due to the production of insulin by the transplanted islets and the need for supplemental insulin therapy. Elevated insulin levels may contribute to the risk of inflammation and rejection, as shown in one study that found a higher risk of islet graft dysfunction in patients with higher insulin levels (139) and further sustained by our recent study reporting that progression to Stages 2 and 3 of T1D increases with HOMA-IR and decreases with the Matsuda Index (140).

2.1.2 Glucose and immunity

Glucose metabolism plays a central role in supporting the functions of innate immune cells (141). High glucose levels can induce the production of ROS (142), which can serve as potent weapons against invading pathogens but can also lead to oxidative stress and inflammation (143). Additionally, high glucose levels can upregulate inflammatory cytokines and chemokines, activate NF-κB, PKC, and p38 MAPK pathways, and alter T-cell activation, differentiation, and functions (144, 145). While existing evidence suggests that persistent high blood glucose levels can induce notable molecular and functional alterations in T cells, resulting from shifts in their proteomic and metabolic profiles (146), it's worth noting that short term elevated blood glucose levels may actually enhance immune responses (147). Additionally, hyperglycemia prompts CD4 T cells to adopt an activated immunophenotype (148). In line with these findings, high blood glucose levels during and after kidney and liver transplantation are associated with higher rates of organ rejection (119). In a study of mice, the timing of islet allograft loss was dependent on the degree of hyperglycemia in the recipient (149). Hyperglycemia is common in islet transplant patients for s reasons, including the underlying diabetic condition, difficulty

controlling blood sugar levels before transplantation, and medications and infections that can occur after transplantation. Hyperglycemia can increase the risk of rejection, so it is important to carefully manage blood glucose levels in these patients.

2.2 The importance of beta cell in the interaction with immune cells

In recent years, significant advancements have emerged in our ability to comprehensively study the interaction between beta cells and immune cells (150). Notably, recent research has reshaped our understanding, highlighting that pancreatic beta cells play an active role rather than remaining passive during the progression of immune recognition (151). It was previously believed that T1D resulted in the complete depletion of beta cells. However, recent studies have uncovered a distinct subset of beta cells that manage to survive, although their functionality is limited (152–154). This revelation suggests that not all beta cells are equally susceptible to immune responses, potentially due to inherent protective mechanisms. As scientific inquiries have revealed a wide spectrum of variations among beta cells (155–158), encompassing genetic expression, physical characteristics, functionality, and communication with neighboring cells, this diversity implies that the unique traits of beta cells themselves could influence their capacity to withstand immune attacks (159). Another intriguing development gaining recent attention proposes that specific stress events affecting beta cells can trigger immune cell activation (160). These pathways include inflammatory stress originating from both innate and adaptive immune responses, as well as endoplasmic reticulum (ER) stress that persists due to the demands of insulin production and intensifies as beta cell mass declines (161, 162). Both pathways are significantly represented in all allogenic beta cell transplantation strategies. The downstream consequences of intrinsic (e.g., ER stress) and extrinsic stressors (e.g., cytokine exposure) on beta cells encompass broad changes in their transcriptomes and proteomes, which can affect the interaction between beta cells and immune cells in a number of ways, including altered expression of surface proteins, secretion of cytokines and chemokines, and changes in metabolic pathways. These changes can alter how they engage with and are perceived by immune cells. For example, a stressed microenvironment plays a crucial role in triggering the overexpression of HLA class I molecules on insulin-producing beta cells (163) and in producing new epitopes (164) formed through various processes, including transpeptidation, disulfide bond formation, deamidation, and citrullination formation of epitopes such as hybrid insulin peptides, alternative splicing, splice variant peptides, and defective ribosomal insulin products (165, 166). Immune recognition of these neoepitopes may be enhanced compared to their native counterparts due to altered HLA binding or increased TCR recognition (167). Adding further complexity to the story, it is now evident that certain gene variants modulate beta cell stress responses, increasing the interindividual variability in how they respond (168–172). Collectively, this evidence suggest that beta cell can be presented to the immune system in a highly individualized and heterogeneous manner, making it difficult to predict and manage the recipient's immune response to the transplanted beta cells.

2.3 The influence of liver site and its significance in the context of immunological challenges

Currently, the liver is the preferred location for clinical Allo Beta Cell Transplantation, despite recent suggestions of alternative implantation sites that might be more advantageous for graft survival (173–181). The intrahepatic site offers benefits: it is a well-established procedure accepted by regulatory agencies and associated with minimal morbidity and a negligible risk of adverse events, such as bleeding and portal thrombosis. Moreover, it allows for the infusion of a substantial tissue volume, up to 20 ml. This site scatters the cells throughout hepatic sinusoids, preventing the formation of clusters that can impede the initial diffusion of oxygen and nutrients. Additionally, it appears to have some immunoprivileged characteristics compared to other sites like the bone marrow and kidney capsule (177, 182, 183). Since the liver is the primary target organ for insulin, intrahepatic islets can mimic physiological pancreatic insulin secretion rather than causing systemic insulin release (184, 185) although there have been suggestions of potential dysfunctional alpha cell function (186). However, the liver presents specific immunological challenges. Monitoring through imaging techniques is not feasible, and routine biopsies are impossible to obtain (187), making it impossible to diagnose rejection promptly. Being an intravascular transplantation, it is prone to the instant blood-mediated inflammatory reaction (IBMIR), an innate immune response that occurs when pancreatic cells encounter ABO-compatible blood. This reaction leads to the release of tissue factor, which activates the coagulation and complement cascades, resulting in leukocyte and macrophage-mediated islet cell death (188–191). Moreover, compared to the native tissue oxygen tension of islets (40 mmHg) and the parenchymal oxygen tension (30 mmHg), the liver provides significantly lower tensions, less than 10 mmHg for both (192) inducing beta cell stress. Amyloid formation (193), associated with type 2 diabetes, has been observed in intraportal islet grafts, and glucolipotoxicity from surrounding hepatocytes has been shown to harm transplanted beta cell (194). Lastly, the liver's endogenous immune system, including Kupffer cells, Liver sinusoidal endothelial cells, Hepatic stellate cells, Resident liver lymphocytes NK, NKT, and CD8+ T cells, and to a lesser extent, CD4+ T cells), and liver dendritic cells, has also been shown to potentially harm allograft survival at this site (195). As an alternative to liver transplantation, subcutaneous transplantation has emerged as an attractive option for Allo Beta Cell Transplantation. This approach offers advantages, including a straightforward surgical procedure, minimal surgical risks, ease of monitoring, and the potential for graft retrieval. However, its efficacy is hampered by the limited blood supply in the subcutaneous space, which leads to insufficient oxygen and nutrient availability. To overcome these challenges and achieve successful subcutaneous transplantation, a comprehensive approach is essential. This approach involves the integration of

bioengineering devices, specialized biomaterials, drug delivery systems, and strategies aimed at promoting early angiogenesis. These components play a crucial role not only in facilitating the incorporation of transplanted insulin-producing cells but also in attaining normoglycemia in recipients. A pivotal aspect of the subcutaneous transplantation's success lies in the development of biomaterials, including hydrogels derived from both natural polymers (such as collagen, fibrin, and alginate) and synthetic polymers (such as polyethylene glycol and polyvinyl alcohol). These biomaterials can be precisely tailored to possess specific mechanical, biological, and biochemical properties. Importantly, they should exhibit pro-angiogenic properties, fostering the formation of blood vessels within the subcutaneous tissue. These biomaterials can be employed in many ways, serving as coatings for islets or forming the basis for implantable bulk scaffolds. Despite promising advancements in subcutaneous transplantation, challenges persist, particularly when using macro and micro devices for Allo Beta Cell encapsulation. Immune and fibrotic responses can encapsulate these devices, limiting the supply of oxygen and nutrients to the transplanted tissue. Clinical studies employing such strategies have not definitively demonstrated superior long-term outcomes compared to intraportal transplantation. The subcutaneous immune response can often lead to fibrotic overgrowth, adversely affecting islet function. Furthermore, immune-protective devices that physically separate islets from immune cells may underestimate the impact of diffusible immune factors on islet functionality (196). When considering other potential transplantation sites, it is worth noting that the testis, thymus, and the anterior chamber of the eye are regarded as immunoprivileged sites and have been explored as locations for allografts or xenografts. However, they typically cannot accommodate a sufficient number of islets to achieve euglycemia (197).

2.4 Recipient and donor matching and their significance in the context of immunological challenges: multiple islet donor preparations and recurrence of autoimmunity

The transplantation of allogeneic beta cells presents specific challenges in adaptive immunology that differ from those encountered in other types of organ or tissue transplants (198). In addition to the innate immune response and issues related to engraftment, transplanted allogeneic beta cells face recognition and rejection by the recipient's immune system, which is further complicated by the recurrent autoimmune responses in individuals with T1D due to preexisting adaptive immune memory. It is challenging to separate and assess the individual impact of these two phenomena (199). One way to gauge the significance of allorecognition is by evaluating the effect of HLA matching on graft outcomes, as the degree of HLA mismatches correlates with the strength of the immune system's response. However, the impact of HLA matching on pancreas transplant outcomes remains a topic of debate (200–205). Allogenic immune recognition may be more relevant in the context of islet transplantation, which presents a unique paradigm in organ transplantation due to its requirement for multiple donors to achieve complete insulin independence. Consequently, HLA matching for islets is often minimal, except for the avoidance of preformed anti-HLA antibodies. Some evidence suggests that HLA-A, -B, and -DR matching (excluding HLA-DR3 and -DR4 matching) is associated with improved islet allograft survival (206–208). Regrettably, due to the more stringent donor selection criteria in islet transplantation relative to other transplant procedures and the substantial risk of manufacturing failures, achieving HLA matching is scarcely feasible in clinical practice. The recurrence of T1D in pancreas transplant recipients was initially reported by Dr David Sutherland in cases where patients received living-related pancreas grafts from twins or HLA-identical siblings and, due to HLA identity, received little to no immunosuppression (209). Observations of relapse of autoimmunity as assessed by autoantibodies and occasionally T cells have also been reported following allogeneic pancreas transplant under immune suppression (210–216).

Although the cases of islet transplants are far fewer than pancreas transplants, there is good evidence to indicate that transplantation of isolated allogeneic islets can cause relapse of autoimmunity in a small but significant portion of patients (199, 217–221). Occasional patients had dramatic rises in islet autoantibodies from around day 5 after transplant that occurred without any sign of allo-immunity (222). Weaker immunosuppression regimes such as MMF plus 1,25 (OH)2 Vit D3 were more frequently associated with a sharp immediate risk in autoantibodies with and without allo-reactivity. Others showed that T cell responses to islet autoantigens are often increased after islet transplants (223, 224). Although associations with reduced graft function have been reported (221), it is not fully proven that the relapsing autoimmune response post islet transplantation equals autoimmune mediated destruction of islet grafts. It's worth mentioning that during immunosuppression and the use of immunodepleting agents, lymphopenia can significantly contribute to the expansion of memory autoreactive T cells (225). This expansion is primarily driven by homeostatic proliferation, which is strongly influenced by the IL-7/IL-7 receptor axis (226). The existence of homeostatic proliferation among effector T cells, including clones of autoreactive T cells, in individuals undergoing islet transplantation (227). Furthermore, it has been demonstrated in various cases, such as the transfer of T1D between siblings after bone marrow transplantation (228) and the development of T1D following islet auto transplantation within the first year after pancreatectomy (229, 230), that autoimmune reactions alone can lead to the destruction of newly transplanted beta cells. With the advent of innovative techniques for producing β cells from readily available pluripotent stem cell sources, concerns pertaining to allorecognition, and HLA matching can be effectively addressed. One approach involves the establishment of master cell banks comprising stem cell-derived β cells that match the major histocompatibility complex (MHC) class I and II alleles commonly found in individuals with T1D. Alternatively, thanks to the capabilities offered by CRISPR-Cas9 gene editing, it becomes feasible to create "stealth" β cells that can evade immune

recognition by disabling endogenous HLA molecules. Additionally, in preparation for the potential resurgence of autoimmune responses, an effective strategy may require the prior reduction of autoreactive memory, along with the conditioning of repopulating lymphocytes to promote enduring immune tolerance. These forthcoming opportunities will be discussed in more detail above.

3 Unanswered questions and persistent knowledge gaps in the immunological challenge of Allo Beta Cell transplantation

Several aspects of the immunological challenge associated with Allo Beta Cell Transplantation continue to elude our complete understanding. These gaps in knowledge raise important questions about the precise mechanisms and factors influencing the success and longevity of beta cell replacement therapies. Addressing these gaps is crucial for advancing our comprehension of the immune response to transplanted beta cells and devising more effective strategies to ensure the sustained function and survival of these cells in individuals with conditions like T1D.

3.1 Lack of evidence on immunosuppression treatment

In contrast to most solid organ transplantations, there is currently no available guidance or formal consensus on the optimal or standard immunosuppressive strategy for Allo Beta Cell Transplantation. This critical gap has led to a significant evolution in immunosuppression approaches over the years, all without the benefit of evidence-based practices (as illustrated in Figure 1). Numerous studies, often conducted on limited patient cohorts, have proposed a variety of immunosuppressive agent combinations (59, 231–233). These encompass agents that deplete T and B cells (such as alemtuzumab, teplizumab, antithymocyte/lymphocyte globulin, rituximab), inhibitors of T-cell activation (like IL2R antagonists daclizumab and basiliximab), replication inhibitors (including azathioprine and mycophenolate mofetil/mycophenolic acid), mTor inhibitors (such as sirolimus and everolimus), lymphocyte tracking inhibitors (like EFA), desensitizing agents (such as intravenous immunoglobulin), co-stimulation inhibitors (including monoclonal antiCD28 belatacept/abatacept), CNIs (cyclosporine and tacrolimus), and anti-inflammatory agents (including corticosteroids, IL1 receptor antagonist, and TNF-alpha inhibitors). It is crucial to emphasize that most of these studies have been observational, consisting of retrospective or prospective single-center single-arm studies. Remarkably, there is only one recently reported randomized controlled trial study that has emerged as an exception, focusing on CXCR1/2 inhibitors (62). Many immunosuppressive drugs used in Allo Beta Cell Transplantation are designed to inhibit specific pathways of alloantigen specific T cell activation, but they ignore the memory autoimmune response, and they were quite ineffective in controlling the IL-7 mediated homeostatic proliferation.

3.2 Lack of reliable method to assess rejection and treatment protocol

Unlike most solid organ transplantations, there is currently no consensus on how to diagnose Allo Beta Cell Transplantation rejection (234). This challenge arises because the traditional gold standard for diagnosing rejection involves tissue biopsy (235). While a whole organ pancreas transplant biopsy can yield valuable insights, particularly for potentially reversible causes of dysfunction, technical challenges limit its routine application. Given that isolated islet transplantation is accomplished by infusing pancreatic islets into the portal circulation, where they disperse throughout the liver, accessing the islet graft for regular biopsies or surveillance becomes unfeasible. Hence, there is a pressing need for standardized clinical diagnostic criteria that can effectively identify ongoing islet allograft rejection. Moreover, there are currently no established treatment protocols in place for Allo Beta Cell Transplantation rejection, which may be related to a paucity of data on diagnostic criteria (236–238). While high-dose steroid therapy is a potential avenue for halting ongoing cellular rejection (234), it's crucial to note that this therapy itself is associated with a possible decline in the functional performance of islet grafts. Furthermore, there have been suggestions for addressing humoral rejection through the utilization of rituximab and IV immunoglobulin therapy, though these recommendations are primarily based on single case reports (237).

3.3 Lack of studies to assess the efficacy of immunologic and metabolic testing to detect early graft dysfunction after Allo Beta Cell transplantation

Undoubtedly, the field of Allo Beta Cell Transplantation faces a conspicuous absence of sensitive, non-invasive serial assays for the early detection of rejection or autoimmune recurrence and the ongoing loss of beta-cell functional mass (239). While a consensus has recently been established for defining clinically successful graft functional outcomes in beta-cell replacement therapies (240), there is still a notable absence of standardized and systematic immunologic and metabolic monitoring protocols following Allo Beta Cell Transplantation. Parameters such as body weight, fasting glucose levels, fasting and random C-peptide concentrations, fasting insulin levels, HbA1c measurements, oral glucose tolerance tests (OGTT), mixed meal tolerance tests (MMTT), insulin clamp studies, continuous glucose monitoring (CGM), assessments for anti-donor human leukocyte antigen antibodies (specifically donor-specific antibodies, DSA), and the monitoring of autoantibodies have been commonly employed by experienced programs worldwide, albeit with varying time schedules and indications (either protocol-driven or initiated "for cause"). However, their effectiveness in detecting early graft dysfunction, particularly at a stage when timely clinical intervention can forestall further deterioration and preserve allograft function, remains unproven (239). Additionally, there remains an ongoing debate regarding the

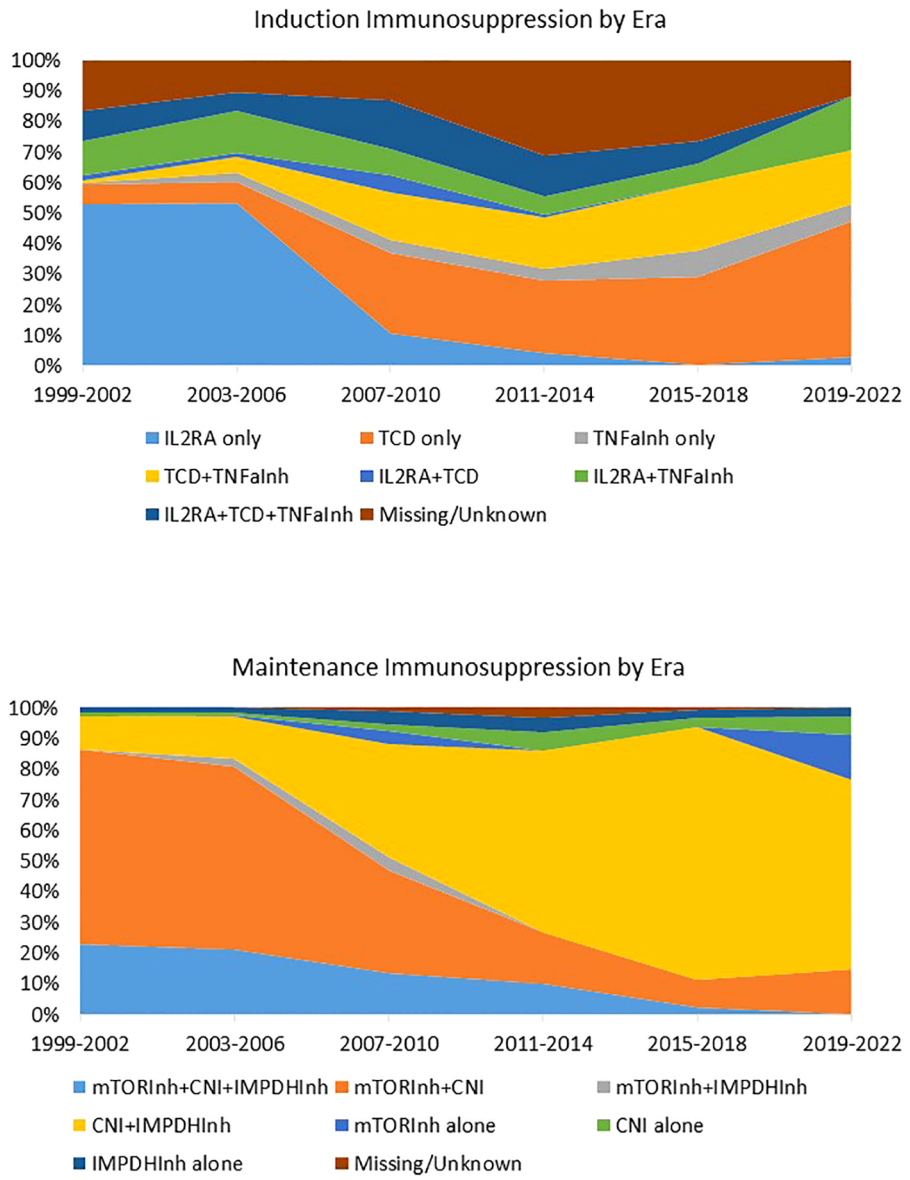

FIGURE 1
Induction and maintenance immunosuppression in islet transplantation by era. Immunosuppression regimen of 1,108 individuals with T1D who received Islet Transplant Alone (n = 992) or Islet after kidney (n = 186) between 1999 and 2022 and were followed by the CITR. Data source: Collaborative Islet Transplant Registry Coordinating Centre: Eleventh allograft report 2022. TCD, T cell depleting agents; Inh, inhibitor; CNI, calcineurin Inhibitor; IMPDH, Inosine-5′-monophosphate dehydrogenase; IL1RA, IL1 receptor antagonist. Reproduced from "Caldara R, Tomajer V, Piemonti L. Enhancing Beta Cell Replacement Therapies: Exploring Calcineurin Inhibitor-Sparing Immunosuppressive Regimens. Transpl Int. 2023 Jun 8;36:11565" with permission from the authors.

predictive role of certain immunological parameters in graft failure. In the broader context of solid organ transplantation, donor-specific antibodies (DSA) are recognized as the primary culprits behind graft failure. Preexisting DSA serves as a relative contraindication to transplantation, and the emergence of *de novo* DSA plays a pivotal role in antibody-mediated rejection, leading to microvascular inflammation and associated with unfavorable outcomes. In the context of islet transplantation, there have been descriptions of the potential adverse effects of *de novo* DSA (199, 241, 242), although not all studies have confirmed this association (243–245). Furthermore, while DSA and assays for islet autoantigen antibodies are well-established and reproducible worldwide, the consistency of other assays and biomarkers remains variable. Emerging assays and platforms designed to assess cellular responses to auto/alloantigens and those focused on donor-derived cell-free deoxyribonucleic acid (dd-cfDNA) are examples of these less established tools that have not yet achieved universal consistency and acceptance.

3.4 Lack of validate biomarker for beta cell death

The current absence of real-time biomarkers for monitoring beta cell death presents a significant challenge in Allo Beta Cell Transplantation. Detecting the loss of islet beta cells after transplantation relies on assessing glycemic control, the need for external insulin supplementation, and measuring insulin secretion, often by evaluating C-peptide levels. The introduction of more sensitive indicators has the potential to facilitate interventions that can prevent clinically significant islet graft loss. Such indicators could be particularly valuable for guiding immune monitoring of humoral and cellular alloimmune and autoimmune markers or for interpreting the potential significance of newly detected alloantigen or autoantigen reactivity in a transplanted islet beta cell graft before any functional deterioration becomes apparent. Various methods have been employed to identify impaired islets in the bloodstream shortly after intraportal infusion, as up to 25% of the transplanted islet mass may be lost. These methods encompass the examination of insulin mRNA (246, 247), glutamic acid decarboxylase-65 (GAD65) (248), miRNA375 (249, 250), and unmethylated insulin DNA (251, 252). These markers have been observed to elevate within 24 hours after islet transplantation, with some being associated with worse islet graft functional outcomes and modulation by anti-inflammatory therapy during the first week post-transplantation. This suggests their potential utility for both predicting early engraftment and assessing interventions aimed at enhancing islet survival during the engraftment period. However, more sensitive, and reproducible assays are needed to detect subtler episodes of cell death that may provide insights into graft rejection or recurrent diabetes.

3.5 Lack of beta cell imaging

Over the past two decades, research in the field of non-invasive beta-cell imaging and beta-cell mass evaluation has witnessed progress (253, 254). This includes the identification of target molecules for imaging probes, the development of chemically modified probes labelled with suitable radioisotopes, and the establishment of analytical methods for signal interpretation through single-photon emission computed tomography and positron emission tomography. Notably, derivatives of exendin-4 designed for imaging show promise as candidates for non-invasive beta-cell mass assessments. However, the non-invasive evaluation of beta-cell mass remains elusive, and practical *in vivo* and clinical techniques for β-cell-specific imaging are yet to be established (253).

4 Emerging directions and game-changing strategies in addressing the immunological challenge of Allo Beta Cell transplantation

The field of Allo Beta Cell Transplantation is witnessing a transformative shift with the emergence of innovative strategies aimed at overcoming the immunological challenges inherent to the procedure. These groundbreaking approaches have the potential to revolutionize the field and significantly enhance the success and sustainability of beta cell replacement therapies.

4.1 Exploring alternatives to conventional immunosuppressive regimens

Allo Beta Cell Transplantation holds great promise as a therapeutic avenue for individuals grappling with T1D, as it offers the potential for achieving insulin independence and markedly improved glycemic control. Nevertheless, the success of this approach is inextricably linked to the adept management of immune responses, a vital factor for thwarting graft rejection and addressing the autoimmune components of the condition. In recent times, the field has witnessed the ascent of various novel approaches within immunosuppression strategies, all geared towards elevating the overall success and accessibility.

4.1.1 Exploring calcineurin inhibitor and depleting agent sparing immunosuppression

Traditional immunosuppressive protocols, while effective in preventing rejection of transplanted organs, often carry the burden of long-term side effects and may not offer comprehensive protection against both alloimmune and autoimmune responses. These customary treatment regimens typically encompass medications like Calcineurin Inhibitors (CNIs), such as cyclosporine and tacrolimus, among others. While these drugs effectively suppress the immune system, achieving their intended objectives, they are not devoid of drawbacks. Prolonged use of CNIs can give rise to complications and their known beta cell (255) and renal toxicities (256) limit their efficacy for pancreas and islet transplantation. These adverse effects can impact a patient's overall well-being and quality of life. Furthermore, CNIs' involvement in the nuclear factor of activated T cells (NFAT) signaling pathway, which is pivotal for the differentiation, maintenance, and suppressive capabilities of Tregs, can have significant repercussions (257). This involvement may hinder the establishment of immune tolerance and impede the effectiveness of potential adoptive therapies employing tolerogenic donor specific Tregs (as discussed below). Additionally, it's important to note that CNIs have no impact on T cell expansion during homeostatic proliferation since they effectively block the IL-2 pathway but are comparatively ineffective in regulating IL-7-mediated homeostatic proliferation (225). Given these considerations, there is a pressing need for research to investigate the safety and feasibility of immunosuppressive regimens that reduce the reliance on CNIs. Equally troublesome is the use of depleting agents like ATG and alemtuzumab (anti-CD52) for induction. These agents can significantly influence the severity of lymphocyte depletion and potentially affect the rate of cell cycling during reconstitution. Lymphocyte depletion therapies with alemtuzumab or ATG can lead to the expansion of alloreactive and autoreactive T cells, in some cases exceeding pretransplant levels (258, 259). Alemtuzumab treatment has been demonstrated to preferentially expand effector-memory T cells in renal transplant recipients, while

induction with ATG expands both effector-memory and central-memory T cell subsets. Furthermore, these agents can pose challenges for the development of adoptive therapy with tolerogenic T regulatory cells, which could be equally recognized and depleted, like conventional T cells. Notably, even non-depleting anti-CD25 monoclonal antibodies could present issues in terms of homeostatic proliferation, as they were specifically designed to prevent the formation of the high-affinity IL-2R complex and block IL-2 signaling. The limited availability of the common gamma chain shared by IL-2 and IL-7 receptors represents a constraint on cytokine signaling. When the formation of the IL-2 receptor is inhibited by non-depleting anti-CD25 monoclonal antibodies, more common gamma chain becomes available for complexing with IL-7R alpha, resulting in increased T cell sensitivity to IL-7 and favoring homeostatic proliferation (260). Considering these considerations, conducting research on immunosuppressive regimens that minimize the use of CNIs and avoid induction with depleting agents will significantly advance beta cell replacement therapies. Some prior small-scale clinical experiences have already demonstrated the value and feasibility of these approaches. Feasibility, safety, and efficacy of CNIs-free and anti-IL-2Ra-free treatments for islet transplantation, which also exclude anti-thymocyte globulin induction during second or third infusions, have been successfully demonstrated (261). More recently, reports have surfaced of 40% insulin independence at 10 years following a single islet infusion with CNI-sparing immunosuppressive regimens, including either belatacept (BELA) or efalizumab (EFA). These regimens have showcased remarkable cases of operational tolerance and substantial expansions of Tregs following islet transplantation (262). Furthermore, the identification of biological and pharmacological controllers of the IL-7/IL-7R axis, which hold promise for potential clinical applications, could be pertinent to the development of advanced immunosuppressive protocols for Allo Beta Cell Transplantation (226).

4.1.2 Rethinking induction therapy and exploring recipient preconditioning

Induction therapy has proven to be an effective strategy for achieving low rates of acute rejection in most allograft situations (263). he necessity for induction immunosuppression arises from the heightened immunogenicity of the allograft during the immediate post-transplant period. Specifically, this vulnerability is attributed to the combined factors of a high frequency of donor-specific T-cell precursors present in most recipients and the activation of the innate immune system during organ transplantation (264). This established approach was developed in a clinical context where organ availability is unpredictable, and the time between organ donation and transplantation falls within a matter of hours. This limitation made it impractical to consider recipient pretreatment longer than 1-2 days or any donor-specific preconditioning strategies. As a result, induction therapy primarily aimed at achieving short-term profound immunosuppression without a focus on long-term sustainability. However, this paradigm could be revolutionized in the realm of Allo Beta Cell Transplantation. The availability of insulin-producing cells derived from replenishable sources like stem cells introduces the possibility of scheduled transplants with known and defined timeframes, along with prior characterization of the donor's MHC profile. This scenario opens new avenues in induction immunosuppression, encompassing approaches such as costimulation-based therapy, mixed chimerism, and adoptive cellular transfer. These innovative strategies aim to restore immunological balance in the context of organ transplantation rather than relying on non-specific immunosuppression. Some experiences have already demonstrated the value and feasibility of these approaches. Some experiences have already demonstrated the value and feasibility of these approaches. For instance, administering apoptotic donor leukocytes around the time of transplant, in conjunction with short-term immunotherapy involving antagonistic anti-CD40 antibody 2C10R4, rapamycin, soluble tumor necrosis factor receptor, and anti-interleukin 6 receptor antibody, has been shown to induce long-term (≥1 year) tolerance to islet allografts in rhesus macaques (265). Similarly, recipient preconditioning with GLP-1 agonists or rapamycin has been proposed as an effective strategy for enhancing graft function in both preclinical and clinical models (266–268). Indeed, this shift in perspective toward induction and recipient preconditioning invites us to reconsider conventional approaches and fosters the exploration of innovative strategies to enhance the field of Allo Beta Cell Transplantation.

4.1.3 Targeting autoimmunity recurrence and beta cell survival

The diabetes community has long anticipated the use of immunosuppressive treatments in individuals with recent-onset T1D and those at risk of developing the disease (269). Currently, aside from the FDA-approved anti-CD3 antibody teplizumab (270), no such treatment is in clinical use. However, recent publications suggest promising strategies in this regard. For instance, low-dose ATG has demonstrated its effectiveness in maintaining C-peptide levels compared to a placebo (271). Teplizumab, in trials involving individuals at high risk of T1D, doubled the time to disease onset compared to a placebo (270). On the other hand, anti-CD3 Otelixizumab failed in its phase III trial. Alefacept, which targets CD2 primarily expressed on CD4+ and CD8+ effector memory T cells, has been tested in recent-onset T1D and displayed C-peptide preservation along with reduced use of exogenous insulin compared to a placebo group (272, 273). Other trials have explored various approaches to combat islet autoimmunity. These include CTLA-4Ig (abatacept) (274), anti-CD20 therapy (rituximab) (275), anti-TNF-α therapies (recombinant TNF-α receptor-IgG fusion protein etanercept and IgG1-κ monoclonal anti-TNF-α antibody golimumab) (276, 277), anti-CD40 therapy (Iscalimab) (278), low-dose IL-2 (279), IL-1 blocker (Anakirna) (280), combination immunomodulatory and beta-cell therapy like anti-IL-21 antibody and liraglutide (281), and Tyrosine Kinase Inhibitors (Imatinib mesylate) (282). While these immunosuppressive regimens have been evaluated to varying degrees of success in recent-onset T1D, exploring these candidates, or future ones, for their ability to attenuate autoimmune responses in beta-cell graft recipients offers new avenues for immune suppression.

4.2 CAR T reg and TCR T reg

The donor beta cells express allogeneic major and minor histocompatibility antigens, traditionally targeted by the host immune response in the setting of organ transplantation. Moreover, the donor beta cells also express a full complement of antigens associated with islet autoimmunity. Of these, glutamic acid decarboxylase 65 (GAD65), insulinoma-associated protein 2, zinc transporter 8 (ZnT8) and (pro)insulin appear to be highly antigenic in humans both for T cells and B cells (166). Beta-cell replacement into a subject with pre-existing autoimmunity is essentially an immunological challenge where conceptually similar immune responses—transplant rejection and tissue-specific autoimmunity—coexist, but with the potential for reactivation of autoreactive memory T and B cells posing an additional set of therapeutic obstacles. Adoptive cell therapy using CD4+CD25+FOXP3+ Tregs, a naturally suppressive immune subset, is a promising approach to achieving localized and specific immune suppression in the site of transplant (283). However, clinical trials testing administration of polyclonal Tregs in recent-onset T1D have observed limited efficacy despite an excellent safety profile (284, 285). Similarly, administration of autologous Tregs together with intraportal allogeneic islet transplantation yielded no severe negative effects (286). These clinical trials have been fundamental to identify barriers to an effective Treg therapy. First, the use of polyclonal Treg for adoptive cell therapy relies on the assumption on the natural existence of rare, disease relevant TCRs in the adoptively transferred Treg population. However, different studies in NOD mice reported that therapy using antigen specific Tregs is far more effective than the one using polyclonal Treg. Notably, one study found that transfer of 2 million antigen-specific (BDC2.5 TCR transgenic) Tregs controlled the rejection of a syngeneic islet transplant in NOD mice, whereas 5 million polyclonal Tregs displayed no effect (287). The recent emergence of advanced gene editing techniques has opened new avenues to engineer Tregs with selected antigen specificity (288). These include the generation of Treg bearing a chimeric antigen receptor (CAR-Treg) as well as T cells bearing a transgenic T cell receptor (TCRtg-Treg) with a selected antigen specificity. CAR are composed by an extracellular antigen-binding domain, usually a single-chain variable fragment (scFv) derived from the variable regions of an antibody linked via hinge and transmembrane domains to an intracellular signaling domain (289). CAR do not need to be MHC-restricted, allowing the use of the same CAR on virtually all subjects independently from their HLAs. Moreover, modern CAR are designed as modular systems in which the signaling pathway activated by antigen recognition can be adapted to the desired effect (290). A notable disadvantage of CARs is the requirement for cell-surface bound target antigen whose expression ideally must be confined to beta-cells. The difficulties in finding a good target antigen on beta-cells has considerably limited the used of CAR-Treg to control autoimmunity in type I diabetes. However, transplanted allogenic beta-cells express mismatched HLA molecules that can be easily targeted by CAR. Human CAR Tregs that target the commonly mismatched HLA-A2 molecules are currently being tested clinically in kidney (NCT04817774) and liver transplantation (NCT052334190). TCRtg-Treg are easier to develop in the context of beta-cell autoimmunity. Indeed, a number of TCRs specific for epitopes of GAD65, preproinsulin, IGRP as well as neo-epitopes have already been identified from patients with type I diabetes (291, 292). While MHC restriction can represent a limitation, the use of target peptides associated to commonly expressed HLAs, such as HLA-A2 or T1D risk associated class II haplotypes, potentially allows to treat a significant proportion of subjects with relatively few different TCRtg-Treg. TCRtg-Treg also requires additional gene editing to be fully functional. Suppression of the endogenous TCR is needed to improve expression of the transgenic TCR but also to avoid mispairing of the endogenous and transgenic TCR alpha and beta chains, potentially impairing beta-cell antigen-specificity and increasing the risk of off-target antigen recognition (293). It has also to be determined whether peptide/HLA class I restricted TCR can efficiently recognize the antigen when transduced into CD4+ Treg and whether transgenic expression of CD8 can improve antigen recognition. With several important issues yet to be determined, Abata Therapeutics have recently announced the development of a beta-cell specific TCRtg-Treg product (ABA-201) that will be clinically tested in 2025. A second important limitation of adoptive Treg therapy is the survival and persistence of Treg transferred in patients that may impact the therapeutic effect. Bluestone et al. (NTC01210664) observed a rapid decline in the number of circulating Treg following adoptive transfer into patients with T1D. Specifically, once infused into patients, the ex vivo expanded Treg population exhibits a biphasic exponential decay kinetic, characterized by a short-lived subset (75-90%) with a half-life of a few days to weeks, and a long-lived subset (10-25%) detectable up to one year post-infusion (284). Notably, although the expanded Treg initially display a CCR7+CD45RO+CD45RA- central-memory phenotype, the subset that survives longer in patients exhibits a CCR7+CD45RA+CD45RO-/+ phenotype, resembling that of conventional naïve or memory stem T cells. Addressing the issue of Treg survival, a second trial involving adoptive transfer of polyclonal Treg cells along with exogenous administration of low doses of recombinant human IL-2 was conducted (279) (NCT02772679). Addition of IL-2 did not improve the survival of adoptively transferred Treg but was associated with increase endogenous Treg numbers and expansion of inflammatory NK and CD8+GMZB+ T cells. Several other strategies to promote Treg survival in patients are under intensive studies. Notably, synthetic orthogonal receptor-ligand pair has been generated. In this approach T cells are transduced with an orthogonal IL-2 receptor that can only be activated by an exogenously administered synthetic ligand (294). An alternative approach is to transduce Treg with a membrane-bound form of IL-2, in which IL-2 is tethered to the membrane by a short linker that only allows cis-interactions between IL-2 and its receptors on the same cell (295). As Treg need to be expanded *in vitro* to achieve a number sufficient to display therapeutic effectiveness, modification to the expansion protocols to improve T cell survival are under consideration. Tregs are traditionally expanded using anti CD3/CD38 microbeads in combination with high doses of interleukin 2 (IL-2) (296). Despite low expression of the IL-7Ralpha (CD127) human naïve Treg have been shown to respond to and proliferate in

response to IL-7 *in vitro* (297). Furthermore, in conditions of Treg depletion, IL-7 contributes to Treg compartment reconstitution in patients treated with the anti-CD25monoclonal antibody basiliximab (226). A novel protocol of expansion of Treg using a combination of IL-2 and IL-7 was shown to improve the survival of Treg in the NSG mouse model (298). As transplanted beta-cells can be targeted by allo-reactive and auto-reactive T cells, adoptive Treg therapy represent an opportunity to keep T cell responses in check. While the clinical testing, especially in T1D, has provided clear results in terms of safety but also highlighted several critical issues that need to be addressed, and effective Treg therapy can be available in the coming years. Specifically in the transplantation setting an additional effort is required to determine T cell survival, persistence, and therapeutic effectiveness when Treg therapy is administered in combination with immune-suppressive drugs.

4.3 Stem stealth cells

Stem cell technology has ushered in a new era in β cell generation for transplantation. "Stem stealth cells" represent a novel concept where stem cells are genetically modified to evade immune recognition. The first and one of the most successful strategies to reduce immunogenicity is the abrogation of the Beta-2 microglobulin (B2M) gene, which encodes a common subunit of HLA class I molecules. Knocking out B2M results in HLA class I-negative iPSCs, which can function as universal donors for the transplantation of cells that do not express HLA class I (299). Several methods have been developed to disrupt the B2M gene in ESCs and iPSCs. For example, one study used Cre-recombinase to ablate two adeno-associated virus (AAV)-inserted cassettes into exon one of the B2M gene. This method successfully silenced B2M expression and resulted in reduced allogeneic responses of T cells (299). A second study employed CRISPR/Cas9 technology to target exons 2 and 3 of the B2M gene, replacing them with other genetic cassettes (300). These cells were resistant to interferon-γ stimulation and alloreactive CD8+ T cells, indicating that they do not express cell surface human leukocyte antigen (HLA) molecules. Additionally, these B2M-/- hESCs do not have any off-target integration or cleavage events, lack stable B2M mRNA, have a normal karyotype, and maintain their self-renewal capacity, genomic stability, and pluripotency. To validate the potential of these strategies, preclinical studies have demonstrated the feasibility of B2M-knockout iPSCs in various transplantation models. B2M-null iPSC-derived cells, such as neurons, cardiomyocytes, and retinal pigment epithelial cells, have been successfully transplanted into animal models, with extended survival and functional integration compared to their HLA-mismatched counterparts (301–303). These promising findings highlight the potential of B2M-knockout cells as universal donors for cell-based therapies. The limit of this approach is that the B2M-null cells are protected from CD8+ T cell responses but become more susceptible to NK cell-mediated destruction (300). To address this issue, new strategies were developed to express specific ligands on the cell surface that interact with inhibitory receptors on NK cells, rendering them less cytotoxic. One such ligand is human leukocyte antigen-E (HLA-E), which interacts with inhibitory receptors such as NKG2A/CD94 on NK cells, leading to their inhibition (304, 305). Expressing HLA-E on the surface of iPSC-derived cells has been shown to protect them from NK cell-mediated lysis (301, 306). In addition to HLA-E, HLA-G, another member of the HLA family with immunosuppressive properties (307) has also been explored (308, 309). Recently, innovative approaches which involve editing iPSC to remove NK-activating ligands, such as CD155 and B7-H3, have been proposed. These ligands, when expressed on the cell surface, can trigger NK cell cytotoxicity (310). By eliminating these ligands, iPSC-derived cells resulted more resistant to NK cell-mediated killing (311). This work also proved that the capacity to differentiate into β cells was not impaired in gene edited iPSC and that iPSC-derived pancreatic cells were able to survive *in vivo* after transplantation in mice, while unedited cells were eliminated by NK cells.

Another component of the immune system involved in rejection is CD4+ T cell, which helps to coordinate the immune response by stimulating other immune cells, such as macrophages, B lymphocytes, and CD8 T lymphocytes. HLA II defected hESC were generated via deleting CIITA, a master regulator of constitutive and IFN-γ inducible expression of HLA II genes. CIITA-/- ESC can differentiate into tissue cells with non-HLA II expression and escape the attack of receptors' CD4+ T cells (302, 312). These strategies and the possibility to combine them hold great promise in enhancing the immune evasion capabilities of transplanted cells.

In addition to approaches that directly aim to escape cytotoxic cell recognition, the induction of tolerogenic genes within transplanted cells to create a more immune-tolerant microenvironment was explored. Several genes have been investigated for their potential to suppress immune responses and promote graft acceptance:

- PD-L1 (Programmed Death-Ligand 1): PD-L1 is an immune checkpoint protein that interacts with the PD-1 receptor on T cells, leading to T cell exhaustion and immune tolerance (313). Studies have shown that overexpressing PD-L1 in iPSC-derived cells can mitigate T cell responses and enhance graft survival (314).
- CTLA4-Ig: Cytotoxic T lymphocyte-associated protein 4-immunoglobulin (CTLA4-Ig) is a fusion protein that binds to CD80 and CD86 on antigen-presenting cells, preventing their interaction with CD28 on T cells. This blockade inhibits T cell activation and promotes immune tolerance (315). Incorporating CTLA4-Ig expression into transplanted cells has demonstrated success in prolonging graft survival (314).
- CD47: CD47 is a cell surface protein that acts as a "don't eat me" signal by binding to the signal-regulatory protein alpha (SIRPα) on phagocytic cells, inhibiting their engulfment of the CD47-expressing cell (316). Enhancing CD47 expression on iPSC-derived cells has been shown to reduce their susceptibility to phagocytic clearance (317, 318).

- IDO (Indoleamine 2,3-Dioxygenase): IDO is an enzyme that plays a role in immunosuppression by degrading tryptophan, an essential amino acid for T cell proliferation (319). By overexpressing IDO in islet cells, researchers have aimed to create a tolerogenic microenvironment that inhibits T cell responses and promotes graft survival (320).

These gene-based strategies aim to create a microenvironment within transplanted cells that is conducive to immune tolerance, thereby improving the long-term survival of grafts. These approaches collectively represent a growing toolbox of strategies to improve the success of islet transplantation without the need for extensive immunosuppressive regimens. To demonstrate this, very recently, gene editing techniques that combine targeting of HLA class I and II and the immunomodulatory gene CD47 were tested in human donor islets, modifying them to become hypoimmune (HIP). It was demonstrated that these human HIP islets could survive, engraft, and improve diabetes in allogeneic, diabetic humanized mice. Furthermore, the HIP islet cells exhibited the ability to evade autoimmune destruction in autologous, diabetic humanized autoimmune mice (318). The same approach of HLA class I and II depletion and CD47 overexpression (B2M-/-CIITA-/-CD47+) was used in rhesus macaque HIP stem cells, which were transplanted into four allogeneic rhesus macaques. The HIP cells demonstrated unrestricted survival for 16 weeks in fully immunocompetent allogeneic recipients and differentiated into various lineages, whereas allogeneic wild-type cells were strongly rejected. Additionally, human HIP cells were differentiated into pancreatic islets and shown to survive in immunocompetent, allogeneic diabetic humanized mice for 4 weeks, effectively ameliorating diabetes. Edited primary rhesus macaque islets with HIP modifications were able to survive for 40 weeks in an allogeneic rhesus macaque recipient without the need for immunosuppression, whereas unedited islets were rapidly rejected (321).

This last evidence supports the strategy to use gene engineering to make stem cell-derived and isolated islet transplants less visible to the host immune system, thereby increasing the likelihood of successful transplantation and reducing the dependence on long-term immunosuppressive therapy. From the abrogation of the B2M and CIITA genes to the modulation of NK ligands, these innovative ways could protect transplanted cells from immune responses (311). Moreover, the induction of tolerogenic genes like PDL-1 and CD47 and the engineering of immune-evasive islets have shown promise in creating a more immunologically tolerant microenvironment within the transplanted cells (322).

Despite these remarkable advancements, challenges remain on the path to clinical implementation. The long-term safety of these immune-evasive strategies need to be rigorously evaluated. In fact, hypoimmunogenic cells may raise potential safety concerns associated with long-term immune surveillance and malignancy risk: a hypoimmunogenic transplant may evade immediate immune responses, but the long-term ability of the recipient's immune system to recognize and respond to potential threats, such as malignancies or chronic infections, may be compromised. Besides, a suppressed immune system may be less effective in preventing the growth and spread of tumor cells. One possible strategy to increase the safety of hypoimmunogenic cell would be to equip the cell with a safety switch, able to induce cell suicide in case of abnormal cell proliferation and tumorigenesis (323, 324).

Finally, the safety of genetic manipulation must be considered, and safety improvements achieved by the thoughtful design of nucleases and repair templates, the analysis of off-target editing, and the careful utilization of viral vectors (325–327). The development of new generations of gene editing tools will hopefully bring to improved targeting of specific sequences while minimizing the risk of unintended outcomes.

In conclusion, if combination of gene editing immunological targets will prove effective and safety requirements will be satisfied, stem stealth cells have the chance of serving as a replenishable and customizable source of βcells for transplantation, mitigating the risks associated with immune rejection.

4.4 Tissue engineering and encapsulation

Tissue engineering and encapsulation technologies have made remarkable progress in creating protective microenvironments for transplanted beta cells, reshaping the landscape of diabetes treatment (328, 329). Micro and macro-encapsulation devices function as essential shields, safeguarding cells from immune attacks while facilitating the crucial exchange of oxygen and nutrients. In this scenario, a valuable lesson has been gleaned from the clinical experience of Viacyte, emphasizing the need for a swift transition from a closed to an open device to facilitate vascular scaffold connection (117). This underscores the importance of considering the mandatory requirements of beta cells in terms of nutrient supply and vascular integration in tissue engineering for beta cell replacement. Concurrently, ongoing initiatives in tissue engineering are focused on the development of bioengineered scaffolds that closely mimic the natural pancreatic microenvironment, thereby enhancing the survival and function of transplanted cells (329). These innovative strategies not only shield beta cells from immune threats but also facilitate seamless integration and sustained functionality within the host environment (328). These technologies, ranging from organ engineering (330) to cutting-edge 3D-bioprinting (331), play a pivotal role in modulating the endocrine niche before transplantation. This modulation is achieved by intricately integrating various cell components within an extracellular matrix (ECM) framework. Dedicated bioreactors enable the repopulation of these constructs with different target cells, matured to acquire new scaffold functions. For example, the repurposing of organ strategies has transformed decellularized rat lungs into structures repopulated with pancreatic islet and endothelial cells, generating a vascularized endocrine pancreas (332, 333). These new devices exhibit matured vascularized endocrine structures, resembling the pancreatic endocrine niche prior the implantation, displaying both

ex vivo and *in vivo* functionality. This versatility in cell selection may allow, in the future, for the design of immunomodulation strategies during the engineering process, reducing device immunogenicity and enabling the delivery of immune-modulatory compounds.

In the first scenario, a viable solution involves selecting autologous endocrine niche cells to create an open vascularized device, significantly reducing immunogenicity from a transplantation perspective. While autologous cells sourced from stem cells are prone to autoimmune responses, recent gene editing advancements have generated cell sources from stem cells or even human islet or pig donors, evading both auto and allo or xeno-immune responses (318, 334, 335). This progress empowers the assembly of innovative open devices, ensuring complete structural integration and genetically engineered immune protection. Although these strategies look promising, they are still evaluated in advanced preclinical stage and further tests will be required to move in clinical arena. In the second scenario, immunomodulatory compounds are delivered within the device and locally released at the transplant site, minimizing compound toxicity, and enhancing local drug efficacy. In this context hydrogels are widely used as cell encapsulation technology, as their mechanical properties, along with the high hydration degree, mimic soft tissues. They can be synthesized in the micro and macro scale, which typically imposes a volume increase that prevents intrahepatic infusion. They have been largely tested within beta cells and have been demonstrated to safely integrate with the recipient allowing vascularization *in vivo* (336, 337). Multiple engineered scaffolds have been developed to deliver immunomodulatory compounds or apoptosis modulators in hydrogel form, dampening or halting the immune-mediated graft response (338, 339). These attempts reported, in preclinical setting promising result in protecting beta cells from recipient immune attack. Additional experimental are on-going to observe long term function of this devices and their efficacy in protecting the graft based on local immunomodulatory compounds with pancreatic islet or stem cell derived beta-cells form immune recognition (340). Alternatively, advanced macro-engineering devices are pre-implanted to foster vascular integration. Subsequently, these devices can be loaded with pancreatic islets and immune-suppressant drugs, shielding engrafted pancreatic islets from inflammatory and immunological reactions. Recent data, in both rodent and human primate model, have demonstrated the effectiveness of this technology in protecting engrafted cells and constraining the immune reaction against the graft in the presence of a reduced early engraftment due to the time of connection of the seeded islet within the new generated vascular network.

In the evolution of tissue engineering approaches for beta cell replacement, a critical role has also the selection of the implantation site that can affect, from oxygen, nutrient supply and immunological activity, the outcome, and the translatability of the results. Despite the agreement that an extrahepatic site for islet transplantation is needed, non a common consensus have been released on the best alternative site for device implantation. The most used is the subcutaneous space considering its exposure and flexibility in case of device retrieval. In this direction a recent study has introduced a cutting-edge computational platform. This platform aims to explore the therapeutic potential of programmable bioartificial pancreas devices (341). The study employed sophisticated software that considered factors such as cell load and site-specific oxygen levels. This analysis allowed for precise adjustments in terms of cell loading and oxygen supply within the device, marking a significant stride in the field of tissue engineering for diabetes treatment. Looking ahead, artificial intelligence (AI) tools are poised to play a pivotal role in advancing beta cell replacement technology (342). By leveraging AI, researchers can amalgamate intricate details such as scaffold designs, transplantation site characteristics (including vascularization and immunoreactivity), and the specific cell types being used (343). These AI-driven tools are anticipated to revolutionize device design, guiding the creation of an ideal technology tailored to individual patient needs. This integration of advanced computational techniques and artificial intelligence heralds a new era in tissue engineering, promising more effective and personalized solutions in the realm of beta cell replacement therapies.

5 Conclusion

In closing, Allo Beta Cell Transplantation represents a beacon of hope in the quest to transform the lives of individuals living with T1D. As we navigate the immunological intricacies that come with this therapeutic approach, innovation, collaboration, and a deep understanding of the interplay between the immune system and beta cells are the keys to success. With each unanswered question, we inch closer to effective solutions, and with each emerging strategy, we gain ground in the battle against T1D. As we move forward, we do so with a shared commitment to improving the lives of those who face the daily challenges of T1D, fuelled by the promise of Allo Beta Cell Transplantation and the resolve to conquer its immunological hurdles.

Author contributions

RoC: Conceptualization, Writing – original draft. VT: Conceptualization, Writing – original draft. PMo: Conceptualization, Writing – original draft. VS: Conceptualization, Writing – original draft. AC: Conceptualization, Data curation, Writing – original draft. RaC: Writing – review & editing. CG: Writing – review & editing. DC: Writing – review & editing. ST: Writing – review & editing. VP: Writing – review & editing. RM: Writing – review & editing. AM: Writing – review & editing. RN: Writing – review & editing. PMa: Writing – review & editing. SP: Conceptualization, Writing – review & editing. LP: Conceptualization, Data curation, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Joslin EP. *The Treatment of Diabetes Mellitus: With Observations Upon the Disease Based Upon Thirteen Hundred Cases*. Philadelphia: Lea & Febiger (1917).

2. Banting FG, Best CH, Collip JB, Campbell WR, Fletcher AA. Pancreatic extracts in the treatment of diabetes mellitus. *Can Med Assoc J* (1922) 12(3):141–6.

3. Piemonti L. Felix dies natalis, insulin … Ceterum autem censeo "Beta is better". *Acta Diabetol* (2021) 58(10):1287–306. doi: 10.1007/s00592-021-01737-3

4. Shah VN, DuBose SN, Li Z, Beck RW, Peters AL, Weinstock RS, et al. Continuous glucose monitoring profiles in healthy nondiabetic participants: A multicenter prospective study. *J Clin Endocrinol Metab* (2019) 104(10):4356–64. doi: 10.1210/jc.2018-027635479355

5. Karageorgiou V, Papaioannou TG, Bellos I, Alexandraki K, Tentolouris N, Stefanadis C, et al. Effectiveness of artificial pancreas in the non-adult population: A systematic review and network meta-analysis. *Metabolism* (2019) 90:20–30. doi: 10.1016/j.metabol.2018.10.002

6. Weisman A, Bai J-W, Cardinez M, Kramer CK, Perkins BA. Effect of artificial pancreas systems on glycaemic control in patients with type 1 diabetes: A systematic review and meta-analysis of outpatient randomised controlled trials. *Lancet Diabetes Endocrinol* (2017) 5(7):501–12. doi: 10.1016/S2213-8587(17)30167-5

7. Bekiari E, Kitsios K, Thabit H, Tauschmann M, Athanasiadou E, Karagiannis T, et al. Artificial pancreas treatment for outpatients with type 1 diabetes: systematic review and meta-analysis. *BMJ* (2018) 361:k1310. doi: 10.1136/bmj.k1310

8. Dai X, Luo Z-c, Zhai L, Zhao W-p, Huang F. Artificial pancreas as an effective and safe alternative in patients with type 1 diabetes mellitus: A systematic review and meta-analysis. *Diabetes Ther* (2018) 9(3):1269–77. doi: 10.1007/s13300-018-0436-y

9. Maahs DM, Buckingham BA, Castle JR, Cinar A, Damiano ER, Dassau E, et al. Outcome measures for artificial pancreas clinical trials: A consensus report. *Diabetes Care* (2016) 39(7):1175–9. doi: 10.2337/dc15-2716

10. Danne T, Nimri R, Battelino T, Bergenstal RM, Close KL, DeVries JH, et al. International consensus on use of continuous glucose monitoring. *Diabetes Care* (2017) 40(12):1631–40. doi: 10.2337/dc17-1600

11. Battelino T, Danne T, Bergenstal RM, Amiel SA, Beck R, Biester T, et al. Clinical targets for continuous glucose monitoring data interpretation: recommendations from the international consensus on time in range. *Diabetes Care* (2019) 42(8):1593–603. doi: 10.2337/dci19-0028dci19-0028

12. Yang J, Zhou Y, Zhang T, Lin X, Ma X, Wang Z, et al. Fasting blood glucose and hba1c correlate with severity of coronary artery disease in elective Pci patients with Hba1c 5.7% to 6.4%. *Angiology* (2020) 71(2):167–74. doi: 10.1177/0003319719887655

13. Verdoia M, Schaffer A, Cassetti E, Barbieri L, Di Ruocco MV, Perrone-Filardi P, et al. Glycosylated hemoglobin and coronary artery disease in patients without diabetes mellitus. *Am J Prev Med* (2014) 47(1):9–16. doi: 10.1016/j.amepre.2014.02.002

14. Xia J, Yin C. Glucose variability and coronary artery disease. *Heart Lung Circ* (2019) 28(4):553–9. doi: 10.1016/j.hlc.2018.10.019

15. Ayhan SS, Tosun M, Ozturk S, Alcelik A, Ozlu MF, Erdem A, et al. Glycated haemoglobin is correlated with the severity of coronary artery disease independently of traditional risk factors in young patients. *Endokrynol Pol* (2012) 63(5):367–71.

16. Dutta B, Neginhal M, Iqbal F. Glycated hemoglobin (Hba1c) correlation with severity of coronary artery disease in non-diabetic patients-a hospital based study from North-Eastern India. *J Clin Diagn research: JCDR* (2016) 10(9):OC20–oc3. doi: 10.7860/jcdr/2016/22378.8525

17. Sarwar N, Aspelund T, Eiriksdottir G, Gobin R, Seshasai SRK, Forouhi NG, et al. Markers of dysglycaemia and risk of coronary heart disease in people without diabetes: reykjavik prospective study and systematic review. *PloS Med* (2010) 7(5):e1000278. doi: 10.1371/journal.pmed.1000278

18. Garg N, Moorthy N, Kapoor A, Tewari S, Kumar S, Sinha A, et al. Hemoglobin A1c in nondiabetic patients: an independent predictor of coronary artery disease and its severity. *Mayo Clin Proc* (2014) 89(7):908–16. doi: 10.1016/j.mayocp.2014.03.017

19. Khaw K-T, Wareham N, Luben R, Bingham S, Oakes S, Welch A, et al. Glycated haemoglobin, diabetes, and mortality in men in Norfolk cohort of European prospective investigation of cancer and nutrition (Epic-norfolk). *BMJ* (2001) 322 (7277):15. doi: 10.1136/bmj.322.7277.15

20. Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (Ukpds 35): prospective observational study. *BMJ* (2000) 321(7258):405–12. doi: 10.1136/bmj.321.7258.405

21. Khaw KT, Wareham N, Bingham S, Luben R, Welch A, Day N. Association of hemoglobin A1c with cardiovascular disease and mortality in adults: the European prospective investigation into cancer in Norfolk. *Ann Intern Med* (2004) 141(6):413–20. doi: 10.7326/0003-4819-141-6-200409210-00006

22. The relationship of glycemic exposure (Hba1c) to the risk of development and progression of retinopathy in the diabetes control and complications trial. *Diabetes* (1995) 44(8):968–83. doi: 10.2337/diab.44.8.968

23. Benhalima K, Standl E, Mathieu C. The importance of glycemic control: how low should we go with hba1c? Start early, go safe, go low. *J Diabetes Complications* (2011) 25 (3):202–7. doi: 10.1016/j.jdiacomp.2010.03.002S1056-8727(10)00041-3

24. McKnight JA, Wild SH, Lamb MJ, Cooper MN, Jones TW, Davis EA, et al. Glycaemic control of type 1 diabetes in clinical practice early in the 21st century: an international comparison. *Diabetes Med* (2015) 32(8):1036–50. doi: 10.1111/dme.12676

25. Shah VN, Grimsmann JM, Foster NC, Dost A, Miller KM, Pavel M, et al. Undertreatment of cardiovascular risk factors in the type 1 diabetes exchange clinic network (United States) and the prospective diabetes follow-up (Germany/Austria) registries. *Diabetes Obes Metab* (2020) 22(9):1577–85. doi: 10.1111/dom.14069

26. Foster NC, Beck RW, Miller KM, Clements MA, Rickels MR, DiMeglio LA, et al. State of type 1 diabetes management and outcomes from the T1d exchange in 2016–2018. *Diabetes Technol Ther* (2019) 21(2):66–72. doi: 10.1089/dia.2018.0384

27. Phelan H, Clapin H, Bruns L, Cameron FJ, Cotterill AM, Couper JJ, et al. The australasian diabetes data network: first national audit of children and adolescents with type 1 diabetes. *Med J Aust* (2017) 206(3):121–5. doi: 10.5694/mja16.00737

28. Carlsen S, Skrivarhaug T, Thue G, Cooper JG, Goransson L, Lovaas K, et al. Glycemic control and complications in patients with type 1 diabetes - a registry-based longitudinal study of adolescents and young adults. *Pediatr Diabetes* (2017) 18(3):188–95. doi: 10.1111/pedi.12372

29. Edridge CL, Dunkley AJ, Bodicoat DH, Rose TC, Gray LJ, Davies MJ, et al. Prevalence and incidence of hypoglycaemia in 532,542 people with type 2 diabetes on oral therapies and insulin: A systematic review and meta-analysis of population based

studies. *PloS One* (2015) 10(6):e0126427. doi: 10.1371/journal.pone.0126427PONE-D-15-00548

30. Alwafi H, Alsharif AA, Wei L, Langan D, Naser AY, Mongkhon P, et al. Incidence and prevalence of hypoglycaemia in type 1 and type 2 diabetes individuals: A systematic review and meta-analysis. *Diabetes Res Clin Pract* (2020) 170:108522. doi: 10.1016/j.diabres.2020.108522

31. Pedersen-Bjergaard U, Thorsteinsson B. Reporting severe hypoglycemia in type 1 diabetes: facts and pitfalls. *Curr Diabetes Rep* (2017) 17(12):131. doi: 10.1007/s11892-017-0965-1

32. Seaquist ER, Anderson J, Childs B, Cryer P, Dagogo-Jack S, Fish L, et al. Hypoglycemia and diabetes: A report of a workgroup of the American diabetes association and the endocrine society. *J Clin Endocrinol Metab* (2013) 98(5):1845–59. doi: 10.1210/jc.2012-4127jc.2012-

33. Khunti K, Alsifri S, Aronson R, Berković MC, Enters-Weijnen C, Forsén T, et al. Impact of hypoglycaemia on patient-reported outcomes from a global, 24-country study of 27,585 people with type 1 and insulin-treated type 2 diabetes. *Diabetes Res Clin Pract* (2017) 130:121–9. doi: 10.1016/j.diabres.2017.05.004

34. Whitmer RA, Karter AJ, Yaffe K, Quesenberry CP, Selby JV. Hypoglycemic episodes and risk of dementia in older patients with type 2 diabetes mellitus. *Jama* (2009) 301(15):1565–72. doi: 10.1001/jama.2009.460

35. Lin CH, Sheu WH. Hypoglycaemic episodes and risk of dementia in diabetes mellitus: 7-year follow-up study. *J Intern Med* (2013) 273(1):102–10. doi: 10.1111/joim.12000

36. Yaffe K, Falvey CM, Hamilton N, Harris TB, Simonsick EM, Strotmeyer ES, et al. Association between hypoglycemia and dementia in a biracial cohort of older adults with diabetes mellitus. *JAMA Internal Med* (2013) 173(14):1300–6. doi: 10.1001/jamainternmed.2013.6176

37. Nilsson M, Jensen N, Gejl M, Bergmann ML, Storgaard H, Zander M, et al. Experimental non-severe hypoglycaemia substantially impairs cognitive function in type 2 diabetes: A randomised crossover trial. *Diabetologia* (2019) 62(10):1948–58. doi: 10.1007/s00125-019-4964-4

38. McCrimmon RJ. Consequences of recurrent hypoglycaemia on brain function in diabetes. *Diabetologia* (2021) 64(5):971–7. doi: 10.1007/s00125-020-05369-0

39. Goto A, Arah OA, Goto M, Terauchi Y, Noda M. Severe hypoglycaemia and cardiovascular disease: systematic review and meta-analysis with bias analysis. *BMJ: Br Med J* (2013) 347:f4533. doi: 10.1136/bmj.f4533

40. Bedenis R, Price AH, Robertson CM, Morling JR, Frier BM, Strachan MW, et al. Association between severe hypoglycemia, adverse macrovascular events, and inflammation in the edinburgh type 2 diabetes study. *Diabetes Care* (2014) 37 (12):3301–8. doi: 10.2337/dc14-0908dc14-0908

41. Giménez M, Gilabert R, Monteagudo J, Alonso A, Casamitjana R, Paré C, et al. Repeated episodes of hypoglycemia as a potential aggravating factor for preclinical atherosclerosis in subjects with type 1 diabetes. *Diabetes Care* (2011) 34(1):198–203. doi: 10.2337/dc10-1371

42. Khunti K, Davies M, Majeed A, Thorsted BL, Wolden ML, Paul SK. Hypoglycemia and risk of cardiovascular disease and all-cause mortality in insulin-treated people with type 1 and type 2 diabetes: A cohort study. *Diabetes Care* (2015) 38 (2):316–22. doi: 10.2337/dc14-0920

43. Hendrieckx C, Halliday J, Bowden J, Colman P, Cohen N, Jenkins A, et al. Severe hypoglycaemia and its association with psychological well-being in Australian adults with type 1 diabetes attending specialist tertiary clinics. *Diabetes Res Clin Pract* (2014) 103(3):430–6. doi: 10.1016/j.diabres.2013.12.005

44. Evans M, Khunti K, Mamdani M, Galbo-Jørgensen CB, Gundgaard J, Bøgelund M, et al. Health-related quality of life associated with daytime and nocturnal hypoglycaemic events: A time trade-off survey in five countries. *Health Qual Life outcomes* (2013) 11(1):1–9. doi: 10.1186/1477-7525-11-90

45. Sturt J, Dennick K, Due-Christensen M, McCarthy K. The detection and management of diabetes distress in people with type 1 diabetes. *Curr Diabetes Rep* (2015) 15(11):101. doi: 10.1007/s11892-015-0660-z

46. Duffus SH, Ta'ani ZA, Slaughter JC, Niswender KD, Gregory JM. Increased proportion of time in hybrid closed-loop "Auto mode" Is associated with improved glycaemic control for adolescent and young patients with adult type 1 diabetes using the minimed 670g insulin pump. *Diabetes Obes Metab* (2020) 22(4):688–93. doi: 10.1111/dom.13912

47. Messer LH, Berget C, Vigers T, Pyle L, Geno C, Wadwa RP, et al. Real world hybrid closed-loop discontinuation: predictors and perceptions of youth discontinuing the 670g system in the first 6 months. *Pediatr Diabetes* (2020) 21(2):319–27. doi: 10.1111/pedi.12971

48. Lung TW, Hayes AJ, Herman WH, Si L, Palmer AJ, Clarke PM. A meta-analysis of the relative risk of mortality for type 1 diabetes patients compared to the general population: exploring temporal changes in relative mortality. *PloS One* (2014) 9(11): e113635. doi: 10.1371/journal.pone.0113635PONE-D-14-37400

49. Centers for Disease Control and Prevention. Diabetes death rates among youths aged </= 19 years–United States, 1968-2009. *MMWR Morb Mortal Wkly Rep* (2012) 61 (43):869–72.

50. Mortality in Type 1 Diabetes in the Dcct/Edic Versus the General Population. *Diabetes Care* (2016) 39(8):1378–83. doi: 10.2337/dc15-2399

51. Secrest AM, Becker DJ, Kelsey SF, LaPorte RE, Orchard TJ. All-cause mortality trends in a large population-based cohort with long-standing childhood-onset type 1 diabetes: the allegheny county type 1 diabetes registry. *Diabetes Care* (2010) 33 (12):2573–9. doi: 10.2337/dc10-1170

52. Soedamah-Muthu S, Fuller J, Mulnier H, Raleigh V, Lawrenson R, Colhoun H. All-cause mortality rates in patients with type 1 diabetes mellitus compared with a non-diabetic population from the Uk general practice research database, 1992–1999. *Diabetologia* (2006) 49(4):660–6. doi: 10.1007/s00125-005-0120-4

53. Jørgensen ME, Almdal TP, Carstensen B. Time trends in mortality rates in type 1 diabetes from 2002 to 2011. *Diabetologia* (2013) 56(11):2401–4. doi: 10.1007/s00125-013-3025-7

54. Petrie D, Lung TW, Rawshani A, Palmer AJ, Svensson A-M, Eliasson B, et al. Recent trends in life expectancy for people with type 1 diabetes in Sweden. *Diabetologia* (2016) 59(6):1167–76. doi: 10.1007/s00125-016-3914-7

55. Huo L, Harding JL, Peeters A, Shaw JE, Magliano DJ. Life expectancy of type 1 diabetic patients during 1997–2010: A national Australian registry-based cohort study. *Diabetologia* (2016) 59(6):1177–85. doi: 10.1007/s00125-015-3857-4

56. Patterson CC, Dahlquist G, Harjutsalo V, Joner G, Feltbower RG, Svensson J, et al. Early mortality in eurodiab population-based cohorts of type 1 diabetes diagnosed in childhood since 1989. *Diabetologia* (2007) 50(12):2439–42. doi: 10.1007/s00125-007-0824-8

57. Livingstone SJ, Levin D, Looker HC, Lindsay RS, Wild SH, Joss N, et al. Estimated life expectancy in a Scottish cohort with type 1 diabetes, 2008-2010. *Jama* (2015) 313(1):37–44. doi: 10.1001/jama.2014.16425

58. Marzorati S, Pileggi A, Ricordi C. Allogeneic islet transplantation. *Expert Opin Biol Ther* (2007) 7(11):1627–45. doi: 10.1517/14712598.7.11.1627

59. Hering BJ, Clarke WR, Bridges ND, Eggerman TL, Alejandro R, Bellin MD, et al. Phase 3 trial of transplantation of human islets in type 1 diabetes complicated by severe hypoglycemia. *Diabetes Care* (2016) 39(7):1230–40. doi: 10.2337/dc15-1988dc15-1988

60. Markmann JF, Rickels MR, Eggerman TL, Bridges ND, Lafontant DE, Qidwai J, et al. Phase 3 trial of human islet-after-kidney transplantation in type 1 diabetes. *Am J Transplant* (2021) 21(4):1477–92. doi: 10.1111/ajt.16174

61. Lablanche S, Vantyghem MC, Kessler L, Wojtusciszyn A, Borot S, Thivolet C, et al. Islet transplantation versus insulin therapy in patients with type 1 diabetes with severe hypoglycaemia or poorly controlled glycaemia after kidney transplantation (Trimeco): A multicentre, randomised controlled trial. *Lancet Diabetes Endocrinol* (2018) 6(7):527–37. doi: 10.1016/S2213-8587(18)30078-0

62. Maffi P, Lundgren T, Tufveson G, Rafael E, Shaw JAM, Liew A, et al. Targeting cxcr1/2 does not improve insulin secretion after pancreatic islet transplantation: A phase 3, double-blind, randomized, placebo-controlled trial in type 1 diabetes. *Diabetes Care* (2020) 43(4):710–8. doi: 10.2337/dc19-1480dc19-1480

63. Vantyghem MC, de Koning EJP, Pattou F, Rickels MR. Advances in beta-cell replacement therapy for the treatment of type 1 diabetes. *Lancet* (2019) 394 (10205):1274–85. doi: 10.1016/S0140-6736(19)31334-0

64. Vantyghem M-C, Chetboun M, Gmyr V, Jannin A, Espiard S, Le Mapihan K, et al. Ten-year outcome of islet alone or islet after kidney transplantation in type 1 diabetes: A prospective parallel-arm cohort study. *Diabetes Care* (2019) 42(11):2042–9. doi: 10.2337/dc19-0401

65. Marfil-Garza BA, Imes S, Verhoeff K, Hefler J, Lam A, Dajani K, et al. Pancreatic islet transplantation in type 1 diabetes: 20-year experience from a single-centre cohort in Canada. *Lancet Diabetes Endocrinol* (2022) 10(7):519–32. doi: 10.1016/S2213-8587(22)00114-0

66. Vantyghem MC, Raverdy V, Balavoine AS, DeFrance F, Caiazzo R, Arnalsteen L, et al. Continuous glucose monitoring after islet transplantation in type 1 diabetes: an excellent graft function (Beta-score greater than 7) is required to abrogate hyperglycemia, whereas a minimal function is necessary to suppress severe hypoglycemia (Beta-score greater than 3). *J Clin Endocrinol Metab* (2012) 97(11): E2078–83. doi: 10.1210/jc.2012-2115jc.2012-2115

67. Johnson JA, Kotovych M, Ryan EA, Shapiro AM. Reduced fear of hypoglycemia in successful islet transplantation. *Diabetes Care* (2004) 27(2):624–5. doi: 10.2337/diacare.27.2.624

68. Poggioli R, Faradji RN, Ponte G, Betancourt A, Messinger S, Baidal DA, et al. Quality of life after islet transplantation. *Am J Transplant* (2006) 6(2):371–8. doi: 10.1111/j.1600-6143.2005.01174.x

69. Toso C, Shapiro AM, Bowker S, Dinyari P, Paty B, Ryan EA, et al. Quality of life after islet transplant: impact of the number of islet infusions and metabolic outcome. *Transplantation* (2007) 84(5):664–6. doi: 10.1097/01.tp.0000280550.01028.8900007890-200709150-00017

70. Leitao CB, Tharavanij T, Cure P, Pileggi A, Baidal DA, Ricordi C, et al. Restoration of hypoglycemia awareness after islet transplantation. *Diabetes Care* (2008) 31(11):2113–5. doi: 10.2337/dc08-0741

71. Tharavanij T, Betancourt A, Messinger S, Cure P, Leitao CB, Baidal DA, et al. Improved long-term health-related quality of life after islet transplantation. *Transplantation* (2008) 86(9):1161–7. doi: 10.1097/TP.0b013e31818a7f4500007890-200811150-00002

72. Radosevich DM, Jevne R, Bellin M, Kandaswamy R, Sutherland DE, Hering BJ. Comprehensive health assessment and five-yr follow-up of allogeneic islet transplant recipients. *Clin Transplant* (2013) 27(6):E715–24. doi: 10.1111/ctr.12265

73. Benhamou PY, Milliat-Guittard L, Wojtusciszyn A, Kessler L, Toso C, Baertschiger R, et al. Quality of life after islet transplantation: data from the gragil 1

and 2 trials. *Diabetes Med* (2009) 26(6):617–21. doi: 10.1111/j.1464-5491.2009.02731.xDME2731

74. Cure P, Pileggi A, Froud T, Messinger S, Faradji RN, Baidal DA, et al. Improved metabolic control and quality of life in seven patients with type 1 diabetes following islet after kidney transplantation. *Transplantation* (2008) 85(6):801–12. doi: 10.1097/TP.0b013e318166a27b00007890-200803270-00005

75. Barshes NR, Vanatta JM, Mote A, Lee TC, Schock AP, Balkrishnan R, et al. Health-related quality of life after pancreatic islet transplantation: A longitudinal study. *Transplantation* (2005) 79(12):1727–30. doi: 10.1097/01.TP.0000160816.21799.F5

76. Thompson DM, Meloche M, Ao Z, Paty B, Keown P, Shapiro RJ, et al. Reduced progression of diabetic microvascular complications with islet cell transplantation compared with intensive medical therapy. *Transplantation* (2011) 91(3):373–8. doi: 10.1097/TP.0b013e31820437f3

77. Lee TC, Barshes NR, O'Mahony CA, Nguyen L, Brunicardi FC, Ricordi C, et al. The effect of pancreatic islet transplantation on progression of diabetic retinopathy and neuropathy. *Transplant Proc* (2005) 37(5):2263–5. doi: 10.1016/j.transproceed.2005.03.011

78. Warnock GL, Thompson DM, Meloche RM, Shapiro RJ, Ao Z, Keown P, et al. A multi-year analysis of islet transplantation compared with intensive medical therapy on progression of complications in type 1 diabetes. *Transplantation* (2008) 86(12):1762–6. doi: 10.1097/TP.0b013e318190b05200007890-200812270-00025

79. Venturini M, Fiorina P, Maffi P, Losio C, Vergani A, Secchi A, et al. Early Increase of Retinal Arterial and Venous Blood Flow Velocities at Color Doppler Imaging in Brittle Type 1 Diabetes after Islet Transplant Alone. *Transplantation* (2006) 81(9):1274–7. doi: 10.1097/01.tp.0000208631.63235.6a00007890-200605150-00009

80. Del Carro U, Fiorina P, Amadio S, De Toni Franceschini L, Petrelli A, Menini S, et al. Evaluation of polyneuropathy markers in type 1 diabetic kidney transplant patients and effects of islet transplantation: neurophysiological and skin biopsy longitudinal analysis. *Diabetes Care* (2007) 30(12):3063–9. doi: 10.2337/dc07-0206

81. Vantyghem MC, Quintin D, Caiazzo R, Leroy C, Raverdy V, Cassim F, et al. Improvement of electrophysiological neuropathy after islet transplantation for type 1 diabetes: A 5-year prospective study. *Diabetes Care* (2014) 37(6):e141–2. doi: 10.2337/dc14-032037/6/e141

82. D'Addio F, Maffi P, Vezzulli P, Vergani A, Mello A, Bassi R, et al. Islet transplantation stabilizes hemostatic abnormalities and cerebral metabolism in individuals with type 1 diabetes. *Diabetes Care* (2014) 37(1):267–76. doi: 10.2337/dc13-1663dc13-1663

83. Gerber PA, Pavlicek V, Demartines N, Zuellig R, Pfammatter T, Wuthrich R, et al. Simultaneous islet-kidney vs pancreas-kidney transplantation in type 1 diabetes mellitus: A 5 year single centre follow-up. *Diabetologia* (2008) 51(1):110–9. doi: 10.1007/s00125-007-0860-4

84. Toso C, Baertschiger R, Morel P, Bosco D, Armanet M, Wojtusciszyn A, et al. Sequential kidney/islet transplantation: efficacy and safety assessment of a steroid-free immunosuppression protocol. *Am J Transplant* (2006) 6(5 Pt 1):1049–58. doi: 10.1111/j.1600-6143.2006.01303.x

85. Fiorina P, Folli F, Zerbini G, Maffi P, Gremizzi C, Di Carlo V, et al. Islet transplantation is associated with improvement of renal function among uremic patients with type I diabetes mellitus and kidney transplants. *J Am Soc Nephrol* (2003) 14(8):2150–8. doi: 10.1097/01.ASN.0000077339.20759.A3

86. Fiorina P, Venturini M, Folli F, Losio C, Maffi P, Placidi C, et al. Natural history of kidney graft survival, hypertrophy, and vascular function in end-stage renal disease type 1 diabetic kidney-transplanted patients: beneficial impact of pancreas and successful islet cotransplantation. *Diabetes Care* (2005) 28(6):1303–10. doi: 10.2337/diacare.28.6.1303

87. Maffi P, Bertuzzi F, De Taddeo F, Magistretti P, Nano R, Fiorina P, et al. Kidney function after islet transplant alone in type 1 diabetes: impact of immunosuppressive therapy on progression of diabetic nephropathy. *Diabetes Care* (2007) 30(5):1150–5. doi: 10.2337/dc06-1794

88. Senior PA, Zeman M, Paty BW, Ryan EA, Shapiro AM. Changes in renal function after clinical islet transplantation: four-year observational study. *Am J Transplant* (2007) 7(1):91–8. doi: 10.1111/j.1600-6143.2006.01573.x

89. Gillard P, Rustandi M, Efendi A, Lee DH, Ling Z, Hilbrands R, et al. Early alteration of kidney function in nonuremic type 1 diabetic islet transplant recipients under tacrolimus-mycophenolate therapy. *Transplantation* (2014) 98(4):451–7. doi: 10.1097/TP.0000000000000086

90. Fung MA, Warnock GL, Ao Z, Keown P, Meloche M, Shapiro RJ, et al. The effect of medical therapy and islet cell transplantation on diabetic nephropathy: an interim report. *Transplantation* (2007) 84(1):17–22. doi: 10.1097/01.tp.0000265502.92321.ab00007890-200707150-00006

91. Leitao CB, Cure P, Messinger S, Pileggi A, Lenz O, Froud T, et al. Stable renal function after islet transplantation: importance of patient selection and aggressive clinical management. *Transplantation* (2009) 87(5):681–8. doi: 10.1097/TP.0b013e31819279a800007890-200903150-

92. Zarinsefat A, Stock PG. Islet vs pancreas transplantation in nonuremic patients with type 1 diabetes. *Transplantation Bioengineering Regeneration Endocrine Pancreas. Elsevier* (2020) . p:417–23. doi: 10.1016/B978-0-12-814833-4.00034-4

93. Gruessner AC, Gruessner RW. Pancreas transplantation for patients with type 1 and type 2 diabetes mellitus in the United States: A registry report. *Gastroenterol Clinics* (2018) 47(2):417–41. doi: 10.1016/j.gtc.2018.01.009

94. Becker BN, Brazy PC, Becker YT, Odorico JS, Pintar TJ, Collins BH, et al. Simultaneous pancreas-kidney transplantation reduces excess mortality in type 1 diabetic patients with end-stage renal disease. *Kidney Int* (2000) 57(5):2129–35. doi: 10.1046/j.1523-1755.2000.00064.x

95. Reddy KS, Stablein D, Taranto S, Stratta RJ, Johnston TD, Waid TH, et al. Long-term survival following simultaneous kidney-pancreas transplantation versus kidney transplantation alone in patients with type 1 diabetes mellitus and renal failure. *Am J Kidney Dis* (2003) 41(2):464–70. doi: 10.1053/ajkd.2003.50057

96. Ojo AO, Meier-Kriesche H-U, Hanson JA, Leichtman A, Magee JC, Cibrik D, et al. The impact of simultaneous pancreas-kidney transplantation on long-term patient survival1. *Transplantation* (2001) 71(1):82–9. doi: 10.1097/00007890-200101150-00014

97. Boggi U, Rosati CM, Marchetti P. Follow-up of secondary diabetic complications after pancreas transplantation. *Curr Opin Organ Transplant* (2013) 18(1):102–10. doi: 10.1097/MOT.0b013e32835c28c5

98. Gremizzi C, Vergani A, Paloschi V, Secchi A. Impact of pancreas transplantation on type 1 diabetes-related complications. *Curr Opin Organ Transplant* (2010) 15(1):119–23. doi: 10.1097/MOT.0b013e32833552bc

99. Jenssen T, Hartmann A, Birkeland KI. Long-term diabetes complications after pancreas transplantation. *Curr Opin Organ Transplant* (2017) 22(4):382–8. doi: 10.1097/MOT.0000000000000436

100. Adler JT, Odorico JS. Chapter 11 - Pancreas after Kidney Transplantation. In: Orlando G, Piemonti L, Ricordi C, Stratta RJ, Gruessner RWG, editors. *Transplantation, Bioengineering, and Regeneration of the Endocrine Pancreas*. Academic Press (2020). p. 125–32.

101. Boggi U, Baronti W, Amorese G, Pilotti S, Occhipinti M, Perrone V, et al. Treating type 1 diabetes by pancreas transplant alone: A cohort study on actual long-term (10 years) efficacy and safety. *Transplantation* (2022) 106(1):145–57. doi: 10.1097/tp.0000000000003627

102. Al-Qaoud TM, Odorico JS, Redfield RR III. Pancreas transplantation in type 2 diabetes: expanding the criteria. *Curr Opin Organ Transplant* (2018) 23(4):454–60. doi: 10.1097/MOT.0000000000000553

103. Scalea JR, Redfield RR III, Arpali E, Leverson G, Sollinger HW, Kaufman DB, et al. Pancreas transplantation in older patients is safe, but patient selection is paramount. *Transplant Int* (2016) 29(7):810–8. doi: 10.1111/tri.12754

104. Stratta RJ, Fridell JA. Pancreas transplantation alone: radical or rationale? *Transplantation* (2021) 106(1):24–5. doi: 10.1097/TP.0000000000003628

105. D'Amour KA, Bang AG, Eliazer S, Kelly OG, Agulnick AD, Smart NG, et al. Production of pancreatic hormone–expressing endocrine cells from human embryonic stem cells. *Nat Biotechnol* (2006) 24(11):1392–401. doi: 10.1038/nbt1259

106. Zhang D, Jiang W, Liu M, Sui X, Yin X, Chen S, et al. Highly efficient differentiation of human es cells and ips cells into mature pancreatic insulin-producing cells. *Cell Res* (2009) 19(4):429–38. doi: 10.1038/cr.2009.28

107. Tateishi K, He J, Taranova O, Liang G, D'Alessio AC, Zhang Y. Generation of insulin-secreting islet-like clusters from human skin fibroblasts. *J Biol Chem* (2008) 283(46):31601–7. doi: 10.1074/jbc.M806597200

108. Nostro MC, Sarangi F, Ogawa S, Holtzinger A, Corneo B, Li X, et al. Stage-specific signaling through Tgfβ Family members and Wnt regulates patterning and pancreatic specification of human pluripotent stem cells. *Development* (2011) 138(5):861–71. doi: 10.1242/dev.055236

109. Hrvatin S, O'Donnell CW, Deng F, Millman JR, Pagliuca FW, DiIorio P, et al. Differentiated human stem cells resemble fetal, not adult, B Cells. *Proc Natl Acad Sci* (2014) 111(8):3038–43. doi: 10.1073/pnas.1400709111

110. Pagliuca FW, Millman JR, Gürtler M, Segel M, Van Dervort A, Ryu JH, et al. Generation of functional human pancreatic B Cells in vitro. *Cell* (2014) 159(2):428–39. doi: 10.1016/j.cell.2014.09.040

111. Rezania A, Bruin JE, Arora P, Rubin A, Batushansky I, Asadi A, et al. Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. *Nat Biotechnol* (2014) 32(11):1121–33. doi: 10.1038/nbt.3033

112. Russ HA, Parent AV, Ringler JJ, Hennings TG, Nair GG, Shveygert M, et al. Controlled induction of human pancreatic progenitors produces functional beta-like cells in vitro. *EMBO J* (2015) 34(13):1759–72. doi: 10.15252/embj.201591058

113. Sambathkumar R, Migliorini A, Nostro MC. Pluripotent stem cell-derived pancreatic progenitors and B-like cells for type 1 diabetes treatment. *Physiology* (2018) 33(6):394–402. doi: 10.1152/physiol.00026.2018

114. Schulz TC. Concise review: manufacturing of pancreatic endoderm cells for clinical trials in type 1 diabetes. *Stem Cells Trans Med* (2015) 4(8):927–31. doi: 10.5966/sctm.2015-0058

115. Agulnick AD, Ambruzs DM, Moorman MA, Bhoumik A, Cesario RM, Payne JK, et al. Insulin-producing endocrine cells differentiated in vitro from human embryonic stem cells function in macroencapsulation devices in vivo. *Stem Cells Trans Med* (2015) 4(10):1214–22. doi: 10.5966/sctm.2015-0079

116. Ramzy A, Thompson DM, Ward-Hartstonge KA, Ivison S, Cook L, Garcia RV, et al. Implanted pluripotent stem-cell-derived pancreatic endoderm cells secrete glucose-responsive C-peptide in patients with type 1 diabetes. *Cell Stem Cell* (2021) 28(12):2047–61.e5. doi: 10.1016/j.stem.2021.10.003

117. Shapiro AJ, Thompson D, Donner TW, Bellin MD, Hsueh W, Pettus J, et al. Insulin expression and C-peptide in type 1 diabetes subjects implanted with stem cell-

derived pancreatic endoderm cells in an encapsulation device. *Cell Rep Med* (2021) 2 (12):100466. doi: 10.1016/j.xcrm.2021.100466

118. Reichman TW, Ricordi C, Naji A, Markmann JF, Perkins BA, Wijkstrom M, et al. 836-P: glucose-dependent insulin production and insulin-independence in type 1 diabetes from stem cell–derived, fully differentiated islet cells—Updated data from the Vx-880 clinical trial. *Diabetes* (2023) 72(Supplement_1). doi: 10.2337/db23-836-P

119. Conte C, Maggiore U, Cappelli G, Ietto G, Lai Q, Salis P, et al. Management of metabolic alterations in adult kidney transplant recipients: A joint position statement of the Italian Society of Nephrology (Sin), the Italian Society for Organ Transplantation (Sito) and the Italian Diabetes Society (Sid). *Nutrition Metab Cardiovasc Dis* (2020) 30 (9):1427–41. doi: 10.1016/j.numecd.2020.05.004

120. Thomas MC, Moran J, Mathew TH, Russ GR, Mohan Rao M. Early peri-operative hyperglycaemia and renal allograft rejection in patients withoutdiabetes. *BMC Nephrol* (2000) 1(1):1–6. doi: 10.1186/1471-2369-1-1

121. Tutone VK, Mark PB, Revanur V, Traynor J, Buist L, Geddes CC, et al. Random blood glucose measurements and survival in nondiabetic renal transplant recipients. In: *Transplantation Proceedings*. Elsevier (2004) 36(10):3006–11. doi: 10.1016/j.transproceed.2004.10.067

122. Wallia A, Parikh ND, Molitch ME, Mahler E, Tian L, Huang JJ, et al. Post-transplant hyperglycemia is associated with increased risk of liver allograft rejection. *Transplantation* (2010) 89(2):222–6. doi: 10.1097/TP.0b013e3181c3c2ff

123. Safronova V, Gabdoulkhakova A, Miller A, Kosarev I, Vasilenko R. Variations of the effect of insulin on neutrophil respiratory burst. The role of tyrosine kinases and phosphatases. . *Biochem (Moscow)* (2001) 66:840–9. doi: 10.1023/A:1011944400908

124. Himpe E, Degaillier C, Coppens A, Kooijman R. Insulin-like growth factor-1 delays fas-mediated apoptosis in human neutrophils through the phosphatidylinositol-3 kinase pathway. *J Endocrinol* (2008) 199(1):69. doi: 10.1677/JOE-08-0028

125. Tessaro FH, Ayala TS, Nolasco EL, Bella LM, Martins JO. Insulin influences lps-induced tnf-B and il-6 release through distinct pathways in mouse macrophages from different compartments. *Cell Physiol Biochem* (2017) 42(5):2093–104. doi: 10.1159/000479904

126. Ratter JM, van Heck JI, Rooijackers HM, Jansen HJ, van Poppel PC, Tack CJ, et al. Insulin acutely activates metabolism of primary human monocytes and promotes a proinflammatory phenotype. *J leukocyte Biol* (2021) 110(5):885–91. doi: 10.1002/JLB.3AB0120-019RR

127. Ferreira SS, Nunes FP, Casagrande FB, Martins JO. Insulin modulates cytokine release, collagen and mucus secretion in lung remodeling of allergic diabetic mice. *Front Immunol* (2017) 8:633. doi: 10.3389/fimmu.2017.00633

128. Lu H, Huang D, Yao K, Li C, Chang S, Dai Y, et al. Insulin enhances dendritic cell maturation and scavenger receptor-mediated uptake of oxidised low-density lipoprotein. *J Diabetes its Complications* (2015) 29(4):465–71. doi: 10.1016/j.jdiacomp.2015.03.005

129. Oherle K, Acker E, Bonfield M, Wang T, Gray J, Lang I, et al. Insulin-like growth factor 1 supports a pulmonary niche that promotes type 3 innate lymphoid cell development in newborn lungs. *Immunity* (2020) 52(2):275–94. doi: 10.1016/j.immuni.2020.01.005

130. Fischer HJ, Sie C, Schumann E, Witte A-K, Dressel R, van den Brandt J, et al. The insulin receptor plays a critical role in T cell function and adaptive immunity. *J Immunol* (2017) 198(5):1910–20. doi: 10.4049/jimmunol.1601011

131. Helderman JH, Strom TB. Specific insulin binding site on T and B lymphocytes as a marker of cell activation. *Nature* (1978) 274(5666):62–3. doi: 10.1038/274062a0

132. DeBenedette M, Snow E. Insulin modulates the interleukin 2 responsiveness of T lymphocytes. *Regional Immunol* (1990) 3(2):82–7.

133. Berman J, Center D. Chemotactic activity of porcine insulin for human T lymphocytes in vitro. *J Immunol (Baltimore Md: 1950)* (1987) 138(7):2100–3. doi: 10.4049/jimmunol.138.7.2100

134. Di Dedda C, Vignali D, Piemonti L, Monti P. Pharmacological targeting of glut1 to control autoreactive T cell responses. *Int J Mol Sci* (2019) 20(19):4962. doi: 10.3390/ijms20194962

135. DiToro D, Harbour SN, Bando JK, Benavides G, Witte S, Laufer VA, et al. Insulin-like growth factors are key regulators of T helper 17 regulatory T cell balance in autoimmunity. *Immunity* (2020) 52(4):650–67. doi: 10.1016/j.immuni.2020.03.013

136. Wu D, Wong CK, Han JM, Orban PC, Huang Q, Gillies J, et al. T reg–specific insulin receptor deletion prevents diet-induced and age-associated metabolic syndrome. *J Exp Med* (2020) 217(8):e20191542. doi: 10.1084/jem.20191542

137. Kavazović I, Krapić M, Beumer-Chuwonpad A, Polić B, Turk Wensveen T, Lemmermann NA, et al. Hyperglycemia and not hyperinsulinemia mediates diabetes-induced memory cd8 T-cell dysfunction. *Diabetes* (2022) 71(4):706–21. doi: 10.2337/db21-0209

138. Baudler S, Baumgartl J, Hampel B, Buch T, Waisman A, Snapper CM, et al. Insulin-like growth factor-1 controls type 2 T cell-independent B cell response. *J Immunol* (2005) 174(9):5516–25. doi: 10.4049/jimmunol.174.9.5516

139. Leitão CB, Bernetti K, Tharavanij T, Cure P, Ricordi C, Alejandro R. Type 2 diabetes mellitus phenotype and graft survival after islet transplantation. *Transplantation* (2009) 88(1):57–61. doi: 10.1097/TP.0b013e3181aa7cc2

140. Petrelli A, Cugnata F, Carnovale D, Bosi E, Libman IM, Piemonti L, et al. Homa-ir and the matsuda index as predictors of progression to type 1 diabetes in autoantibody-positive relatives. *Diabetologia* (2023). doi: 10.1007/s00125-023-06034-y

141. Jafar N, Edriss H, Nugent K. The effect of short-term hyperglycemia on the innate immune system. *Am J Med Sci* (2016) 351(2):201–11. doi: 10.1016/j.amjms.2015.11.011

142. González P, Lozano P, Ros G, Solano F. Hyperglycemia and oxidative stress: an integral, updated and critical overview of their metabolic interconnections. *Int J Mol Sci* (2023) 24(11):9352. doi: 10.3390/ijms24119352

143. Morey M, O'Gaora P, Pandit A, Hélary C. Hyperglycemia acts in synergy with hypoxia to maintain the pro-inflammatory phenotype of macrophages. *PloS One* (2019) 14(8):e0220577. doi: 10.1371/journal.pone.0220577

144. Shomali N, Mahmoudi J, Mahmoodpoor A, Zamiri RE, Akbari M, Xu H, et al. Harmful effects of high amounts of glucose on the immune system: an updated review. *Biotechnol Appl Biochem* (2021) 68(2):404–10. doi: 10.1002/bab.1938

145. Pahwa R, Jialal I. Hyperglycemia induces toll-like receptor activity through increased oxidative stress. *Metab syndrome related Disord* (2016) 14(5):239–41. doi: 10.1089/met.2016.29006.pah

146. Giese I-M, Schilloks M-C, Degroote RL, Weigand M, Renner S, Wolf E, et al. Chronic hyperglycemia drives functional impairment of lymphocytes in diabetic ins C94y transgenic pigs. *Front Immunol* (2021) 11:607473. doi: 10.3389/fimmu.2020.607473

147. Recino A, Barkan K, Wong FS, Ladds G, Cooke A, Wallberg M. Hyperglycaemia does not affect antigen-specific activation and cytolytic killing by cd8+ T cells in *vivo*. *Bioscience Rep* (2017) 37(4):BSR20171079. doi: 10.1042/BSR20171079

148. Iwai N, Steib C, Marzo A, Lerret NM. The role of hyperglycemia in cd4 T cell survival and differentiation. *Am Soc Clin Lab Sci* (2018). doi: 10.29074/ascls.2018000331

149. Melzi R, Battaglia M, Draghici E, Bonifacio E, Piemonti L. Relevance of hyperglycemia on the timing of functional loss of allogeneic islet transplants: implication for mouse model. *Transplantation* (2007) 83(2):167–73. doi: 10.1097/01.tp.0000250659.24286.43

150. Toren E, Burnette KS, Banerjee RR, Hunter CS. Tse HM. Partners in crime: beta-cells and autoimmune responses complicit in type 1 diabetes pathogenesis. *Front Immunol* (2021) 12:756548. doi: 10.3389/fimmu.2021.756548

151. James EA, Joglekar AV, Linnemann AK, Russ HA, Kent SC. The beta cell-immune cell interface in type 1 diabetes (T1d). *Mol Metab* (2023) 78:101809. doi: 10.1016/j.molmet.2023.101809

152. Oram RA, McDonald TJ, Shields BM, Hudson MM, Shepherd MH, Hammersley S, et al. Most people with long-duration type 1 diabetes in a large population-based study are insulin microsecretors. *Diabetes Care* (2015) 38(2):323–8. doi: 10.2337/dc14-0871

153. Wasserfall C, Nick HS, Campbell-Thompson M, Beachy D, Haataja L, Kusmartseva I, et al. Persistence of pancreatic insulin mrna expression and proinsulin protein in type 1 diabetes pancreata. *Cell Metab* (2017) 26(3):568–75. doi: 10.1016/j.cmet.2017.08.013

154. Gianani R, Campbell-Thompson M, Sarkar S, Wasserfall C, Pugliese A, Solis J, et al. Dimorphic histopathology of long-standing childhood-onset diabetes. *Diabetologia* (2010) 53:690–8. doi: 10.1007/s00125-009-1642-y

155. Dorrell C, Schug J, Canaday PS, Russ HA, Tarlow BD, Grompe MT, et al. Human islets contain four distinct subtypes of B Cells. *Nat Commun* (2016) 7(1):11756. doi: 10.1038/ncomms11756

156. Dror E, Fagnocchi L, Wegert V, Apostle S, Grimaldi B, Gruber T, et al. Epigenetic dosage identifies two major and functionally distinct B Cell subtypes. *Cell Metab* (2023) 35(5):821–36. doi: 10.1016/j.cmet.2023.03.008

157. Johnston NR, Mitchell RK, Haythorne E, Pessoa MP, Semplici F, Ferrer J, et al. Beta cell hubs dictate pancreatic islet responses to glucose. *Cell Metab* (2016) 24 (3):389–401. doi: 10.1016/j.cmet.2016.06.020

158. Salem V, Silva LD, Suba K, Georgiadou E, Neda Mousavy Gharavy S, Akhtar N, et al. Leader B-cells coordinate Ca2+ Dynamics across pancreatic islets in vivo. *Nat Metab* (2019) 1(6):615–29. doi: 10.1038/s42255-019-0075-2

159. Benninger RK, Kravets V. The physiological role of B-cell heterogeneity in pancreatic islet function. *Nat Rev Endocrinol* (2022) 18(1):9–22. doi: 10.1038/s41574-021-00568-0

160. Marré ML, James EA, Piganelli JD. B Cell er stress and the implications for immunogenicity in type 1 diabetes. *Front Cell Dev Biol* (2015) 3:67. doi: 10.3389/fcell.2015.00067

161. Leenders F, Groen N, de Graaf N, Engelse MA, Rabelink TJ, de Koning EJ, et al. Oxidative stress leads to B-cell dysfunction through loss of B-cell identity. *Front Immunol* (2021) 12:690379. doi: 10.3389/fimmu.2021.690379

162. Hasnain SZ, Prins JB, McGuckin MA. Oxidative and endoplasmic reticulum stress in B-cell dysfunction in diabetes. *J Mol Endocrinol* (2016) 56(2):R33–54. doi: 10.1530/JME-15-0232

163. Marroqui L, Dos Santos RS, Op De Beeck A, Coomans de Brachène A, Marselli L, Marchetti P, et al. Interferon-B Mediates human beta cell Hla class I overexpression, endoplasmic reticulum stress and apoptosis, three hallmarks of early human type 1 diabetes. *Diabetologia* (2017) 60:656–67. doi: 10.1007/s00125-016-4201-3

164. James EA, Pietropaolo M, Mamula MJ. Immune recognition of B-cells: neoepitopes as key players in the loss of tolerance. *Diabetes* (2018) 67(6):1035–42. doi: 10.2337/dbi17-0030

165. Rodriguez-Calvo T, Johnson JD, Overbergh L, Dunne JL. Neoepitopes in type 1 diabetes: etiological insights, biomarkers and therapeutic targets. *Front Immunol* (2021) 12:667989. doi: 10.3389/fimmu.2021.667989

166. James EA, Mallone R, Kent SC, DiLorenzo TP. T-cell epitopes and neo-epitopes in type 1 diabetes: A comprehensive update and reappraisal. *Diabetes* (2020) 69 (7):1311–35. doi: 10.2337/dbi19-0022

167. Gonzalez-Duque S, Azoury ME, Colli ML, Afonso G, Turatsinze J-V, Nigi L, et al. Conventional and neo-antigenic peptides presented by B Cells are targeted by circulating naïve Cd8+ T cells in type 1 diabetic and healthy donors. *Cell Metab* (2018) 28(6):946–60. doi: 10.1016/j.cmet.2018.07.007

168. Colli ML, Moore F, Gurzov EN, Ortis F, Eizirik DL. Mda5 and Ptpn2, two candidate genes for type 1 diabetes, modify pancreatic B-cell responses to the viral by-product double-stranded rna. *Hum Mol Genet* (2010) 19(1):135–46. doi: 10.1093/hmg/ddp474

169. Marroqui L, Dos Santos RS, Fløyel T, Grieco FA, Santin I, Op de Beeck A, et al. Tyk2, a candidate gene for type 1 diabetes, modulates apoptosis and the innate immune response in human pancreatic B-cells. *Diabetes* (2015) 64(11):3808–17. doi: 10.2337/db15-0362

170. Santin I, Moore F, Colli ML, Gurzov EN, Marselli L, Marchetti P, et al. Ptpn2, a candidate gene for type 1 diabetes, modulates pancreatic B-cell apoptosis via regulation of the Bh3-only protein bim. *Diabetes* (2011) 60(12):3279–88. doi: 10.2337/db11-0758

171. Chandra V, Ibrahim H, Halliez C, Prasad RB, Vecchio F, Dwivedi OP, et al. The type 1 diabetes gene tyk2 regulates B-cell development and its responses to interferon-B. *Nat Commun* (2022) 13(1):6363. doi: 10.1038/s41467-022-34069-z

172. Roca-Rivada A, Marín-Cañas S, Colli ML, Vinci C, Sawatani T, Marselli L, et al. Inhibition of the type 1 diabetes candidate gene Ptpn2 aggravates Tnf-B-induced human beta cell dysfunction and death. *Diabetologia* (2023) 66(8):1544–56. doi: 10.1007/s00125-023-05908-5

173. Bertuzzi F, Colussi G, Lauterio A, De Carlis L. Intramuscular islet allotransplantation in type 1 diabetes mellitus. *Eur Rev Med Pharmacol Sci* (2018) 22 (6):1731–6. doi: 10.26355/eurrev_201803_14588

174. Christoffersson G, Henriksnäs J, Johansson L, Rolny C, Ahlström H, Caballero-Corbalan J, et al. Clinical and experimental pancreatic islet transplantation to striated muscle: establishment of a vascular system similar to that in native islets. *Diabetes* (2010) 59(10):2569–78. doi: 10.2337/db10-0205

175. Stice MJ, Dunn TB, Bellin MD, Skube ME, Beilman GJ. Omental pouch technique for combined site islet autotransplantation following total pancreatectomy. *Cell Transplant* (2018) 27(10):1561–8. doi: 10.1177/0963689718798627

176. Maffi P, Balzano G, Ponzoni M, Nano R, Sordi V, Melzi R, et al. Autologous pancreatic islet transplantation in human bone marrow. *Diabetes* (2013) 62(10):3523–31. doi: 10.2337/db13-0465

177. Maffi P, Nano R, Monti P, Melzi R, Sordi V, Mercalli A, et al. Islet allotransplantation in the bone marrow of patients with type 1 diabetes: A pilot randomized trial. *Transplantation* (2019) 103(4):839–51. doi: 10.1097/TP.0000000000002416

178. Wszola M, Berman A, Gorski L, Ostaszewska A, Serwanska-Swietek M, Krajewska M, et al. Endoscopic islet autotransplantation into gastric submucosa—1000-day follow-up of patients. In: *Transplantation Proceedings*. Elsevier (2018) 50 (7):2119–23. doi: 10.1016/j.transproceed.2018.02.138

179. Jindal RM, Sidner RA, McDaniel HB, Johnson MS, Fineberg SE. Intraportal vs kidney subcapsular site for human pancreatic islet transplantation. In: *Transplantation Proceedings* (1998) 30(2):398–9. doi: 10.1016/s0041-1345(97)01327-4

180. White SA, London NJ, Johnson PR, Davies JE, Pollard C, Contractor HH, et al. The risks of total pancreatectomy and splenic islet autotransplantation. *Cell Transplant* (2000) 9(1):19–24. doi: 10.1177/096368970000900103

181. Shishido A, Caicedo A, Rodriguez-Diaz R, Pileggi A, Berggren P-O, Abdulreda M. Clinical intraocular islet transplantation is not a number issue. *CellR4–repair replacement regeneration reprogramming* (2016) 4(4):e2120.

182. Cantarelli E, Citro A, Pellegrini S, Mercalli A, Melzi R, Dugnani E, et al. Transplant site influences the immune response after islet transplantation: bone marrow versus liver. *Transplantation* (2017) 101(5):1046–55. doi: 10.1097/TP.0000000000001462

183. Kamei T, Yasunami Y. Demonstration of donor specific unresponsiveness in rat islet allografts: importance of transplant site for induction by cyclosporin a and maintenance. *Diabetologia* (1989) 32:779–85. doi: 10.1007/BF00264907

184. Guan J, Zucker PF, Behme MT, Zhong R, Atkison P, Dupré J. Insulin resistance prevented by portal delivery of insulin in rats with renal subcapsular islet grafts. *Diabetes* (1997) 46(3):372–8. doi: 10.2337/diab.46.3.372

185. Rickels MR, Fuller C, Dalton-Bakes C, Markmann E, Palanjian M, Cullison K, et al. Restoration of glucose counterregulation by islet transplantation in long-standing type 1 diabetes. *Diabetes* (2015) 64(5):1713–8. doi: 10.2337/db14-1620

186. Kendall DM, Teuscher AU, Robertson RP. Defective glucagon secretion during sustained hypoglycemia following successful islet allo-and autotransplantation in humans. *Diabetes* (1997) 46(1):23–7. doi: 10.2337/diab.46.1.23

187. Toso C, Isse K, Demetris AJ, Dinyari P, Koh A, Imes S, et al. Histologic graft assessment after clinical islet transplantation. *Transplantation* (2009) 88(11):1286–93. doi: 10.1097/TP.0b013e3181bc06b0

188. Moberg L, Johansson H, Lukinius A, Berne C, Foss A, Källén R, et al. Production of tissue factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation. *Lancet* (2002) 360(9350):2039–45. doi: 10.1016/S0140-6736(02)12020-4

189. Piemonti L, Leone BE, Nano R, Saccani A, Monti P, Maffi P, et al. Human pancreatic islets produce and secrete Mcp-1/Ccl2: relevance in human islet transplantation. *Diabetes* (2002) 51(1):55–65. doi: 10.2337/diabetes.51.1.55

190. Melzi R, Mercalli A, Sordi V, Cantarelli E, Nano R, Maffi P, et al. Role of ccl2/mcp-1 in islet transplantation. *Cell Transplant* (2010) 19(8):1031–46. doi: 10.3727/096368910X514639

191. Piemonti L, Calori G, Mercalli A, Lattuada G, Monti P, Garancini M, et al. Fasting plasma leptin, monocyte chemoattracting protein-1 (Mcp-1/ccl2) and B-tumor necrosis factor receptor 2 (B-tnfr-2) concentrations in glucose tolerant and intolerant women. *Impact Cardiovasc Mortality. Diabetes Care* (2003) 26(10):2883–9. doi: 10.2337/diacare.26.10.2883

192. Carlsson P-O, Palm F, Andersson A, Liss P. Markedly decreased oxygen tension in transplanted rat pancreatic islets irrespective of the implantation site. *Diabetes* (2001) 50(3):489–95. doi: 10.2337/diabetes.50.3.489

193. Westermark GT, Westermark P, Berne C, Korsgren O. Widespread amyloid deposition in transplanted human pancreatic islets. *New Engl J Med* (2008) 359(9):977–9. doi: 10.1056/NEJMc0802893

194. LEITao CB, Bernetti K, Tharavanij T, Cure P, Lauriola V, Berggren P-O, et al. Lipotoxicity and decreased islet graft survival. *Diabetes Care* (2010) 33(3):658–60. doi: 10.2337/dc09-1387

195. Delaune V, Berney T, Lacotte S, Toso C. Intraportal islet transplantation: the impact of the liver microenvironment. *Transplant Int* (2017) 30(3):227–38. doi: 10.1111/tri.12919

196. Wolanin M, Stachyra B, Stawikowski C, Zielonka B, Osińska A, Wolanin I, et al. Pancreatic islet transplantation in patients suffering from type 1 diabetes-indications, methods, possible risks and future directions: A literature review. *J Education Health Sport* (2023) 32(1):47–63. doi: 10.12775/JEHS.2023.32.01.004

197. Pellegrini S. Alternative transplantation sites for islet transplantation. *Transplantation Bioengineering Regeneration Endocrine Pancreas. Elsevier* (2020). p:833–47. doi: 10.1016/B978-0-12-814833-4.00065-4

198. Sordi V, Pellegrini S, Piemonti L. Immunological issues after stem cell-based B Cell replacement. *Curr Diabetes Rep* (2017) 17:1–8. doi: 10.1007/s11892-017-0901-4

199. Piemonti L, Everly MJ, Maffi P, Scavini M, Poli F, Nano R, et al. Alloantibody and autoantibody monitoring predicts islet transplantation outcome in human type 1 diabetes. *Diabetes* (2013) 62(5):1656–64. doi: 10.2337/db12-1258db12-1258

200. Gruessner A, Sutherland D, Gruessner R. Matching in pancreas transplantation-a registry analysis. In: *Transplantation Proceedings*. Orlando, FL: Grune & Stratton (2001). p. 1969.

201. Berney T, Malaise J, Morel P, Toso C, Demuylder-Mischler S, Majno P, et al. Impact of hla matching on the outcome of simultaneous pancreas–kidney transplantation. *Nephrol Dialysis Transplant* (2005) 20(suppl_2):ii48–53. doi: 10.1093/ndt/gfh1082

202. Rudolph E, Dunn T, Mauer D, Noreen H, Sutherland D, Kandaswamy R, et al. Hla-a,-B,-C,-Dr, and-Dq matching in pancreas transplantation: effect on graft rejection and survival. *Am J Transplant* (2016) 16(8):2401–12. doi: 10.1111/ajt.13734

203. Malaise J, Berney T, Morel P, Toso C, Demuylder-Mischler S, Majno P, et al. Effect of Hla Matching in Simultaneous Pancreas–Kidney Transplantation. *Transplantation Proceedings* (2005) 37(6):2846–7. doi: 10.1016/j.transproceed.2005.05.023

204. Mancini M, Connors A, Wang X, Nock S, Spencer C, Mccullough C, et al. Hla matching for simultaneous pancreas-kidney transplantation in the United States: A multivariable analysis of the unos data. *Clin Nephrol* (2002) 57(1):27–37. doi: 10.5414/CNP57027

205. Parajuli S, Kaufman DB, Djamali A, Welch BM, Sollinger HW, Mandelbrot DA, et al. Association of human leukocyte antigen mismatches between donor-recipient and donor-donor in pancreas after kidney transplant recipients. *Transplant Int* (2021) 34(12):2803–15. doi: 10.1111/tri.14138

206. Ballou C, Barton F, Payne EH, Berney T, Villard J, Meier RP, et al. Matching for hla-dr excluding diabetogenic Hla-Dr3 and Hla-Dr4 predicts insulin independence after pancreatic islet transplantation. *Front Immunol* (2023) 14:1110544. doi: 10.3389/fimmu.2023.1110544

207. Lemos JRN, Baidal DA, Poggioli R, Fuenmayor V, Chavez C, Alvarez A, et al. Hla-B Matching Prolongs Allograft Survival in Islet Cell Transplantation. *Cell Transplant* (2023) 32:9636897231166529. doi: 10.1177/09636897231166529

208. Lemos JR, Baidal DA, Poggioli R, Fuenmayor V, Chavez C, Alvarez A, et al. Hla-B matching prolongs allograft survival in islet cell transplantation. *Cell Transplant* (2023) 32:09636897231166529. doi: 10.1177/09636897231166529

209. Sutherland D, Sibley R, Xu X, Michael A, Srikanta A, Taub F, et al. Twin-to-twin pancreas transplantation: reversal and reenactment of the pathogenesis of type I diabetes. *Trans Assoc Am Physicians* (1984) 97:80–7.

210. Burke GW, Vendrame F, Virdi SK, Ciancio G, Chen L, Ruiz P, et al. Lessons from pancreas transplantation in type 1 diabetes: recurrence of islet autoimmunity. *Curr Diabetes Rep* (2015) 15:1–9. doi: 10.1007/s11892-015-0691-5

211. Bosi E, Bottazzo GF, Secchi A, Pozza G, Shattock M, Saunders A, et al. Islet cell autoimmunity in type I diabetic patients after hla-mismatched pancreas transplantation. *Diabetes* (1989) 38(Supplement_1):82–4. doi: 10.2337/diab.38.1.S82

212. Esmatjes E, Rodríguez-Villar C, Ricart MJ, Casamitjana R, Martorell J, Sabater L, et al. Recurrence of immunological markers for type 1 (Insulin-dependent) diabetes mellitus in immunosuppressed patients after pancreas transplantation. *Transplantation* (1998) 66(1):128–31. doi: 10.1097/00007890-199807150-00022

213. Thivolet C, Abou-Amara S, Martin X, Lefrancois N, Petruzzo P, McGregor B, et al. Serological markers of recurrent beta cell destruction in diabetic patients undergoing pancreatic transplantation. *Transplantation* (2000) 69(1):99. doi: 10.1097/00007890-200001150-00018

214. Braghi S, Bonifacio E, Secchi A, Di Carlo V, Pozza G, Bosi E. Modulation of humoral islet autoimmunity by pancreas allotransplantation influences allograft outcome in patients with type 1 diabetes. *Diabetes* (2000) 49(2):218–24. doi: 10.2337/diabetes.49.2.218

215. Assalino M, Genevay M, Morel P, Demuylder-Mischler S, Toso C, Berney T. Recurrence of type 1 diabetes after simultaneous pancreas–kidney transplantation in the absence of gad and ia-2 autoantibodies. *Am J Transplant* (2012) 12(2):492–5. doi: 10.1111/j.1600-6143.2011.03844.x

216. Occhipinti M, Lampasona V, Vistoli F, Bazzigaluppi E, Scavini M, Boggi U, et al. Zinc transporter 8 autoantibodies increase the predictive value of islet autoantibodies for function loss of technically successful solitary pancreas transplant. *Transplantation* (2011) 92(6):674–7. doi: 10.1097/TP.0b013e31822ae65f

217. Sharma V, Andersen D, Thompson M, Woda BA, Stoff JS, Hartigan C, et al. Autoimmunity after Islet-Cell Allotransplantation. *N Engl J Med* (2006) 355 (13):1397–9. doi: 10.1056/NEJMc061530

218. Stegall MD, Lafferty KJ, Kam I, Gill RG. Evidence of recurrent autoimmunity in human allogeneic islet transplantation. *Transplantation* (1996) 61(8):1272–4. doi: 10.1097/00007890-199604270-00027

219. Korutla L, Rickels MR, Hu RW, Freas A, Reddy S, Habertheuer A, et al. Noninvasive diagnosis of recurrent autoimmune type 1 diabetes after islet cell transplantation. *Am J Transplant* (2019) 19(6):1852–8. doi: 10.1111/ajt.15322

220. Kizilgul M, Wilhelm J, Dunn T, Beilman G, Pruett T, Chinnakotla S, et al. The prognostic significance of glutamic acid decarboxylase antibodies in patients with chronic pancreatitis undergoing total pancreatectomy with islet autotransplantation. *Diabetes Metab* (2019) 45(3):301–5. doi: 10.1016/j.diabet.2018.01.001

221. Jaeger C, Brendel MD, Hering BJ, Eckhard M, Bretzel RG. Progressive islet graft failure occurs significantly earlier in autoantibody-positive than in autoantibody-negative iddm recipients of intrahepatic islet allografts. *Diabetes* (1997) 46(11):1907–10. doi: 10.2337/diab.46.11.1907

222. Bosi E, Braghi S, Maffi P, Scirpoli M, Bertuzzi F, Pozza G, et al. Autoantibody response to islet transplantation in type 1 diabetes. *Diabetes* (2001) 50(11):2464–71. doi: 10.2337/diabetes.50.11.2464

223. Roep BO, Stobbe I, Duinkerken G, van Rood JJ, Lernmark A, Keymeulen B, et al. Auto- and alloimmune reactivity to human islet allografts transplanted into type 1 diabetic patients. *Diabetes* (1999) 48(3):484–90. doi: 10.2337/diabetes.48.3.484

224. Velthuis JH, Unger WW, Abreu JR, Duinkerken G, Franken K, Peakman M, et al. Simultaneous detection of circulating autoreactive Cd8+ T-cells specific for different islet cell-associated epitopes using combinatorial mhc multimers. *Diabetes* (2010) 59(7):1721–30. doi: 10.2337/db09-1486db09-1486

225. Monti P, Scirpoli M, Maffi P, Ghidoli N, De Taddeo F, Bertuzzi F, et al. Islet transplantation in patients with autoimmune diabetes induces homeostatic cytokines that expand autoreactive memory T cells. *J Clin Invest* (2008) 118(5):1806–14. doi: 10.1172/JCI35197

226. Vignali D, Gürth C-M, Pellegrini S, Sordi V, Sizzano F, Piemonti L, et al. Il-7 mediated homeostatic expansion of human Cd4+ Cd25+ Foxp3+ Regulatory T cells after depletion with anti-Cd25 monoclonal antibody. *Transplantation* (2016) 100 (9):1853–61. doi: 10.1097/TP.0000000000001276

227. Monti P, Piemonti L. Homeostatic T Cell Proliferation after Islet Transplantation. *Clin Dev Immunol* (2013) 2013:217934. doi: 10.1155/2013/217934

228. Lampeter EF, McCann SR, Kolb H. Transfer of diabetes type 1 by bone-marrow transplantation. *Lancet* (1998) 351(9102):568–9. doi: 10.1016/S0140-6736(05)78555-X

229. Bellin M, Moran A, Wilhelm J, O'Brien T, Gottlieb P, Yu L, et al. Development of autoimmune-mediated B Cell failure after total pancreatectomy with autologous islet transplantation. *Am J Transplant* (2015) 15(7):1991–4. doi: 10.1111/ajt.13216

230. Ali KF, San Martin VT, Stevens T, Walsh RM, Bottino R, Trucco M, et al. Autoimmunity in autologous islet transplantation. *OBM Transplant* (2018) 2 (3):10.21926/obm.transplant.1803014. doi: 10.21926/obm.transplant.1803014

231. Shapiro AJ, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *New Engl J Med* (2000) 343(4):230–8. doi: 10.1056/NEJM200007273430401

232. Onaca N, Takita M, Levy MF, Naziruddin B. Anti-inflammatory approach with early double cytokine blockade (Il-1B and Tnf-B) is safe and facilitates engraftment in islet allotransplantation. *Transplant direct* (2020) 6(3):e530. doi: 10.1097/TXD.0000000000000977

233. Scharp DW, Lacy PE, Santiago JV, McCullough CS, Weide LG, Falqui L, et al. Insulin Independence after Islet Transplantation into Type I Diabetic Patient. *Diabetes* (1990) 39(4):515–8. doi: 10.2337/diab.39.4.515

234. Landstra CP, Nijhoff MF, Roelen DL, de Vries AP, de Koning EJ. Diagnosis and treatment of allograft rejection in islet transplantation. *Am J Transplant* (2023) 23 (9):1425–33. doi: 10.1016/j.ajt.2023.05.035

235. Nakano SJ, Everitt MD. Beyond the biopsy: is it time to change the gold standard for rejection diagnosis in clinical decision-making and scientific discovery? *J Heart Lung Transplant* (2022) 41(10):1414–6. doi: 10.1016/j.healun.2022.04.008

236. Rickels MR, Kamoun M, Kearns J, Markmann JF, Naji A. Evidence for allograft rejection in an islet transplant recipient and effect on B-cell secretory capacity. *J Clin Endocrinol Metab* (2007) 92(7):2410–4. doi: 10.1210/jc.2007-0172

237. Kessler L, Parissiadis A, Bayle F, Moreau F, Pinget M, Froelich N, et al. Evidence for humoral rejection of a pancreatic islet graft and rescue with rituximab and Iv immunoglobulin therapy. *Am J Transplant* (2009) 9(8):1961–6. doi: 10.1111/j.1600-6143.2009.02711.x

238. Moreau F, Toti F, Bayle F, Berney T, Egelhofer H, Chastre M, et al. Rescue of a pancreatic islet graft after steroid therapy. *Transplantation* (2012) 93(3):e10–e1. doi: 10.1097/TP.0b013e31823f7fb2

239. Ward C, Odorico JS, Rickels MR, Berney T, Burke GW III, Kay TW, et al. International survey of clinical monitoring practices in pancreas and islet transplantation. *Transplantation* (2022) 106(8):1647–55. doi: 10.1097/TP.0000000000004058

240. Rickels MR, Stock PG, De Koning EJ, Piemonti L, Pratschke J, Alejandro R, et al. Defining outcomes for B-cell replacement therapy in the treatment of diabetes: A consensus report on the igls criteria from the Ipita/Epita opinion leaders workshop. *Transplant Int* (2018) 31(4):343–52. doi: 10.1111/tri.13138

241. Brooks A, Carter V, Liew A, Marshall H, Aldibbiat A, Sheerin N, et al. *De novo* donor-specific hla antibodies are associated with rapid loss of graft function following islet transplantation in type 1 diabetes. *Am J Transplant* (2015) 15(12):3239–46. doi: 10.1111/ajt.13407

242. Maanaoui M, Chetboun M, Top I, Elsermans V, Kerr-Conte J, Le Mapihan K, et al. The challenge of Hla donor specific antibodies in the management of pancreatic islet transplantation: an illustrative case-series. *Sci Rep* (2022) 12(1):12463. doi: 10.1038/s41598-022-16782-3

243. Pouliquen E, Baltzinger P, Lemle A, Chen C-C, Parissiadis A, Borot S, et al. Anti-donor Hla antibody response after pancreatic islet grafting: characteristics, risk factors, and impact on graft function. *Am J Transplant* (2017) 17(2):462–73. doi: 10.1111/ajt.13936

244. Chaigne B, Geneugelijk K, Bédat B, Ahmed MA, Hönger G, De Seigneux S, et al. Immunogenicity of anti-Hla antibodies in pancreas and islet transplantation. *Cell Transplant* (2016) 25(11):2041–50. doi: 10.3727/096368916X691673

245. Chen C-C, Pouliquen E, Broisat A, Andreata F, Racapé M, Bruneval P, et al. Endothelial chimerism and vascular sequestration protect pancreatic islet grafts from antibody-mediated rejection. *J Clin Invest* (2018) 128(1):219–32. doi: 10.1172/jci93542

246. Ritz-Laser B, Oberholzer J, Toso C, Brulhart M-C, Zakrzewska K, Ris F, et al. Molecular detection of circulating B-cells after islet transplantation. *Diabetes* (2002) 51 (3):557–61. doi: 10.2337/diabetes.51.3.557

247. Berney T, Mamin A, Shapiro AJ, Ritz-Laser B, Brulhart M-C, Toso C, et al. Detection of insulin mrna in the peripheral blood after human islet transplantion predicts deterioration of metabolic control. *Am J Transplant* (2006) 6(7):1704–11. doi: 10.1111/j.1600-6143.2006.01373.x

248. Ling Z, De Pauw P, Jacobs-Tulleneers-Thevissen D, Mao R, Gillard P, Hampe CS, et al. Plasma Gad65, a marker for early B-cell loss after intraportal islet cell transplantation in diabetic patients. *J Clin Endocrinol Metab* (2015) 100(6):2314–21. doi: 10.1210/jc.2015-1216

249. Piemonti L, Correa-Tapia M, Lampasona V, Ferrer J. Circulating Mir-375 levels after islet transplantation in humans: A biomarker for ongoing beta cell injury. *CellR4* (2013) 1(1):e35.

250. Kanak MA, Takita M, Shahbazov R, Lawrence MC, Chung WY, Dennison AR, et al. Evaluation of microrna375 as a novel biomarker for graft damage in clinical islet transplantation. *Transplantation* (2015) 99(8):1568–73. doi: 10.1097/TP.0000000000000625

251. Bellin MD, Clark P, Usmani-Brown S, Dunn TB, Beilman GJ, Chinnakotla S, et al. Unmethylated insulin DNA is elevated after total pancreatectomy with islet autotransplantation: assessment of a novel beta cell marker. *Am J Transplant* (2017) 17 (4):1112–8. doi: 10.1111/ajt.14054

252. Gala-Lopez BL, Neiman D, Kin T, O'Gorman D, Pepper AR, Malcolm AJ, et al. Beta cell death by cell-free DNA and outcome after clinical islet transplantation. *Transplantation* (2018) 102(6):978–85. doi: 10.1097/TP.0000000000002083

253. Murakami T, Fujimoto H, Inagaki N. Non-invasive beta-cell imaging: visualization, quantification, and beyond. *Front Endocrinol* (2021) 12:714348. doi: 10.3389/fendo.2021.714348

254. Cheung P, Eriksson O. The current state of beta-cell-mass pet imaging for diabetes research and therapies. *Biomedicines* (2021) 9(12):1824. doi: 10.3390/biomedicines9121824

255. Penfornis A, Kury-Paulin S. Immunosuppressive drug-induced diabetes. *Diabetes Metab* (2006) 32(5):539–46. doi: 10.1016/S1262-3636(06)72809-9

256. Naesens M, Kuypers DR, Sarwal M. Calcineurin inhibitor nephrotoxicity. *Clin J Am Soc Nephrol* (2009) 4(2):481–508. doi: 10.2215/CJN.04800908

257. Furukawa A, Wisel SA, Tang Q. Impact of Immune-Modulatory Drugs on Regulatory T Cell. *Transplantation* (2016) 100(11):2288–300. doi: 10.1097/TP.0000000000001379

258. Gurkan S, Luan Y, Dhillon N, Allam S, Montague T, Bromberg J, et al. Immune reconstitution following rabbit antithymocyte globulin. *Am J Transplant* (2010) 10 (9):2132–41. doi: 10.1111/j.1600-6143.2010.03210.x

259. Trzonkowski P, Zilvetti M, Chapman S, Więckiewicz J, Sutherland A, Friend P, et al. Homeostatic repopulation by Cd28– Cd8+ T cells in alemtuzumab-depleted kidney transplant recipients treated with reduced immunosuppression. *Am J Transplant* (2008) 8(2):338–47. doi: 10.1111/j.1600-6143.2007.02078.x

260. Monti P, Brigatti C, Heninger A, Scirpoli M, Bonifacio E. Disengaging the il-2 receptor with daclizumab enhances Il-7–mediated proliferation of Cd4+ and Cd8+ T cells. *Am J Transplant* (2009) 9(12):2727–35. doi: 10.1111/j.1600-6143.2009.02825.x

261. Maffi P, Berney T, Nano R, Niclauss N, Bosco D, Melzi R, et al. Calcineurin inhibitor-free immunosuppressive regimen in type 1 diabetes patients receiving islet transplantation: single-group phase 1/2 trial. *Transplantation* (2014) 98(12):1301–9. doi: 10.1097/TP.0000000000000396

262. Wisel SA, Posselt AM, Szot GL, Nunez M, Santos-Parker K, Gardner JM, et al. A multi-modal approach to islet and pancreas transplantation with calcineurin-sparing immunosuppression maintains long-term insulin independence in patients with type I diabetes. *Transplant Int* (2023) 36:11367. doi: 10.3389/ti.2023.11367

263. Hawksworth JS, Leeser D, Jindal RM, Falta E, Tadaki D, Elster EA. New directions for induction immunosuppression strategy in solid organ transplantation. *Am J Surg* (2009) 197(4):515–24. doi: 10.1016/j.amjsurg.2008.04.025

264. Kirk AD. Induction immunosuppression. *Transplantation* (2006) 82(5):593–602. doi: 10.1097/01.tp.0000234905.56926.7f

265. Singh A, Ramachandran S, Graham ML, Daneshmandi S, Heller D, Suarez-Pinzon WL, et al. Long-term tolerance of islet allografts in nonhuman primates induced by apoptotic donor leukocytes. *Nat Commun* (2019) 10(1):1–16. doi: 10.1038/s41467-019-11338-y

266. Buss JL, Rajab A, Essig ED, Bergdall VK, Wang J, Osei K. Exenatide pretreatment improved graft function in nonhuman primate islet recipients compared to treatment after transplant only. *J Transplant* (2012) 2012:382518. doi: 10.1155/2012/382518

267. Monti P, Scirpoli M, Maffi P, Piemonti L, Secchi A, Bonifacio E, et al. Rapamycin monotherapy in patients with type 1 diabetes modifies Cd4+ Cd25+ Foxp3+ Regulatory T-cells. *Diabetes* (2008) 57(9):2341–7. doi: 10.2337/db08-0138

268. Melzi R, Maffi P, Nano R, Sordi V, Mercalli A, Scavini M, et al. Rapamycin does not adversely affect intrahepatic islet engraftment in mice and improves early islet engraftment in humans. *Islets* (2009) 1(1):42–9. doi: 10.4161/isl.1.1.8881

269. Nagy G, Szekely TE, Somogyi A, Herold M, Herold Z. New therapeutic approaches for type 1 diabetes: disease-modifying therapies. *World J Diabetes* (2022) 13(10):835. doi: 10.4239/wjd.v13.i10.835

270. Herold KC, Bundy BN, Long SA, Bluestone JA, DiMeglio LA, Dufort MJ, et al. An anti-Cd3 antibody, teplizumab, in relatives at risk for type 1 diabetes. *New Engl J Med* (2019) 381(7):603–13. doi: 10.1056/NEJMoa1902226

271. Haller MJ, Gitelman SE, Gottlieb PA, Michels AW, Rosenthal SM, Shuster JJ, et al. Anti-thymocyte globulin/G-csf treatment preserves B Cell function in patients with established type 1 diabetes. *J Clin Invest* (2015) 125(1):448–55. doi: 10.1172/JCI78492

272. Rigby MR, DiMeglio LA, Rendell MS, Felner EI, Dostou JM, Gitelman SE, et al. Targeting of memory T cells with alefacept in new-onset type 1 diabetes (T1dal study): 12 month results of a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet Diabetes Endocrinol* (2013) 1(4):284–94. doi: 10.1016/S2213-8587(13)70111-6

273. Rigby MR, Harris KM, Pinckney A, DiMeglio LA, Rendell MS, Felner EI, et al. Alefacept provides sustained clinical and immunological effects in new-onset type 1 diabetes patients. *J Clin Invest* (2015) 125(8):3285–96. doi: 10.1172/jci81722

274. Orban T, Bundy B, Becker DJ, DiMeglio LA, Gitelman SE, Goland R, et al. Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: A randomised, double-blind, placebo-controlled trial. *Lancet* (2011) 378(9789):412–9. doi: 10.1016/S0140-6736(11)60886-6

275. Pescovitz MD, Greenbaum CJ, Krause-Steinrauf H, Becker DJ, Gitelman SE, Goland R, et al. Rituximab, B-lymphocyte depletion, and preservation of beta-cell function. *New Engl J Med* (2009) 361(22):2143–52. doi: 10.1056/NEJMoa0904452

276. Quattrin T, Haller MJ, Steck AK, Felner EI, Li Y, Xia Y, et al. Golimumab and beta-cell function in youth with new-onset type 1 diabetes. *New Engl J Med* (2020) 383 (21):2007–17. doi: 10.1056/NEJMoa2006136

277. Mastrandrea L, Yu J, Behrens T, Buchlis J, Albini C, Fourtner S, et al. Etanercept treatment in children with new-onset type 1 diabetes: pilot randomized, placebo-controlled, double-blind study. *Diabetes Care* (2009) 32(7):1244–9. doi: 10.2337/dc09-0054

278. Smigoc Schweiger D. Recent advances in immune-based therapies for type 1 diabetes. *Hormone Res Paediatrics* (2022) 631–45. doi: 10.1159/000524866

279. Dong S, Hiam-Galvez KJ, Mowery CT, Herold KC, Gitelman SE, Esensten JH, et al. The effect of low-dose il-2 and treg adoptive cell therapy in patients with type 1 diabetes. *JCI Insight* (2021) 6(18):e147474. doi: 10.1172/jci.insight.147474

280. Moran A, Bundy B, Becker DJ, DiMeglio LA, Gitelman SE, Goland R, et al. Interleukin-1 antagonism in type 1 diabetes of recent onset: two multicentre, randomised, double-blind, placebo-controlled trials. *Lancet* (2013) 381(9881):1905–15. doi: 10.1016/S0140-6736(13)60023-9

281. von Herrath M, Bain SC, Bode B, Clausen JO, Coppieters K, Gaysina L, et al. Anti-interleukin-21 antibody and liraglutide for the preservation of B-cell function in adults with recent-onset type 1 diabetes: A randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet Diabetes Endocrinol* (2021) 9(4):212–24. doi: 10.1016/S2213-8587(21)00019-X

282. Gitelman SE, Bundy BN, Ferrannini E, Lim N, Blanchfield JL, DiMeglio LA, et al. Imatinib therapy for patients with recent-onset type 1 diabetes: A multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet Diabetes Endocrinol* (2021) 9(8):502–14. doi: 10.1016/S2213-8587(21)00139-X

283. Sanders JM, Jeyamogan S, Mathew JM, Leventhal JR. Foxp3+ Regulatory T cell therapy for tolerance in autoimmunity and solid organ transplantation. *Front Immunol* (2022) 13:1055466. doi: 10.3389/fimmu.2022.1055466

284. Bluestone JA, Buckner JH, Fitch M, Gitelman SE, Gupta S, Hellerstein MK, et al. Type 1 diabetes immunotherapy using polyclonal regulatory T cells. *Sci Trans Med* (2015) 7(315):315ra189–315ra189. doi: 10.1126/scitranslmed.aad4134

285. Marek-Trzonkowska N, Myśliwiec M, Dobyszuk A, Grabowska M, Techmańska I, Juścińska J, et al. Administration of Cd4+ Cd25highcd127– regulatory T cells preserves B-cell function in type 1 diabetes in children. *Diabetes Care* (2012) 35(9):1817–20. doi: 10.2337/dc12-0038

286. Bergström M, Yao M, Müller M, Korsgren O, von Zur-Mühlen B, Lundgren T. Autologous regulatory T cells in clinical intraportal allogenic pancreatic islet transplantation. *Transplant Int* (2021) 34(12):2816–23. doi: 10.1111/tri.14163

287. Tang Q, Henriksen KJ, Bi M, Finger EB, Szot G, Ye J, et al. *In vitro*–expanded antigen-specific regulatory T cells suppress autoimmune diabetes. *J Exp Med* (2004) 199(11):1455–65. doi: 10.1084/jem.20040139

288. Bettini M, Bettini ML. Function, failure, and the future potential of tregs in type 1 diabetes. *Diabetes* (2021) 70(6):1211–9. doi: 10.2337/dbi18-0058

289. Rosado-Sanchez I, Levings MK. Building a car-treg: going from the basic to the luxury model. *Cell Immunol* (2020) 358:104220. doi: 10.1016/j.cellimm.2020.104220

290. Roybal KT, Lim WA. Synthetic immunology: hacking immune cells to expand their therapeutic capabilities. *Annu Rev Immunol* (2017) 35:229–53. doi: 10.1146/annurev-immunol-051116-052302

291. Nakayama M, Michels AW. Using the T cell receptor as a biomarker in type 1 diabetes. *Front Immunol* (2021) 12:777788. doi: 10.3389/fimmu.2021.777788

292. Nguyen H, Arribas-Layton D, Chow I, Speake C, Kwok WW, Hessner MJ, et al. Characterizing T cell responses to enzymatically modified beta cell neo-epitopes. *Front Immunol* (2023) 13:1015855. doi: 10.3389/fimmu.2022.1015855

293. Manfredi F, Cianciotti BC, Potenza A, Tassi E, Noviello M, Biondi A, et al. Tcr redirected T cells for cancer treatment: achievements, hurdles, and goals. *Front Immunol* (2020) 11:1689. doi: 10.3389/fimmu.2020.01689

294. Sockolosky JT, Trotta E, Parisi G, Picton L, Su LL, Le AC, et al. Selective targeting of engineered T cells using orthogonal Il-2 cytokine-receptor complexes. *Science* (2018) 359(6379):1037–42. doi: 10.1126/science.aar3246

295. Kremer J, Henschel P, Simon D, Riet T, Falk C, Hardtke-Wolenski M, et al. Membrane-bound Il-2 improves the expansion, survival, and phenotype of car tregs and confers resistance to calcineurin inhibitors. *Front Immunol* (2022) 13:1005582. doi: 10.3389/fimmu.2022.1005582

296. Hoffmann P, Eder R, Kunz-Schughart LA, Andreesen R, Edinger M. Large-scale in vitro expansion of polyclonal human Cd4+ Cd25high regulatory T cells. *Blood* (2004) 104(3):895–903. doi: 10.1182/blood-2004-01-0086

297. Heninger A-K, Theil A, Wilhelm C, Petzold C, Huebel N, Kretschmer K, et al. Il-7 abrogates suppressive activity of human Cd4+ Cd25+ Foxp3+ Regulatory T cells and allows expansion of alloreactive and autoreactive T cells. *J Immunol* (2012) 189 (12):5649–58. doi: 10.4049/jimmunol.1201286

298. Cosorich I, Filoni J, Di Dedda C, Ferrari A, Jofra T, Cesarano S, et al. Interleukin-7 improves the fitness of regulatory T cells for adoptive transfer. *Immunology* (2023) 170(4):540–52. doi: 10.1111/imm.13690

299. Riolobos L, Hirata RK, Turtle CJ, Wang P-R, Gornalusse GG, Zavajlevski M, et al. Hla engineering of human pluripotent stem cells. *Mol Ther* (2013) 21(6):1232–41. doi: 10.1038/mt.2013.59

300. Wang D, Quan Y, Yan Q, Morales JE, Wetsel RA. Targeted disruption of the B 2-microglobulin gene minimizes the immunogenicity of human embryonic stem cells. *Stem Cells Trans Med* (2015) 4(10):1234–45. doi: 10.5966/sctm.2015-0049

301. Gornalusse GG, Hirata RK, Funk SE, Riolobos L, Lopes VS, Manske G, et al. Hla-E-expressing pluripotent stem cells escape allogeneic responses and lysis by Nk cells. *Nat Biotechnol* (2017) 35(8):765–72. doi: 10.1038/nbt.3860

302. Han X, Wang M, Duan S, Franco PJ, Kenty JH-R, Hedrick P, et al. Generation of hypoimmunogenic human pluripotent stem cells. *Proc Natl Acad Sci* (2019) 116 (21):10441–6. doi: 10.1073/pnas.1902566116

303. Chen Y, Zhou Y, Zhou Z, Fang Y, Ma L, Zhang X, et al. Hypoimmunogenic human pluripotent stem cells are valid cell sources for cell therapeutics with normal self-renewal and multilineage differentiation capacity. *Stem Cell Res Ther* (2023) 14 (1):11. doi: 10.1186/s13287-022-03233-z

304. Lee N, Llano M, Carretero M, Ishitani A, Navarro F, López-Botet M, et al. Hla-E is a major ligand for the natural killer inhibitory receptor Cd94/Nkg2a. *Proc Natl Acad Sci* (1998) 95(9):5199–204. doi: 10.1073/pnas.95.9.5199

305. Goodall KJ, Nguyen A, Sullivan LC, Andrews DM. The expanding role of murine class Ib Mhc in the development and activation of natural killer cells. *Mol Immunol* (2019) 115:31–8. doi: 10.1016/j.molimm.2018.05.001

306. Cross-Najafi AA, Farag K, Isidan A, Li W, Zhang W, Lin Z, et al. Co-expression of Hla-E and Hla-G on genetically modified porcine endothelial cells attenuates human Nk cell-mediated degranulation. *Front Immunol* (2023) 14. doi: 10.3389/fimmu.2023.1217809

307. Rouas-Freiss N, Gonçalves RM-B, Menier C, Dausset J, Carosella ED. Direct evidence to support the role of Hla-G in protecting the fetus from maternal uterine natural killer cytolysis. *Proc Natl Acad Sci* (1997) 94(21):11520–5. doi: 10.1073/pnas.94.21.11520

308. Pazmany L, Mandelboim O, Vales-Gomez M, Davis DM, Reyburn HT, Strominger JL. Protection from natural killer cell-mediated lysis by Hla-G expression on target cells. *Science* (1996) 274(5288):792–5. doi: 10.1126/science.274.5288.792

309. Shi L, Li W, Liu Y, Chen Z, Hui Y, Hao P, et al. Generation of hypoimmunogenic human pluripotent stem cells via expression of membrane-bound and secreted B2m-Hla-G fusion proteins. *Stem Cells* (2020) 38(11):1423–37. doi: 10.1002/stem.3269

310. Long EO, Sik Kim H, Liu D, Peterson ME, Rajagopalan S. Controlling natural killer cell responses: integration of signals for activation and inhibition. *Annu Rev Immunol* (2013) 31:227–58. doi: 10.1146/annurev-immunol-020711-075005

311. Chimienti R, Baccega T, Torchio S, Manenti F, Pellegrini S, Cospito A, et al. Engineering of immune checkpoints B7-H3 and Cd155 enhances immune compatibility of Mhc-I–/– Ipscs for B Cell replacement. *Cell Rep* (2022) 40 (13):111423. doi: 10.1016/j.celrep.2022.111423

312. Chen H, Li Y, Lin X, Cui D, Cui C, Li H, et al. Functional disruption of human leukocyte antigen ii in human embryonic stem cell. *Biol Res* (2015) 48:1–9. doi: 10.1186/s40659-015-0051-6

313. Fife BT, Bluestone JA. Control of peripheral T-cell tolerance and autoimmunity via the Ctla-4 and Pd-1 pathways. *Immunol Rev* (2008) 224(1):166–82. doi: 10.1111/j.1600-065X.2008.00662.x

314. Rong Z, Wang M, Hu Z, Stradner M, Zhu S, Kong H, et al. An effective approach to prevent immune rejection of human esc-derived allografts. *Cell Stem Cell* (2014) 14(1):121–30. doi: 10.1016/j.stem.2013.11.014

315. Walker LS, Abbas AK. The enemy within: keeping self-reactive T cells at bay in the periphery. *Nat Rev Immunol* (2002) 2(1):11–9. doi: 10.1038/nri701

316. Jaiswal S, Jamieson CH, Pang WW, Park CY, Chao MP, Majeti R, et al. Cd47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. *Cell* (2009) 138(2):271–85. doi: 10.1016/j.cell.2009.05.046

317. Deuse T, Hu X, Gravina A, Wang D, Tediashvili G, De C, et al. Hypoimmunogenic derivatives of induced pluripotent stem cells evade immune rejection in fully immunocompetent allogeneic recipients. *Nat Biotechnol* (2019) 37 (3):252–8. doi: 10.1038/s41587-019-0016-3

318. Hu X, Gattis C, Olroyd AG, Friera AM, White K, Young C, et al. Human hypoimmune primary pancreatic islets avoid rejection and autoimmunity and alleviate diabetes in allogeneic humanized mice. *Sci Trans Med* (2023) 15(691):eadg5794. doi: 10.1126/scitranslmed.adg5794

319. Munn DH, Sharma MD, Baban B, Harding HP, Zhang Y, Ron D, et al. Gcn2 kinase in T cells mediates proliferative arrest and anergy induction in response to indoleamine 2, 3-dioxygenase. *Immunity* (2005) 22(5):633–42. doi: 10.1016/j.immuni.2005.03.013

320. Paul PK, Das R, Drow T, Nylen EA, de Souza AH, Wang Z, et al. Islet allografts expressing a pd-L1 and ido fusion protein evade immune rejection and reverse preexisting diabetes in immunocompetent mice without systemic immunosuppression. *Am J Transplant* (2022) 22(11):2571–85. doi: 10.1111/ajt.17162

321. Hu X, White K, Olroyd AG, DeJesus R, Dominguez AA, Dowdle WE, et al. Hypoimmune induced pluripotent stem cells survive long term in fully immunocompetent, allogeneic rhesus macaques. *Nat Biotechnol* (2023), 1–11. doi: 10.1038/s41587-023-01784-x

322. Gerace D, Zhou Q, Kenty JH-R, Veres A, Sintov E, Wang X, et al. Engineering human stem cell-derived islets to evade immune rejection and promote localized immune tolerance. *Cell Rep Med* (2023) 4(1):100879. doi: 10.1016/j.xcrm.2022.100879

323. Greco R, Oliveira G, Stanghellini MTL, Vago L, Bondanza A, Peccatori J, et al. Improving the safety of cell therapy with the Tk-suicide gene. *Front Pharmacol* (2015) 6:95. doi: 10.3389/fphar.2015.00095

324. Wunderlich S, Haase A, Merkert S, Jahn K, Deest M, Frieling H, et al. Targeted biallelic integration of an inducible caspase 9 suicide gene in Ipscs for safer therapies. *Mol Therapy-Methods Clin Dev* (2022) 26:84–94. doi: 10.1016/j.omtm.2022.05.011

325. Wienert B, Cromer MK. Crispr nuclease off-target activity and mitigation strategies. *Front Genome Editing* (2022) 4:1050507. doi: 10.3389/fgeed.2022.1050507

326. Yin H, Kauffman KJ, Anderson DG. Delivery technologies for genome editing. *Nat Rev Drug Discovery* (2017) 16(6):387–99. doi: 10.1038/nrd.2016.280

327. LaFountaine JS, Fathe K, Smyth HD. Delivery and therapeutic applications of gene editing technologies Zfns, Talens, and Crispr/Cas9. *Int J pharmaceutics* (2015) 494 (1):180–94. doi: 10.1016/j.ijpharm.2015.08.029

328. Pignatelli C, Campo F, Neroni A, Piemonti L, Citro A. Bioengineering the vascularized endocrine pancreas: A fine-tuned interplay between vascularization, extracellular-matrix-based scaffold architecture, and insulin-producing cells. *Transplant Int* (2022) 35:10555. doi: 10.3389/ti.2022.10555

329. Bowers DT, Song W, Wang L-H, Ma M. Engineering the vasculature for islet transplantation. *Acta biomaterialia* (2019) 95:131–51. doi: 10.1016/j.actbio.2019.05.051

330. Guyette JP, Gilpin SE, Charest JM, Tapias LF, Ren X, Ott HC. Perfusion decellularization of whole organs. *Nat Protoc* (2014) 9(6):1451–68. doi: 10.1038/nprot.2014.097

331. Ribezzi D, Gueye M, Florczak S, Dusi F, de Vos D, Manente F, et al. Shaping synthetic multicellular and complex multimaterial tissues via embedded extrusion-volumetric printing of microgels. *Adv Mater* (2023) 35(36):e2301673. doi: 10.1002/adma.202301673

332. Citro A, Moser PT, Dugnani E, Rajab TK, Ren X, Evangelista-Leite D, et al. Biofabrication of a vascularized islet organ for type 1 diabetes. *Biomaterials* (2019) 199:40–51. doi: 10.1016/j.biomaterials.2019.01.035

333. Citro A, Neroni A, Pignatelli C, Campo F, Policardi M, Monieri M, et al. Directed self-assembly of a xenogeneic vascularized endocrine pancreas for type 1 diabetes. *Nat Commun* (2023) 14(1):878. doi: 10.1038/s41467-023-36582-1

334. Kharbikar BN, Mohindra P, Desai TA. Biomaterials to enhance stem cell transplantation. *Cell Stem Cell* (2022) 29(5):692–721. doi: 10.1016/j.stem.2022.04.002

335. Griffith BP, Goerlich CE, Singh AK, Rothblatt M, Lau CL, Shah A, et al. Genetically modified porcine-to-human cardiac xenotransplantation. *New Engl J Med* (2022) 387(1):35–44. doi: 10.1056/NEJMoa2201422

336. Nicodemus GD, Bryant SJ. Cell encapsulation in biodegradable hydrogels for tissue engineering applications. *Tissue Eng Part B: Rev* (2008) 14(2):149–65. doi: 10.1089/ten.teb.2007.0332

337. Teramura Y, Asif S, Ekdahl KN, Nilsson B. Cell surface engineering for regulation of immune reactions in cell therapy. *Immune Responses to Biosurfaces: Mech Ther Interventions* (2015) 865:189–209. doi: 10.1007/978-3-319-18603-0_12

338. Samojlik MM, Stabler CL. Designing biomaterials for the modulation of allogeneic and autoimmune responses to cellular implants in type 1 diabetes. *Acta biomaterialia* (2021) 133:87–101. doi: 10.1016/j.actbio.2021.05.039

339. Headen DM, Woodward KB, Coronel MM, Shrestha P, Weaver JD, Zhao H, et al. Local immunomodulation with fas ligand-engineered biomaterials achieves allogeneic islet graft acceptance. *Nat materials* (2018) 17(8):732–9. doi: 10.1038/s41563-018-0099-0

340. Li F, Crumley K, Bealer E, King JL, Saito E, Grimany-Nuno O, et al. Fas ligand-modified scaffolds protect stem cell derived B-cells by modulating immune cell numbers and polarization. *ACS Appl Materials Interfaces* (2022). doi: 10.1021/acsami.2c12939

341. Ernst AU, Wang L-H, Worland SC, Marfil-Garza BA, Wang X, Liu W, et al. A predictive computational platform for optimizing the design of bioartificial pancreas devices. *Nat Commun* (2022) 13(1):6031. doi: 10.1038/s41467-022-33760-5

342. Nosrati H, Nosrati M. Artificial intelligence in regenerative medicine: applications and implications. *Biomimetics* (2023) 8(5):442. doi: 10.3390/biomimetics8050442

343. Mukherjee S, Yadav G, Kumar R. Recent trends in stem cell-based therapies and applications of artificial intelligence in regenerative medicine. *World J Stem Cells* (2021) 13(6):521. doi: 10.4252/wjsc.v13.i6.521

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Advancements in innate immune regulation strategies in islet transplantation

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As a newly emerging organ transplantation technique, islet transplantation has shown the advantages of minimal trauma and high safety since it was first carried out. The proposal of the Edmonton protocol, which has been widely applied, was a breakthrough in this method. However, direct contact between islets and portal vein blood will cause a robust innate immune response leading to massive apoptosis of the graft, and macrophages play an essential role in the innate immune response. Therefore, therapeutic strategies targeting macrophages in the innate immune response have become a popular research topic in recent years. This paper will summarize and analyze recent research on strategies for regulating innate immunity, primarily focusing on macrophages, in the field of islet transplantation, including drug therapy, optimization of islet preparation process, islet engineering and Mesenchymal stem cells cotransplantation. We also expounded the heterogeneity, plasticity and activation mechanism of macrophages in islet transplantation, providing a theoretical basis for further research.

KEYWORDS

islet transplantation, innate immune response, immunoregulation, macrophage, diabetes

Abbreviations: IBMIR, Immediate blood-mediated inflammatory response; ECM, Extracellular matrix; TNF-α, Tumor necrosis factor α; IFN-γ, Interferon-γ; STAT1, Signal transducers and activators of transcription 1; KCs, Kupffer cells; IL, Interleukin; VEGF-A, Vascular endothelial growth factor A; LPS, Lipopolysaccharide; ROS, Reactive oxygen species; TLRs, Toll-like receptors; IL-1Ra, Interleukin-1 receptor antagonist; hAAT, Human alpha1-antitrypsin; EPO, Erythropoietin; KO, Knockout; MSCs, Mesenchymal stem cells; ASCs, Adipose-derived mesenchymal stem cells; hIAPP, Human intestinal amyloid precursor protein; ATF3, Activation transcription factor 3; Del-1, Developmental endothelial locus-1; HO-1, Heme oxygenase-1; sTNF-αR, Soluble TNF-α type I receptor; Dex, Dexamethasone; IDO, Indoleamine 2,3-dioxygenase; TA, Tannic acid; BAT, Brown adipose tissue.

1 Introduction

Since the Edmonton protocol was proposed in 2000, islet transplantation has developed rapidly as a new technology. This protocol proposed a glucocorticoid-free immunosuppressive regimen consisting of sirolimus, tacrolimus, and daclizumab and a scheme of sequential transplantation of islets from at least two donor pancreases. During the follow-up period (with a median follow-up time of 1 year), all seven patients who received this protocol achieved insulin independence (1). Subsequent clinical trials verified the protocol's effectiveness and noted the necessity of minimizing the interval between 2 islet infusions (2). The outcomes of islet transplantation over the past two decades were assessed in a large cohort study conducted by the Edmonton group. Among 255 patients, the 5-year insulin independence rate after operation was 32%, and 8% still had insulin independence 20 years later (3). Islet transplantation has the advantages of minimal trauma, high safety, a short hospital stay, less patient suffering, and repeatability. It is especially suitable for "brittle diabetes" type I diabetes patients, type II diabetes patients with pancreatic islet dysfunction, diabetes patients after liver and kidney transplantation, and patients with nonmalignant pancreatic resection for the prevention of postoperative diabetes. Compared with traditional drug and insulin therapy, animal experiments have proven that islet transplantation can increase the body and muscle weight of diabetic rats, more effectively reduce proteinuria, significantly improve the conduction velocity of the tail nerve, restore thermal and ameliorate mechanical nociceptive thresholds, and improve the residual β-cell state in the recipient pancreas (4). In a clinical control experiment, islet transplantation slowed the progression of diabetic microvascular complications, such as a declining renal glomerular filtration rate and retinal changes (5); compared with no transplantation, islet transplantation resulted in near-normal platelet activation and prothrombotic factor levels, cerebral metabolism and function, and neuropsychological test results (6).

The most common and ideal transplantation route is through the transhepatic portal vein (7). Nevertheless, islet cells implanted in the portal vein are often lost within three days due to hypoxia, nutritional deficiency, the mechanical pressure of the portal vein, and the immediate blood-mediated inflammatory response (IBMIR). The early loss of implanted islets is an essential factor in the failure to achieve insulin independence in single transplant patients. The IBMIR was first proposed by the W Bennet team in 1999 and was validated in *in vivo* and *in vitro* experiments. The reaction is defined as a rapid, strong, and nonspecific immune inflammatory response induced by tissue factors exposed on the surfaces of islets, with characteristics such as platelet activation, aggregation, coagulation, and complement system activation, as well as an infiltration of neutrophils, monocytes and macrophages and release of inflammatory factors. Usually, within 15 minutes after transplantation, the islets are encircled by a thrombus; after 1 hour, the islets undergo massive apoptosis due to the infiltration of white blood cells (8). This reaction also leads to early apoptosis of autologous islets in patients who undergo total pancreatectomy and autologous islet transplantation (9). At present, the standard clinical therapy relies on anticoagulants such as heparin, soluble complement receptor 1, and protease inhibitors such as ulinastatin. However, the IBMIR is a complex reaction involving multiple factors, and 50-70% of transplanted islets still undergo early apoptosis due to the IBMIR in existing therapeutic schemes. Therefore, the inhibition of the IBMIR to avoid early apoptosis of transplanted islets is an urgent issue in clinical work (10).

Because macrophages are essential in the IBMIR and subsequent adaptive immunity and are plastic and heterogeneous in inflammatory reactions (11, 12), This article reviews the strategy of innate immune regulation with macrophages as the primary target in recent years in islet transplantation to provide references for subsequent basic research and improve the survival rate of islets in clinical islet transplantation (Tables 1, 2).

2 Activation and role of macrophages in the IBMIR

Clinical islet transplantation requires four steps: perfusion of the donor pancreas, digestion of the pancreas to separate the islets from exocrine glands, purification of the islets, and transplantation into recipients through the portal vein (7). When the prepared islets are infused into patients via the portal vein, it will trigger IBMIR.

IBMIR is initiated by a strong activation of the coagulation cascade. After contact with blood in the portal vein, islet tissue factor expression induces the extrinsic coagulation pathway. The negative charge on the islet surface triggers the intrinsic coagulation pathway. At the same time, islets secrete inflammatory factors such as IL-8 and MCP-1, which have chemotactic and proinflammatory effects on macrophages and neutrophils (46, 47).

Activated platelets may attach through binding to extracellular matrix (ECM) and collagen on the surface of the islet. Meanwhile, owing to the fast and transient expression of p-selectin on the membrane of activated platelet alpha granules and vascular endothelial Weibel-Palade bodies, the p-selectin lectin-like domain binds to sialyl Lewis x and the p-selectin glycoprotein ligand 1 in the neutrophils and mononuclear cells, thus mediating the rolling of neutrophils and mononuclear cells on the endothelial cell surface and the adhesion of neutrophils and mononuclear cells to platelets (48, 49). On the other hand, vascular endothelial cells secrete IL-6 and IL-8 to promote the aggregation of neutrophils and macrophages (46).

Complement activation is triggered by the natural immunoglobulins IgG and IgM. When isolated islets are exposed to blood, the complement system is quickly activated, leading to the lysis of islet cells. At the same time, the production of the allergic toxins C3a and C5a further induces the aggregation of macrophages and neutrophils and promotes mononuclear cells to release cytokines such as IL-1, IL-6, IL-8, and TNF-α (50).

Granulocytes appear 8 hours after islet transplantation and extensively infiltrate the transplants after 12 hours. Neutrophils are predominant members of the granulocyte family and the first line of defense of innate immunity. They contain many cytokines, which are released when activated and have destructive effects on islets;

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TABLE 1 *In vivo* and *in vitro* experiments for all therapeutic strategy.

Therapeutic strategy	vitro studies	vivo studies in mammals	Donor	Receptor	Transplantation type
PTD (13)	+	+	mice	mice	Allotransplantation
CO (14)	+	+	mice	mice	Allotransplantation
NOX-A12 (15)	–	+	mice	mice	Isotransplantation
mNOX-E36 (15)	–	+	mice	mice	Isotransplantation
MCC950 (16)	+	+	mice	mice	Isotransplantation
hAAT (17) (18)	+	+	mice	mice	Allotransplantation
			mice	mice	Allotransplantation
			human	mice	Xenotransplantation
1,25(OH)2D3 (19–21)	+	+	rat	rat	Isotransplantation
DHMEQ (22)	+	+	mice	mice	Isotransplantation
ARA290 (23)	+	+	mice	mice	Isotransplantation
liraglutide (24) (25)	+	+	rat	rat	Isotransplantation
teduglutide (26)	+	–	N/A	N/A	N/A
captopril (27)	+	+	pig	mice	Xenotransplantation
Diannexin (28)	+	+	mice	mice	Isotransplantation
CP-ASCs (29)	+	+	mice	mice	Isotransplantation
			human	mice	Xenotransplantation
autologous MSCs (30)	+	+	human	human	Allotransplantation
MSCs-derived exosomes (31)	+	+	rat	mice	Xenotransplantation
OptiPrep (32)	+	+	human	mice	Xenotransplantation
APT070 (33)	+	+	human	mice	Xenotransplantation
anakinra (34)	+	–	N/A	N/A	N/A
ATF3 KO (35)	+	+	mice	mice	Isotransplantation
overexpress Del-1 (36)	+	+	mice	mice	Isotransplantation
Overexpress sTNF-αR-Fc/HO-1 (37)	+	+	pig	mice	Xenotransplantation
MHC I and II KO and overexpress CD47 (38)	+	+	human	mice	Xenotransplantation
			mice	mice	Allotransplantation
			mice	mice	Isotransplantation
thermoplastic polyurethane-based nanofiber capsules (39)	+	+	mice	mice	Isotransplantation
	+	+	mice	mice	Allotransplantation
Dexa (40)	+	+	pig	mice	Xenotransplantation
bilirubin (41)	+	+	mice	mice	Isotransplantation
IDO (42)	+	+	rat	mice	Xenotransplantation
TA (43–45)	+	+	mice	mice	Allotransplantation

PTD, protein transduction domain proteins; 1,25(OH)2D3, 1,25-Dihydroxy vitamin D3; DHMEQ, Dehydroxymethylepoxyquinomicin.

neutrophils make significant contributions to the activation and recruitment of macrophages in acute inflammation sites. After activation, they produce various chemokines to attract mononuclear cells and macrophages; in addition, the infiltration of neutrophils leads to the release of cytokines from T cells and macrophages, such as tumor necrosis factor α (TNF-α) and macrophage inflammatory protein 1α. The mobilization of this immune effector could not only expand IBMIR but also induce

TABLE 2 Mechanisms and limitations of all therapeutic strategy.

Therapeutic strategy	mechanism	limitations
PTD (13)	Block TLR4 signaling	Risk of affecting islet vascularization; neglect the impact of clinical immunosuppression schemes
CO (14)	Block TLR4 signaling	
NOX-A12 (15)	Bind and antagonize CCL2/MCP-1	neglect the impact of clinical immunosuppression schemes
mNOX-E36 (15)	Bind and antagonize CXCL12/SDF-1	
MCC950 (16)	Inhibit the activation of NLRP3 inflammasome	Risk of affecting islet vascularization; neglect the impact of clinical immunosuppression schemes
hAAT (17) (18)	Increase IL-1Ra expression and secretion Inhibit IFN-γ-induced STAT1 phosphorylation; Inhibit iNOS production	neglect the impact of clinical immunosuppression schemes
1,25(OH)2D3 (19–21)	Reduce TNF-α/NF-κB pathway activation; Reduce macrophage recruitment; Promote the polarization of M1 macrophages into M2 macrophages via the VDR-PPARγ pathway	neglect the impact of clinical immunosuppression schemes; Risk of graft fibrosis
DHMEQ (22)	Inhibit NF-κB activation at the nuclear translocation level of macrophage-based immune cells	neglect the impact of clinical immunosuppression schemes
ARA290 (23)	Inhibit NF-κB pathway by activating EPOR-βcR/PI3K-Akt signaling pathway	neglect the impact of clinical immunosuppression schemes
liraglutide (24) (25)	Inhibit the expression of proinflammatory cytokines; Inhibit macrophage recruitment; modulate macrophages M2 polarization via the cAMP-PKA-STAT3 signaling pathway	Risk of affecting islet vascularization; neglect the impact of clinical immunosuppression schemes
teduglutide (26)	Inhibit M1 macrophages polarization by mediating the crosstalk between endocrine cells and macrophages	
captopril (27)	Protect ECM by inhibiting gelatinase activity to reduce macrophage infiltration	Risk of affecting islet vascularization
Diannexin (28)	Inhibit leukocyte and platelet aggregation attachment by binding externalized phosphatidylserine residues on the surface of early apoptotic cells	Risk of affecting islet vascularization
CP-ASCs (29)	Mediate the expression of TNF receptor superfamily member 11b through paracrine IGF-1; Reduce the infiltration of macrophages into the graft	Risk of induced thrombosis in the liver; difficult to ensure the close contact between MSCs and islets
MSCs-derived exosomes (31)	Regulate macrophages by regulating NF-κB signaling pathway	The total amount of drugs carried by microcapsules is limited
OptiPrep (32)	Reduce the production of cytokines/chemokines during islet preparation.	Compared with Ficoll-based purification, Islet yields decreased slightly (have no statistical differences)
APT070 (33)	Reduce iC3b production in islets; Reduce C4d and C5b-9 deposition in islets	Unable to target graft administration posttransplantation
anakinra (34)	Reduce the formation of hIAPP	Risk of subcutaneous amyloidosis caused by long-term subcutaneous injection
ATF3 KO (35)	Inhibit the expression of proinflammatory cytokines and chemokines in islets	Potential cytotoxicity and tumorigenicity
overexpress Del-1 (36)	Inhibit platelet-monocyte aggregate formation by blocking the interaction between monocyte Mac-1-integrin and platelet GPIb	
overexpress sTNF-αR-Fc/HO-1 (37)	Inhibit the expression of proinflammatory cytokines and chemokines in islets; Inhibit macrophage recruitment	
MHC I and II KO and overexpress CD47 (38)	Inhibit innate and adaptive immunity	
thermoplastic polyurethane-based nanofiber capsules (39)	Block macrophages activation	Potential cytotoxicity and tumorigenicity
Dexa (40)	Reduce macrophage-dominant inflammatory cell infiltration and pericapsular fibrosis	The total amount of drugs carried by microcapsules is limited
bilirubin (41)	Activate the Nrf2 pathway to polarize macrophages to the	

TABLE 2 Continued

Therapeutic strategy	mechanism	limitations
	M2 phenotype; Inhibit the NF-κB pathway to inhibit M1 polarization	
IDO (42)	Induce tryptophan deficiency; Reduce the proinflammatory activity and viability of macrophages; Reduce the infiltration of macrophages in the graft	
TA (43–45)	Regulate macrophages polarization by reducing the production of ROS	

PTD, protein transduction domain proteins; 1,25(OH)2D3, 1,25-Dihydroxy vitamin D3; DHMEQ, Dehydroxymethylepoxyquinomicin.

subsequent adaptive immunity, inducing and enhancing cellular rejection responses (50, 51) (Figure 1).

The gathered macrophages continue to secrete cytokines such as IL-6 and IL-8 to maintain the inflammatory response and release proinflammatory factors such as IL-1β, IFN-γ, and TNF-α. The IL-1β secreted by macrophages and neutrophils binds to the IL-1β receptor on the surface of islet cells, activating the IL-1 receptor-associated kinase to activate TNF receptor-associated factor 6, which leads to the phosphorylation and degradation of IκB, releasing NF-κB from the inhibitory IκB, and entering the nucleus of cells to regulate the transcription of various genes, including IL-1, IL-6, TNF-α and iNOS. The TNF-α produced by macrophages and islet cells binds to the TNF receptor, activating the NF-kB and MAPK pathways and inducing cell apoptosis. Apoptosis is activated by the activation of caspase-3, mediated by the MAPK pathway, or by activating effector caspases, including caspase-3, which FADD mediates. The interferon-γ (IFN-γ) produced by macrophages binds to IFN-γ receptors on the surface of the islets, activating JAK1 and JAK2. Activated JAK2 activates signal transducers and activators of transcription 1 (STAT1). Then, STAT1 is transferred to the nucleus for gene regulation, eventually leading to islet apoptosis. The apoptosis-promoting effect of STAT1 may be partially mediated by the activation of caspase-2, caspase-3 and caspase-7 (52). Under the combined effects of the cytokines IL-1β, TNF-α, and IFN-γ, overexpression of iNOS in β cells and macrophages leads to excessive NO synthesis. Subsequently, NO loses an electron to combine with superoxide free radicals, forming a highly active free radical peroxynitrite (ONOO-). The cytotoxicity of ONOO- then induces apoptosis in islet cells. On the other hand,

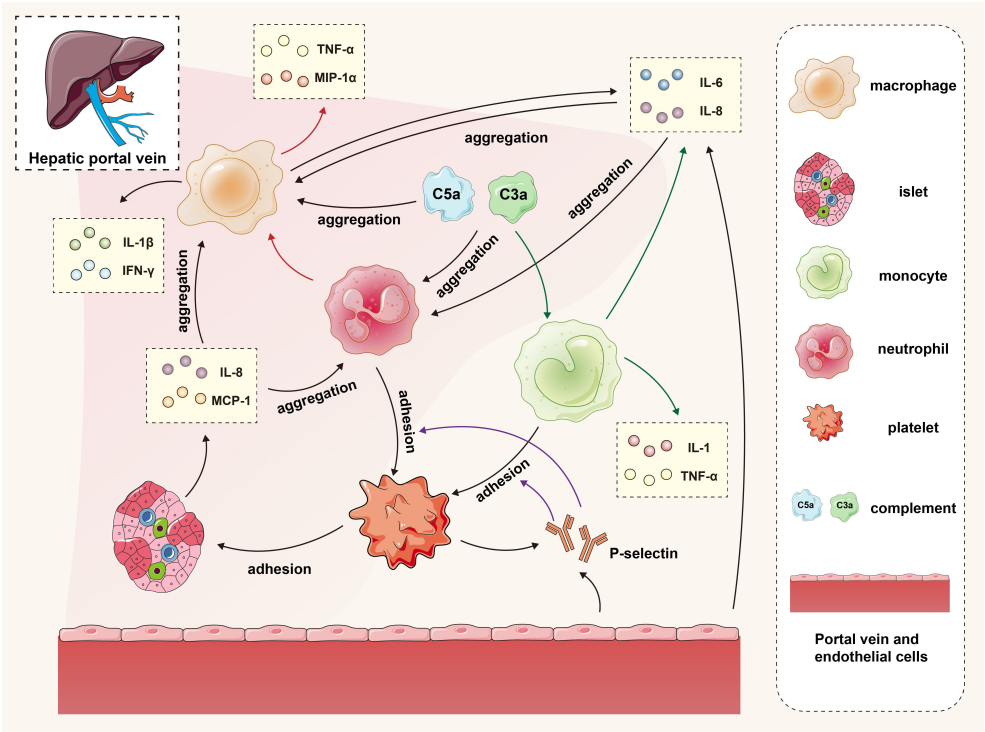

FIGURE 1
The interaction of islets, macrophages, monocytes, neutrophils, platelets, complement and endothelial cells in the IBMIR. In the early stage of transplantation, islets secrete proinflammatory factors and activate the complement system, promoting the recruitment of platelets, neutrophils, and mononuclear macrophages to the graft. Vascular endothelial cells secrete cytokines while releasing P-selectin, facilitating adhesion between monocytes/neutrophils and platelets. The accumulated mononuclear macrophages and neutrophils further contribute to macrophage recruitment and cytokine secretion. The green arrows represent the process by which complement promotes monocytes to secrete cytokines, the red arrows depict the process by which neutrophils promote macrophages to secrete cytokines, and the purple arrows illustrate the procedure in which P-selectin facilitates adherence of monocytes and neutrophils to platelets.

macrophages play an antigen-presenting role, promoting the activation of T cells into CD8+ T cells and CD4+ T cells. Activated T cells produce cytokines such as IFN-γ, TNF-α, and lymphotoxin, thereby inducing β-cell apoptosis. T cells also express Fas receptor-associated ligands and TNF-related apoptosis-inducing ligands, activating effector caspases to cause cellular apoptosis. In addition, CD8+ T cells directly contact and promote the release of granzyme B into the cytoplasm of target cells through perforin, thereby activating nucleases and caspases to kill target cells (11, 50, 53).

3 Macrophage origins in islet transplantation

Macrophages play crucial roles in the IBMIR after islet transplantation. Generally, they aggregate around the islets at 8 hours and infiltrate them at 12 hours posttransplantation. By 24 hours posttransplantation, the transplanted islets are entirely infiltrated (51). In islet transplantation, macrophages usually come from three sources: Kupffer cells (KCs) in the recipient's liver, mononuclear macrophages from the recipient's bone marrow, and macrophages resident in the donor's islets.

For donor-derived macrophages, on the one hand, islet-resident macrophages mediate the production of islet interleukin (IL)-1β and impair the function of beta cells induced by islet amyloid-like polypeptides (54). On the other hand, some studies have shown that during islet compensation in the early stage of diabetes, islet-resident macrophages contribute to angiogenesis by supporting islet vascular endothelial growth factor A (VEGF-A) secretion during islet remodeling, suggesting their critical role in supporting islet compensation during diabetes (55). Further animal experiments confirmed that in a mouse autoimmune diabetes model, islet resident macrophages showed different phenotypes, such as maturation, self-replication, proinflammation, and immune tolerance, throughout the disease course, demonstrating heterogeneity in the inflammatory process (56). Although the number of donor-derived macrophages is usually small, their diverse functions in islet cells during the diabetes stage prove their importance. However, relevant research on donor-derived macrophages in islet transplantation is still lacking.

KCs are resident macrophages in the liver. KCs in the sinusoids can phagocytose pathogens from the arterial and venous systems, playing an essential role in innate immunity. Single-cell RNA sequencing analysis of freshly isolated human liver demonstrates the presence of two distinct intrahepatic CD68+ macrophage subsets in the steady state: one is an inflammatory macrophage subset enriched in the expression of LYZ, CSTA, and CD74; the other is a tolerogenic macrophage subset distinguished by high expression of CD5L, MARCO, and VSIG4. Two distinct cell populations under the same lipopolysaccharide (LPS)/IFN-γ stimulation conditions showed that the tolerogenic function subset secreted less TNF-α (57). In another study, F4/80+ KCs in the mouse liver were divided into CD68+ subsets with phagocytic activity and CD11b+ subsets with cytokine generation capability (58). KCs preferentially induce tolerogenic immunity under noninflammatory conditions and perceive the condition of liver tissue, and their response to environmental changes plays an essential role in the pathogenesis of liver diseases (59). In the development of alcohol-related liver disease, KCs induce oxidative stress and inflammation in the liver and promote the progression of alcohol-related liver disease by participating in reactive oxygen species (ROS) production and activating pathways leading to cytokine and chemokine production. The liver stellate cells activated by KCs contribute to the progression of liver fibrosis. In islet transplantation, implanted islet cells, acinar cells, and secreted soluble factors activate KCs to secrete proinflammatory cytokines, such as IL-1β and TNF-α. The inhibition of KCs may extend the survival of implanted islets (60, 61). In summary, KCs exhibit significant heterogeneity and strong plasticity under physiological and inflammatory conditions.

Monocytes are generally thought to be derived from myeloid progenitors derived from pluripotent hematopoietic stem cells in the bone marrow. Monocytes further differentiate into dendritic cells, macrophages, and osteoclasts. In humans, monocytes can be divided into the CD14hiCD16- classic subtype, which is mainly responsible for innate perception, immune response, migration, and differentiation into macrophages at the injury site, and CD14loCD16+ nonclassic monocytes, which are mainly responsible for vessel system monitoring and tissue repair. The two subtypes are analogous to the CX3CR1intCCR2+CD62L+CD43loLy6Chi inflammatory subtype and CX3CR1hiCCR2-CD62L-CD43hiLy6Clo patrolling monocytes found in mouse tissues. In addition, a small number of CD14+CD16+ "intermediate" subtype monocytes are mainly responsible for antigen presentation and cytokine secretion during the immune response and play an essential role in the inflammatory cascade, also known as transitional inflammatory monocytes (62–64). Under inflammatory conditions, such as islet transplantation within the portal vein, islets and endothelial cells release inflammatory factors such as CCR2, recruiting "classic" monocytes out of the bone marrow to the inflammatory sites and differentiating into dendritic cells and inflammatory macrophages, producing TNF, iNOS and ROS to trigger and expand the inflammatory response; "nonclassic" monocytes usually differentiate into M2 immunomodulatory phenotype macrophages while suppressing inflammation, thus promoting the vascularization of the transplanted islets and allowing the graft to colonize the hepatic sinuses and survive for a long time to perform islet functions (41). As essential participants in the innate immune response, monocyte-derived macrophages are also highly plastic and show "cross-differentiation" under the influence of different environments, which has been confirmed in the inflammatory environment of different diseases (62). In islet transplantation, depleting dendritic cells derived from recipient monocytes can enhance the early graft function (65). Another study demonstrated that bone marrow-derived mononuclear cells can be cultured into spheroids, with CXCR4+CD31+ myeloid cells being the main cell components. In the islet transplantation model under the renal capsule of syngeneic mice, cotransplantation of bone marrow–derived spheroids improves the blood supply reconstruction and graft function (66). Although research on macrophages from different sources in innate immune response in

the field of islet transplantation remains limited, existing studies have provided initial insights into the influence of monocytes with different functions on islet transplantation.

4 Advances in macrophage studies in islet transplantation

Due to the importance of macrophages in the immune response to islet transplants, research on macrophages has always been a popular topic in the field of islet transplantation, and considerable progress and achievements have been made. Most of them focus on traditional drug therapy, interstitial cell coculture or cotransplantation, the optimization of islet isolation and culture methods, islet modification and engineering before transplantation, etc. The most common and effective strategy is to directly regulate the innate immune response of the recipient using traditional drugs to reduce damage to the graft caused by immune cells, including macrophages.

4.1 Drug therapies targeting macrophages in the innate immune response

Toll-like receptors (TLRs) are important sensors for innate immunity and bridges between innate and adaptive immunity (67). Mammalian TLRs that occupy the plasma membrane include those that detect microbial cell surface components, such as TLR4 (LPS), TLR5 (flagellin), and TLRs 1, 2 and 6 (bacterial lipoproteins). TLRs found in endosomes detect nucleic acids, such as TLR3 (double stranded (ds) RNA), TLR7 and 8 (single stranded (ss) RNA), and TLR9 (unmethylated CpG containing ssDNA) (68). TLR4 is a highly representative TLR, and its expression in islets is controversial. Studies have shown that TLR4 is not expressed in mouse islet β cells and that islet resident macrophages are its major source and mediate the TLR4 pathway to induce proinflammatory factor secretion in the islets (69). Other related studies suggest that TLR4 and its related molecules, myeloid differentiation protein-2 and the endotoxin receptor CD14, are expressed in islet β cells (70, 71). Regardless of the source of TLR4, the islet isolation and transplantation process can lead to the upregulation of TLR4 expression in the islets, the activation of the TLR4/MyD88 pathway, and the production of chemokines that recruit mononuclear cells and macrophages to the islets. By blocking TLR4 activation with carbon monoxide, protein transduction domain proteins, etc., inflammation and macrophage infiltration during transplantation can be suppressed (13, 14). Inhibiting the functions of the chemokines produced by islet cells can effectively reduce macrophage infiltration of the graft.

L-selectin, also known as Spiegelmers, is a new class of oligonucleotide drug. Two specific L-selectins, mNOX-E36 and NOX-A12, bind and antagonize CCL2/MCP-1 and CXCL12/SDF-1, respectively. In a syngeneic intraportal transplant mouse model, mNOX-E36 and NOX-A12 decreased the hepatic recruitment of inflammatory monocytes, CD11b+/Ly6Chi/CCR2+ cells and CD11b+/Ly6Chi/CXCR4+ cells; prevented inflammation-mediated islet destruction; and significantly improved islet function after transplantation (15).

In the islet isolation process, detrimental factors such as damage-associated molecular patterns, ROS, oxidative stress, and mitochondrial dysfunction can activate the NLRP3 inflammasome within islet cells, and the activated inflammasome cleaves caspase-1 and activates pro-IL-1β to IL-1β. IL-1β can induce macrophage recruitment to the transplant, upregulate the Fas receptor, activate the NF-κB pathway, and cause β-cell apoptosis and functional impairment. MCC950 is a specific inhibitor of the NLRP3 inflammasome that can inhibit IL-1β expression in transplant islets, the infiltration of macrophages around the islets, and fluctuations in blood glucose in the recipient (16).

Another strategy to target the proinflammatory effects of IL-1β is to block the activation of its corresponding receptor. Interleukin-1 receptor antagonist (IL-1Ra) is an endogenous IL-1 inhibitor that can bind to IL-1R1, prevent IL-1R accessory protein recruitment, and inhibit the activation of IL-1R. Human alpha1-antitrypsin (hAAT) is a serine protease inhibitor with tissue protection, anti-inflammatory, and immunoregulation activities. hAAT increased IL-1Ra expression and secretion both in primary islet and macrophages. In a mouse model of renal subcapsular islet allotransplantation, hAAT pretreatment significantly increased insulin transcription levels, while the transcription levels of IL-1β, TNF-α, and other inflammatory factors in islet grafts significantly decreased (17). Moreover, hAAT partially inhibits M1-type macrophage activation by inhibiting IFN-γ-induced STAT1 phosphorylation and iNOS production (18).

1,25-Dihydroxy vitamin D3, also known as calcitriol, is commonly converted from vitamin D3 in the human body. Its effects on the innate and adaptive immune systems are manifested as the induction of immunological tolerance and the activation of anti-inflammatory pathways. Its main functions are as follows: 1. to inhibit the synthesis of proinflammatory cytokines by monocytes and macrophages; 2. To reduce the expression of major histocompatibility complex-II class molecules on the surface of macrophages, thereby reducing the antigen presentation and T-cell stimulation ability of macrophages; and 3. to promote the polarization of macrophages from the M1 phenotype to the M2 phenotype via the VDR-PPARγ pathway. Calcitriol may prolong the survival of homologous islet grafts by reducing TNF-α/NF-kB pathway activation and macrophage recruitment in the grafts in syngeneic rat intraportal islet transplantation models (19–21).

The NF-κB signaling pathway downstream of TNFR, IL-1R, and TLR4 plays an important role in the innate immune response. Macrophages are activated through the NF-κB pathway and release proinflammatory cytokines such as TNF-α, IL-1, IL-6, and IL-12. The NF-κB signaling pathway is regulated by TNFR1, IL-1R, TLR, and other receptors, so the regulation of the NF-κB signaling pathway is the focus of research on immune tolerance after islet transplantation. Dehydroxymethylepoxyquinomicin acts as an inhibitor of NF-κB to inhibit NF-κB activation at the nuclear translocation level. It can inhibit the activation of TNF-α, IL-6, and serum high mobility complex-1 on macrophage-based immune cells through the NF-κB pathway and protect islet grafts from the injury caused by transplantation via the portal vein (22, 72).

ARA290, a pyroglutamate helix B surface peptide composed of 11 amino acids particular to EPOR-βcR without the side effects of erythropoietin (EPO), promotes endothelial cell activation and platelet reactivity. Through the activation of the EPOR-βcR/PI3K-Akt signaling pathway, ARA290 can inhibit the transcription of proinflammatory factors driven by the NF-κB pathway and the activation of macrophages; reduce the transcription levels of IL-6, MCP-1, MIP-1β, TNF-α and IL-1β in the graft; and thus prohibit the damage of proinflammatory factors on islets and the proapoptotic effect, thereby improving blood glucose levels and islet graft function (23). Similarly, the application of liraglutide, an analog of GLP-1, and teduglutide, a glucagon-like peptide-2 receptor agonist, can inhibit the expression of proinflammatory cytokines, macrophage recruitment, and M1 phenotype polarization during and after transplantation and can thus improve graft function. Its ability to polarize macrophages may be mediated by the cAMP-PKA-STAT3 signaling pathway (24–26) (Figure 2).

Currently, significant progress has been made in drug trials for macrophages. However, most experiments often use experimental drugs individually, neglecting the potential impact of current clinical immunosuppression schemes on macrophages. After years of development, the immunomodulation protocol improved by the Edmonton protocol, which includes tacrolimus and mycophenolate mofetil combined with dalizumab or baliximab, has been widely applied to islet transplantation in most transplant centers around

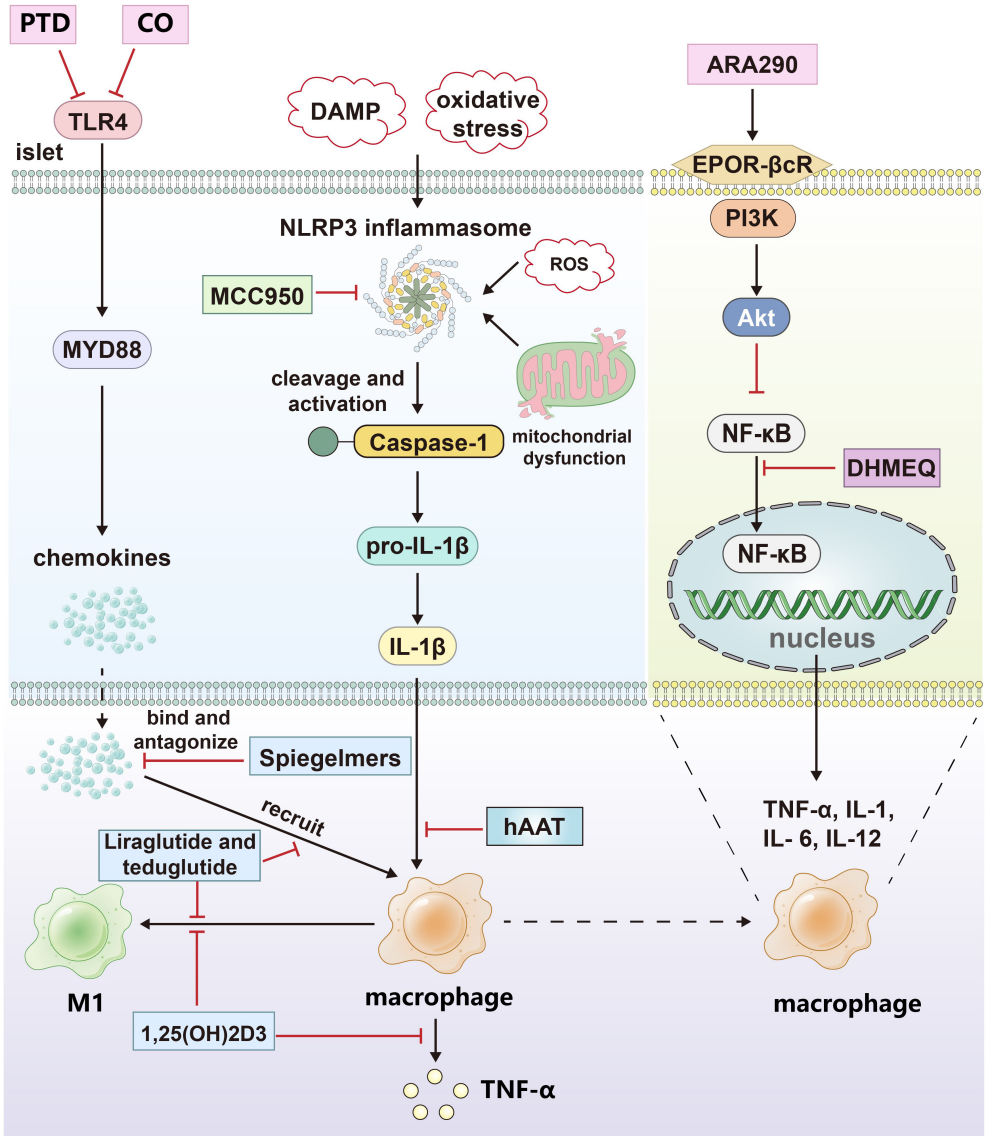

FIGURE 2
The drug treatment strategies targeting islets and macrophages. CO and protein transduction domain proteins (PTD) blocked the signal pathway of TLR4; Spiegelmers binds to and antagonizes chemokines; MCC950 inhibits the secretion of IL-1β by inhibiting NLRP3 inflammasome. hAAT inhibits the effect of IL-1β by increasing the expression and secretion of IL-1Ra in primary islets and macrophages. Calcitriol inhibited the secretion of proinflammatory factors and M1 polarization of macrophages; Dehydroxymethylepoxyquinomicin (DHMEQ) inhibited the nuclear translocation of NF-κB; ARA290 blocked NF-κB pathway by activating EPOR-βcR/PI3K-Akt signal pathway; Liraglutide and teduglutide inhibit the expression of proinflammatory factors and M1 polarization in macrophages. The black arrows indicate promotion and the red inhibitors indicate inhibition.

the world (73). As a calcineurin inhibitor, Tacrolimus can inhibit T cell activation and cytokine production. It has been found that tacrolimus inhibits the activation of the JAK2/STAT3 signal by targeting JAK2, thereby inhibiting the polarization of M2 macrophages and exerting its anti-fibrosis function (74). After human islets were transplanted into NSG mice, tacrolimus activates islet resident macrophages by inhibiting the NFAT pathway and stimulates them to produce IL-1β by increasing amyloid deposition in transplanted islets, thus inhibiting β cell function. The application of Exendin-4 reduces this effect (75). In recent years, the potential effects of mycophenolate mofetil on monocytes have also been discovered, including the inhibition of proinflammatory factor secretion and adhesion molecule expression. Baliximab can upregulate the percentage of CD14 +CD163+ monocytes (76). At present, there are few studies on the influence of existing immunomodulation schemes for islet transplantation on macrophages. Ignoring this potential influence may pose risks in clinical trials and lead to inconsistencies between therapeutic effects and animal experiments. Therefore, fully integrating drug research on macrophage immunomodulation into clinical practice is imperative.

4.2 Regulation of macrophages in islet transplantation by ECM and interstitial cells

Islet transplantation, unlike liver, kidney, and pancreas transplantation, falls more into the category of cell transplantation. Therefore, in addition to regulating the recipient's immune system, research on islets has also become a focus of immune regulation.

4.2.1 The role of ECM in immune regulation

The islet mass typically accounts for 2% of the total pancreatic mass, and a boundary composed mainly of the ECM usually exists between the endocrine islet cells and the exocrine acinus. During the islet isolation process, the digestion of enzymes can disrupt the ECM, thus destroying the structure and function of the islet. In addition, the concept of the "capsule" of the islet, which describes the stromal structures surrounding the islets, has recently arisen. There is a population of resident macrophages enriched in the peri-islet capsular area that play a barrier role in preventing the infiltration of other immune cells in the disease state, so maintaining the integrity of the interstitial structure around the islets is vital for maintaining the therapeutic efficacy of islet transplants (77).

Type IV and VI collagen proteins and laminin are essential components of the islet ECM. Matrix metalloproteases are a family of zinc-dependent endopeptidases involved in ECM turnover under several conditions. MMP-2 (gelatinase A) and MMP-9 (gelatinase B) interact with elastin and types I, III, and IV gelatins when activated, facilitating their degradation within connective tissue matrices. In islet transplantation, macrophages and neutrophils can secrete gelatinases, such as MMP-9, which can degrade ECM. The knockdown of MMP-9 and application of the MMP inhibitor captopril can significantly reduce macrophage infiltration into the islets, thus protecting the function of the graft and prolonging its survival time (27). However, another study showed that after mouse islets were transplanted into recipient muscle tissue, VEGF-A, expressed at high levels, could recruit CD11b+/Gr-1+/CXCR4hi neutrophils and induce this neutrophil subset to secrete a large amount of the effector protein MMP-9. However, islet revascularization was impaired in MMP-9-deficient mice. Thus, MMP-9 is essential for vascular reconstruction and functional integration following islet transplantation (78). Although therapeutic strategies targeting MMP-9 can reduce the degradation of ECM and the infiltration of inflammatory cells, mainly macrophages in the early stage, MMP-9 knockdown or inhibitors affect the vascularization of graft islets in the late stage. Therefore, the application of MMP-9 knockout (KO) or inhibition is highly controversial. The protection of ECM integrity and reduction of inflammatory cell infiltration without affecting the process of islet vasculogenesis remain challenging.

In addition to maintaining ECM integrity, the inhibition of the attachment of leukocyte and platelet aggregates to islets after ECM destruction is also a promising research direction. Diannexin is a recombinant homodimer of the endogenous anticoagulant molecule annexin V, which binds externalized phosphatidylserine residues on the surface of early apoptotic cells, thereby suppressing the attachment of leukocytes and platelet aggregates. In the syngeneic mouse renal subepithelial transplantation model, diannexin application reduced the recruitment of macrophages and T cells to the islet periphery, significantly reduced the mRNA expression level of the apoptotic marker Bid, reduced islet cell apoptosis, and improved the early function of islet transplants (28). Although diannexin has already undergone phase II clinical trials in kidney transplantation and achieved outstanding results in the early stage of posttransplantation, further exploration is needed to determine its efficacy after portal vein transplantation due to the graft in the portal vein comes into direct contact with a large amount of blood.

Additionally, this study did not demonstrate any advantage in using diannexin during the late stage of posttransplantation. Is this due to a reduction in macrophage recruitment during the early stage, which may potentially damage the vascularization process of the graft? The validation of this issue necessitates further investigation in subsequent studies.

4.2.2 Cotransplantation of islets and MSCs

Islet transplantation offers an additional advantage in that the islet preparation can be augmented with supplementary cells possessing immunomodulatory properties, thereby regulating the immune response following transplantation. Mesenchymal stem cells (MSCs) are self-renewing multipotent mesenchymal stromal cells that can be isolated from tissues of mesodermal origin and can differentiate into a cell lineage. MSCs have been widely used in the field of autoimmune diseases and transplantation due to their low immunogenicity and ability to affect innate and specific immune cells through the release of various immunomodulatory factors. The low immunogenicity of MSCs is due to the lack of expression of

major histocompatibility complex-II molecules on their cell surface and the lack of expression of costimulatory molecules (CD40, CD86, or CD80) important for immune recognition. Therefore, the application of allogeneic MSCs would not cause severe immune reactions in recipients (79). By far, the most prevalent source of MSCs in clinical trials is adult bone marrow, followed by adipose tissue and puerperal discards such as umbilical cord tissue and placental cells (80). In islet transplantation, MSCs can promote the polarization of mononuclear cells/macrophages to the M2 phenotype by secreting exosomes and cytokines including Indoleamine 2,3-dioxygenase (IDO), IL-4 and IL-10 (81), while inhibiting their differentiation to the M1 phenotype, reducing the production of IL-12 and blocking the maturation of dendritic cells; the ability of immature dendritic cells to present antigen will be diminished and ultimately weaken T-cell function, thus improving the survival rate of the transplanted islet (82). At present, the MSC research directions are mainly the selection of subgroups under different types and states and MSC engineering.

Adipose-derived mesenchymal stem cells (ASCs) have advantages such as easy acquisition, more extensive multipotential differentiation ability, a better immunoregulatory effect, and more secretion of angiogenic factors (83, 84). Innate immune responses are the leading cause of transplant failure for patients undergoing total pancreas resection and autologous islet transplantation. ASCs derived from chronic pancreatitis patients show no significant differences in phenotype, differentiation capacity, or secretion of growth factors compared to those derived from healthy donors. The cotransplantation of mouse islets and chronic pancreatitis-ASCs can inhibit the expression of TNF-α and Bcl-2 modifying factor. Chronic pancreatitis-ASCs mediate the expression of the graft anti-apoptotic gene TNF receptor superfamily member 11b through paracrine IGF-1 and reduce the infiltration of macrophages into the graft and β-cell death, ultimately improve islet function (29). This study has some implications for clinical islet autotransplantation because, although MSCs have low immunogenicity, they also express MHC I molecules and MHC II will be expressed under the influence of IFN-γ (85). Therefore, cotransplantation of the recipient's own ASCs would be a better choice if clinical conditions permit. More encouragingly, the clinical trial of autologous MSCs and islet cotransplantation has been initially carried out, improving transplant patients' prognosis based on sound safety (30).

Given the potent immunomodulatory effects of hAAT, the cotransplantation of hAAT-engineered mesenchymal stromal cells and islets was shown to inhibit macrophage migration, significantly reduce the infiltration of CD11c+ and F4/80+ cells, and increase the number of CD206+ cells. By transforming macrophages into a protective state favoring islet survival, hAAT-engineered mesenchymal stromal cells significantly improved the survival of cotransplanted islets (86).

However, there are limitations in the application of MSCs at present, and the protective effect of MSCs on the graft is partly achieved through direct contact with the graft and the cytokines it secretes (87). Ensuring close contact between MSCs and islets during transplantation into the recipient's liver is challenging, and there is also a risk of MSC-induced thrombosis in the liver (88). Cotransplantation of MSCs and islets under the renal capsule can solve this problem. Still, this transplantation method has the disadvantages of difficult transplantation and systemic insulin release, so it is difficult to apply to clinical work. Therefore, a team has proposed a method of carrying immunomodulatory components derived from MSCs on islet microcapsules. The MSCs-derived exosomes have the immunoregulatory ability to multiply immune cells. After being loaded on the islet microcapsule, they can exert their immunoregulatory ability on macrophages by regulating the NF-κB signaling pathway, thus alleviating pericapsular growth and fibrosis and significantly delaying the rejection of xenogeneic islets in mice recipients (31). Although this study did not adopt a portal vein transplantation model, it still provides insights into the cotransplantation of MSCs.

4.3 Optimization of islet cells

Islet transplantation is a minimally invasive organ transplantation technology. The technical key and difficulty lie in the isolation and optimization of islet cells and the development of and breakthroughs in transplantation technology.

4.3.1 Optimization of islet isolation and culture techniques

The islet preparation protocol used in clinical practice is becoming increasingly mature; however, research on its optimization is ongoing. Reducing islet damage during preparation can yield high-quality islets and minimize the innate immune response after transplantation.

4.3.1.1 Optimization of islet isolation

Islets are cell masses with a diameter of 100 to 400 μm, and the size of the islet cell mass can affect the outcome after transplantation. In one study, islets with a mean diameter of 250 μm were divided into a small islet group (mean diameter <250 μm) and a large islet group (mean diameter >250 μm). The small islet group showed higher insulin secretion and viability and lower levels of microthrombosis, inflammatory cytokine expression, and inflammatory cell infiltration after transplantation (89). Although further selection of isolated islets will waste clinical resources in the context of the shortage of donor pancreas, this study provides some enlightenment for the strategy of differentiation of islet cells by inducing iPSCs.

Islet purification is the process of separating isolated islet cells from other cells in the pancreatic parenchyma, such as exocrine acinar cells and interstitial cells, to obtain a higher purity islet cell preparation. High-purity islet cell preparation can reduce complications such as increased portal vein pressure during transplantation, the innate immune response after transplantation, and the infiltration of neutrophils and macrophages. In clinical practice, islet purification is usually carried out by continuous gradient density centrifugation using Ficoll solution with different densities and a COBE2991 centrifuge. The purification method based on iodixanol (OptiPrep) has been applied in experimental

research and clinical applications. Compared with Ficoll purification, the efficiency of islet purification by this method was not significantly different. The production of cytokines/chemokines such as IFN-γ, TNF-α, IL-1β, IL-6, IL-8, RANTES, MCP-1, and MIP-1β in the supernatants of islet preparations in the OptiPrep group was significantly reduced after 48 hours of culture (32). The reduction in cytokines/chemokines can significantly attenuate the infiltration of immune cells, such as macrophages, after transplantation.

4.3.1.2 Optimization of islet culture

Another procedure before islet transplantation is islet culturing and preparation of islet preparations, which is of particular importance for the transportation of islet preparations to interregional clinical transplant centers. On the other hand, the culture before transplantation creates conditions for the quality control of the preparation and the immune induction of the recipients before transplantation. Most importantly, a period of culture can improve purification quality, reduce the number of apoptotic cells and byproducts, and thus attenuate the innate immune response after transplantation (7).

The optimization of culture methods can further reduce the damage to isolated islets. For example, an islet culture medium with fibrin as a scaffold and perfluorodecalin as an oxygen diffusion-enhancing medium was shown to improve islet function, islet viability, and islet cell hypoxia caused by three-dimensional medium encapsulation (90). However, the islets cultured by this method caused an infiltration of macrophages around the graft in the early stage of transplantation into the portal vein of rats, which may have been caused by the attachment of matrix residues to integrins and the acceleration and enhancement of the IBMIR (91). Therefore, developing an alternative scheme to current *in vitro* islet culturing to increase the survival rate of islets while minimizing the introduction of additional antigens to reduce the infiltration of macrophages around the transplant is a promising research direction.

Adding drugs to the islet culture process to inhibit islet activation and thereby reduce macrophage recruitment after transplantation is also viable.

The complement cascade plays an amplifying role in the IBMIR, with the core step being the cleavage of C3 into C3b by the C3 convertase. This ultimately leads to the assembly of the membrane attack complex, the release of soluble C3a and C5a, and thus the activation and recruitment of inflammatory cells. APT070, also known as mirococept, is a modified fragment of complement receptor 1 (CD35) that can protect cells against complement activation. By preincubation with islets, C-peptide release, iC3b production, and C4d and C5b-9 deposition in islets embedded with thrombi were reduced *in vitro*. In a humanized mouse renal subcapsular islet transplantation model, APT070 reduced the infiltration of human CD45+ cells, macrophages (CD11b+), and neutrophils (CD66b+) into the islets (33).

Human intestinal amyloid precursor protein (hIAPP) is a 37-amino-acid peptide cosecreted with insulin by β cells (92) that can promote the recruitment of macrophages by inducing the secretion of chemokines, such as CCL2 and CXCL1, and induce macrophages to secrete proinflammatory factors such as TNF-α through the IL-1R/MyD88 pathway. hIAPP is cytotoxic and induces β-cell apoptosis through the IL-1β/Fas/caspase-8 apoptotic pathway, which is an important reason for long-term transplant failure (93, 94). In the pretransplantation culturing process, applying the IL-1Ra anakinra can reduce the formation of hIAPP, reduce macrophage infiltration and antagonize hIAPP-induced β-cell apoptosis (34). Although anakinra has been used in the clinical treatment of rheumatoid arthritis and, when combined with etanercept, can improve islet function (3), there have been clinical reports of subcutaneous amyloidosis caused by long-term subcutaneous injection of anakinra (95). Therefore, further observation is still needed to determine its long-term efficacy. Optimized islet culture techniques can reduce the infiltration of inflammatory cells such as macrophages in clinical transplantation, prolong the *in vitro* survival time of separated islets, reduce the preparation frequency of islet cells for research, and provide excellent convenience for experimental research on islet cells.

4.3.2 Related studies on the engineering of islet cells

In addition to optimizing the preparation process of islets to reduce their immunogenicity, another strategy to reduce inflammation and macrophage regulation after transplantation is to engineer islet cells.

4.3.2.1 Gene modification of islets

Gene modification technology has been widely used in the field of organ transplantation, especially in the field of xenotransplantation. Specific gene KO animal-derived islets can achieve better transplantation results in islet transplantation.

Activation transcription factor 3 (ATF3) is a stress-induced apoptotic gene whose expression is upregulated by various signals during islet isolation and transplantation, such as cytokines, nutritional deficiency, serum stimulation, and calcium signals. After transplantation, the infiltration of macrophages into the grafts was found to be significantly reduced in islets taken from ATF3 KO mice. The expression of caspase-3 and apoptotic factors (Noxa, bNIP3) in transplants was significantly reduced, and the grafts had better glucose homeostasis (35).

KO corresponds to a specific immunosuppressive gene overexpression strategy. Developmental endothelial locus-1 (Del-1) is an endothelium-derived anti-inflammatory glycoprotein that regulates β2 integrin-dependent leukocyte adhesion. In a syngeneic portal vein graft model using mice with endothelial cell-specific overexpression of Del-1 as recipients, the overexpression of Del-1 inhibited platelet-monocyte aggregate formation by the binding of the leukocyte β2-integrin Mac-1 to cognate counterreceptors on platelets, predominantly glycoprotein Ib. The infiltration of Ly6G-CD11b+ cells (monocytes) in the liver was reduced, thereby reducing the intensity of the IBMIR and protecting the islets from damage (36). Similarly, the knock-in of immunosuppressive genes is a promising way to modify islets.

Heme oxygenase-1 (HO-1) has been identified as a ubiquitous stress protein with antioxidant, anti-apoptotic and anti-

inflammatory effects and improved outcomes of islet allografts and xenografts. Soluble TNF-α type I receptor (sTNF-αR) can inhibit TNF-α-induced cell activation. An adenovirus vector was used to overexpress the fusion protein sTNF-αR-Fc/HO-1 of human HO-1, sTNF-αR, and human IgG1Fc in porcine islets. The modified porcine islets transplanted into the subrenal capsule of humanized mice significantly inhibited the infiltration of macrophages and T cells into the grafts. It also reduced the expression of RANTES, TNF-α, and IL-6 and inhibited the apoptosis of grafts (37).

Due to the shortage of donor pancreases, inducing iPSCs to differentiate into islets has become a prominent research topic. However, stem cell-derived islets' potential cytotoxicity or oncogenicity is the main problem. Additionally, for patients with autoimmune diabetes, islets differentiated from autologous stem cells are also at risk of being attacked by autoimmunity. Gene modification technology serves as an effective means to address these issues (96). In recent research, primary human islet cells can escape the killing effect of macrophages, NK cells and subsequent adaptive immune response in humanized mouse model through KO the genes encoding class I and II MHC and over express CD47. Furthermore, through gene modification of T1DM patients' iPSC-derived islets, autoimmune escape was realized in autologous, diabetic humanized mice. By blocking CD47, the islet in the recipient can be eliminated, which ensures the safety of clinical application in the future (38). This study suggests that reserving the "switch" to remove the modified cells can greatly improve the safety of gene editing technology.

4.3.2.2 The application of material chemistry in islet transplantation

With the continuous development of materials science and chemistry technology, breakthrough progress has been made in its application in the medical field, including but not limited to drug delivery and tumor targeting. Choosing a suitable material to encapsulate islet cells and isolate islets from the portal microenvironment can block the innate immune response and improve the patient's long-term dependence on immunosuppressants. However, the difficulty of islet microencapsulation is avoiding contact between islets and the blood while ensuring the exchange of oxygen and nutrients and allowing the hormones released by islets to enter the circulation. Another problem of microencapsulation is that although the capsule of the islet prevents the infiltration of inflammatory cells such as macrophages into the graft, the capsule itself as a foreign body will also cause the infiltration of inflammatory cells, and long-term macrophage infiltration will cause fibrosis around the capsule. The capsule structure itself is also an obstacle to vascularizing the islet. The hypovascularization of the graft will interfere with the local clearance of hIAPP. The presence of hIAPP in the encapsulated transplant will lead to graft failure (97).

In previous studies, alginate has always been a focal point in the research on microcapsule materials due to its excellent biocompatibility and ease of preparation. However, the variability in alginate production results in inconsistent endotoxin content and purity, which can affect its biocompatibility as microcapsules (98). Moreover, microcapsules formed by alginate-based hydrogels will allow unnecessary cells to enter and exit due to their intrinsic softness and open network structure. In contrast, microcapsules made of traditional polymers such as polytetrafluoroethylene or polycaprolactone can effectively prevent cell escape but may lead to pericapsular fibrosis (99). Unlike the microcapsules made from the materials mentioned above, thermoplastic polyurethane-based nanofiber capsules can minimize foreign body reactions and block unwanted macrophage activation (39). Another advantage of nano-materials is that the shape of microcapsules, the size of pores and the density of pores can be designed. Generally, globular proteins have a diameter ranging from 2 nm to 10 nm, while organic metabolites have a diameter between 0.5 nm and 1 nm. Immune cells like macrophages and leukocytes are approximately 6-10 microns in diameter; therefore, microcapsules with a pore size of 20 nm can maintain cell function and reduce essential immune components (100). However, the potential cytotoxicity of nano-material microcapsules is a problem that cannot be ignored, and further large-scale non-human primate experiments and clinical trials are necessary to verify their safety (101).

Another strategy for islet encapsulation is drug loading of the capsule. Dexamethasone (Dex) is an immunosuppressive glucocorticoid that effectively inhibits inflammatory pathways and can polarize human blood-derived monocytes to the M2 phenotype while preserving their migratory function. However, in contrast to other organ transplants, higher concentrations of Dex in islet transplants severely impair cell mobility and lead to impaired engraftment and angiogenesis. It also impairs the glucose responsiveness of β cells. A polydimethylsiloxane scaffold equipped with Dex can locally deliver immunoregulatory Dex in a controlled manner and polarize macrophages into the M2 phenotype without affecting islet function, thus creating a protective microenvironment for transplanted islets (102). Similarly, the transplantation of dexamethasone 21-phosphate (Dexa)-containing chitosan-coated alginate microencapsulated porcine islets into the enterocoelia of diabetic mice can reduce macrophage-dominant inflammatory cell infiltration and pericapsular fibrosis (40). These results suggest that microencapsulated islet transplantation strategies have great potential in islet xenotransplantation.

In recent years, bilirubin, as the end product of heme metabolism, has been found to inhibit the infiltration of KCs into transplanted islets in the liver (103). On the other hand, bilirubin can activate the Nrf2 pathway to polarize macrophages to the M2 phenotype and inhibit the NF-κB pathway to inhibit M1 polarization and enhance the function of M2 macrophages, thus playing an antioxidant and anti-inflammatory role. ϵ-Polylysine-bilirubin conjugate-encapsulated islets can effectively promote the polarization of macrophages to the M2 phenotype, optimize the immune microenvironment for islet survival and function, and maintain the normal blood glucose level of recipients for more than 35 days (41).

IDO is a cytosolic, heme-containing enzyme that catalyzes the first and rate-limiting step in metabolizing the essential amino acid L-tryptophan to N-formyl kynurenine. Its high expression is one of the causes of tumor immune escape, and its immunoprotective effect on T-cell-mediated allogeneic rejection has been widely

studied in organ transplantation. An islet-fibroblast composite graft composed of Indoleamine 2,3-dioxygenase-expressing fibroblast-populated collagen gel matrices and islet cells can induce tryptophan deficiency, reduce the viability of macrophages, inhibit their proinflammatory activity, and reduce the infiltration of macrophages in the graft (42). Tannic acid (TA) is a natural polyphenol with antioxidant activity that can scavenge free radicals, inhibit free radical-induced oxidation, and elicit immunomodulation. A novel cytoprotective nanothin multilayer coating for islet encapsulation consisting of TA and poly(N-vinylpyrrolidone) can reduce M1 macrophage polarization and the chemokine synthesis involved in leukocyte recruitment and increase the expression of alternatively activated M2 macrophage-associated mRNAs, such as Arg1, Retnla and Ccl17, in the graft. The frequency and cell number of Arg-1+ and CD206+ macrophages are significantly increased, alleviating islet transplantation rejection (43–45). In summary, drug encapsulation is not only used as a supplementary method for islet microencapsulation but also has the advantages of reducing the immunogenicity of the capsule material itself and delivering drugs to the target area in a localized and controlled manner, which can regulate the polarization of macrophages while minimizing the systemic adverse reactions and side effects of drugs. However, loading drugs onto microcapsules limits the total amount of drugs that can be loaded. How do we deliver drugs targeted when the drugs loaded are exhausted? Furthermore, verifying whether grafts that lose the immunomodulatory effect of drugs can maintain long-term function is necessary. Additionally, most related studies do not utilize portal vein transplantation, and different transplantation sites may result in variations in graft function (Figure 3).

5 Discussion and outlook

Islet transplantation, an emerging organ transplantation technology, solves the problem of insufficient insulin secretion

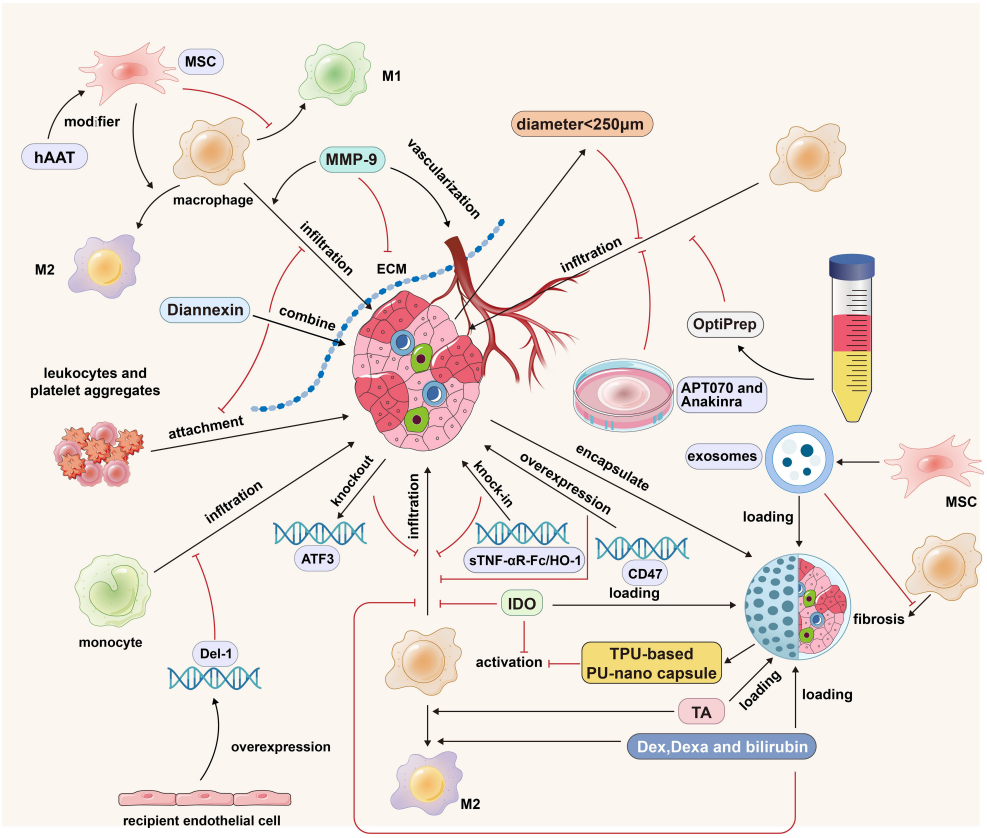

FIGURE 3
The strategies of islet cells engineering, islet isolation optimization, islet culture optimization, ECM regulation and MSCs cotransplantation in islet transplantation. MMP-9 promotes the infiltration of macrophages into the graft by degrading ECM, but it also has the function of promoting the vascularization of the graft; Diannexin inhibits the adhesion of leukocytes and platelet aggregates by binding to the externalized phosphatidylserine residues on the islet surface; MSCs promote the polarization of M1 macrophages into M2 macrophages; The modification of hAAT strengthened the anti-inflammatory effect of MSCs; The islet diameter less than 250 microns and the purification method based on OptiPrep can reduce the expression of graft cytokines/chemokines and reduce the infiltration of immune cells; APT070 can inhibit the activation of complement system and the infiltration of immune cells; Anakinra can reduce the formation of hIAPP, thus reducing macrophage infiltration; Donor ATF3 KO, sTNF-αR-Fc/HO-1 overexpression and receptor Del-1 overexpression can reduce the infiltration of mononuclear macrophages; Thermoplastic polyurethane-based nanofiber capsules can inhibit the activation of macrophages; Microcapsule-loaded Dex/Dexa/bilirubin can promote the M2 polarization of macrophages and reduce the infiltration of macrophages into the graft. The black arrows indicate promotion and the red inhibitors indicate inhibition.

and dramatically improves the quality of life of diabetic patients. Inhibiting the proinflammatory effect of macrophages in the IBMIR directly or indirectly through different therapeutic means can significantly improve the survival rate of early grafts. However, macrophages have a "double-edged sword" role in the inflammatory response. M2 macrophages have the potential to promote the proliferation of β cells and, at the same time, play an active role in tissue repair and blood supply reconstruction of the graft (104). Simply inhibiting the infiltration of macrophages into grafts is not conducive to graft repair and the vascularization process. Regulating the M1/M2 phenotype of macrophages is the most prevalent treatment strategy currently employed, which can mitigate the detrimental effects of inflammatory responses on grafts without compromising the functional capacity of M2 macrophages. However, on the one hand, the potential inhibitory effects of this approach on subsets of cells that are beneficial to graft function remain unclear. On the other hand, many studies have confirmed the role of M2 macrophages in renal and pulmonary fibrosis. M2 macrophages promote the proliferation and activation of fibroblasts by secreting cytokines and differentiate into αSMA+ myofibroblasts through a process called macrophage-to-myofibrolast transition mediated by TGFβ1–Smad3 signaling. Therefore, it is uncertain whether regulating macrophage polarization will lead to fibrosis of transplanted islets (105–107). In the latest research, mice's kidney macrophages were divided into monocyte-derived macrophages and kidney resident macrophages by single-cell RNA sequencing technology after renal ischemia-reperfusion. More importantly, S100a9hiLy6chi monocyte subsets were successfully defined by analyzing tissues and performing RNA velocity analysis during disease progression after renal ischemia-reperfusion, which played a role in initiating and amplifying inflammatory injury throughout the acute phase of acute kidney injury, and were verified in tissue samples from clinical patients. In the mouse model, targeting this subgroup to block S100a8/a9 signaling can effectively prevent acute kidney injury caused by ischemia-reperfusion (108). Further exploration of the heterogeneity of macrophages in inflammation and the targeting of different functional subsets to obtain the most ideal immune tolerance or modulation strategies are promising research directions.

Intraportal islet transplantation is the most common route of islet transplantation in clinical practice. However, in animal experiments, there are other sites that could be used, such as the renal capsule, peritoneal cavity and anterior chamber. These sites may mitigate the IBMIR by reducing islet contact with blood. For example, for patients with T1DM and End-Stage Renal Disease, a new transplantation strategy is to transplant prevascularized islets under the renal capsule of the donor's kidney. In the non-human primate model, this strategy will not affect the renal function of the donor's kidney, and it results in better islet function compared to Intraportal transplantation and renal capsule transplantation without prevascularization. Maintaining proper islet function benefits the long-term survival of the donor kidney (109). Compared with the cotransplantation of islets and MSCs, another popular area of research in recent years is the transplantation of islets into MSC-rich tissues. Brown adipose tissue (BAT) is densely vascularized and innervated and is rich in MSCs, M2 macrophages, and immunosuppressive regulatory T cells. BAT is a potential efficacious site for islet transplantation (110, 111). Bone marrow is associated with advantages similar to those of BAT (112). Spleen, an organ rich in MSC, has unique advantages as a transplantation site. On the one hand, insulin produced by transplanted islets can flow into the portal vein through the splenic vein, which is closer to physiological insulin release profiles. On the other hand, spleen has the potential to promote islet regeneration. Although some clinical transplantation centers have performed intrasplenic islet transplantation, there is a risk of arteriovenous thrombosis and subcapsular hematoma (113). A more ideal transplantation site and microenvironment are conducive to the survival and function of the graft. Although these sites have different advantages, none can meet the characteristics of minimal trauma, rich blood supply, and an immune-tolerant environment; at the same time, their safety and effectiveness still need to be verified. Additionally, they are associated with problems such as poor oxygenation and inconsistent outcomes in rodent and large animal islet transplantation models (114). Therefore, exploring new transplantation sites still requires a significant amount of time.

The strategy of combining medical and materials chemistry/engineering techniques can obtain outstanding research results in the field. Islet microencapsulation technology has been a popular research direction in recent years, and the ability of systems created with this technology to carry drugs and release drugs locally highlights its superiority, especially in the field of xenogeneic islet transplantation. Such systems can significantly reduce macrophage-mediated islet destruction and protect graft function. However, pericapsular fibrotic overgrowth is the main problem in this field, and proinflammatory and anti-inflammatory macrophages may mediate this adverse reaction. Only by clarifying the action mechanism of macrophages can we further promote the application of islet microencapsulation technology in clinical work (115). Another advantage of materials chemistry as a delivery system lies in its ability to enhance drug delivery and tissue absorption, while its specific absorption by target tissues solves the side effects of some drugs when administered systemically (116). The strategy of utilizing nano-materials to target liver tissues and regulate the maturation, activation, and polarization of macrophages within the liver has lately garnered significant attention in academic circles.

With the progression of single-cell sequencing and spatial transcriptomics technologies, the identification of previously unknown cell subsets with distinct functions has become feasible. In the future, through a deeper understanding of the role of macrophages in the inflammatory response and the continuous optimization of corresponding immunomodulatory techniques, a more suitable microenvironment for graft survival and function can be constructed, and the prognosis of patients with islet transplants can be gradually improved.

Author contributions

KD: Writing – original draft. JL: Writing – original draft. JZ: Writing – review & editing. TC: Writing – original draft. HL: Writing – original draft. FL: Writing – original draft. ZL: Writing – review & editing. BG: Writing – original draft. SW: Writing – review & editing. FW: Writing – review & editing.

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Conflict of interest

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References

1. Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *New Engl J Med* (2000) 343(4):230–8. doi: 10.1056/NEJM200007273430401

2. Forbes S, Flatt AJ, Bennett D, Crookston R, Pimkova M, Birtles L, et al. The impact of islet mass, number of transplants, and time between transplants on graft function in a national islet transplant program. *Am J Transplant* (2022) 22(1):154–64. doi: 10.1111/ajt.16785

3. Marfil-Garza BA, Imes S, Verhoeff K, Hefler J, Lam A, Dajani K, et al. Pancreatic islet transplantation in type 1 diabetes: 20-year experience from a single-centre cohort in Canada. *Lancet Diabetes endocrinol* (2022) 10(7):519–32. doi: 10.1016/S2213-8587(22)00114-0

4. Figliuzzi M, Bianchi R, Cavagnini C, Lombardi R, Porretta-Serapiglia C, Lauria G, et al. Islet transplantation and insulin administration relieve long-term complications and rescue the residual endogenous pancreatic β cells. *Am J pathol* (2013) 183(5):1527–38. doi: 10.1016/j.ajpath.2013.07.032

5. Thompson DM, Meloche M, Ao Z, Paty B, Keown P, Shapiro RJ, et al. Reduced progression of diabetic microvascular complications with islet cell transplantation compared with intensive medical therapy. *Transplantation* (2011) 91(3):373–8. doi: 10.1097/TP.0b013e31820437f3

6. D'Addio F, Maffi P, Vezzulli P, Vergani A, Mello A, Bassi R, et al. Islet transplantation stabilizes hemostatic abnormalities and cerebral metabolism in individuals with type 1 diabetes. *Diabetes Care* (2014) 37(1):267–76. doi: 10.2337/dc13-1663

7. Shapiro AM, Pokrywczynska M, Ricordi C. Clinical pancreatic islet transplantation. *Nat Rev Endocrinol* (2017) 13(5):268–77. doi: 10.1038/nrendo.2016.178

8. Bennet W, Sundberg B, Groth CG, Brendel MD, Brandhorst D, Brandhorst H, et al. Incompatibility between human blood and isolated islets of Langerhans: a finding with implications for clinical intraportal islet transplantation? *Diabetes* (1999) 48(10):1907–14. doi: 10.2337/diabetes.48.10.1907

9. Naziruddin B, Iwahashi S, Kanak MA, Takita M, Itoh T, Levy MF. Evidence for instant blood-mediated inflammatory reaction in clinical autologous islet transplantation. *Am J Transplant* (2014) 14(2):428–37. doi: 10.1111/ajt.12558

10. Takaki T, Shimoda M. Pancreatic islet transplantation: toward definitive treatment for diabetes mellitus. *Global Health Med* (2020) 2(4):200–11. doi: 10.35772/ghm.2020.01057

11. Chen P, Yao F, Lu Y, Peng Y, Zhu S, Deng J, et al. Single-cell landscape of mouse islet allograft and syngeneic graft. *Front Immunol* (2022) 13:853349. doi: 10.3389/fimmu.2022.853349

12. Hu M, Hawthorne WJ, Yi S, O'Connell PJ. Cellular immune responses in islet xenograft rejection. *Front Immunol* (2022) 13:893985. doi: 10.3389/fimmu.2022.893985

13. Dong H, Zhang Y, Song L, Kim DS, Wu H, Yang L, et al. Cell-permeable peptide blocks TLR4 signaling and improves islet allograft survival. *Cell transplantation* (2016) 25(7):1319–29. doi: 10.3727/096368916X690449

14. Goldberg A, Parolini M, Chin BY, Czismadia E, Otterbein LE, Bach FH, et al. Toll-like receptor 4 suppression leads to islet allograft survival. *FASEB J* (2007) 21(11):2840–8. doi: 10.1096/fj.06-7910com

15. Citro A, Pellegrini S, Dugnani E, Eulberg D, Klussmann S, Piemonti L. CCL2/MCP-1 and CXCL12/SDF-1 blockade by L-aptamers improve pancreatic islet engraftment and survival in mouse. *Am J Transplant* (2019) 19(11):3131–8. doi: 10.1111/ajt.15518

16. Matsuoka T, Yoshimatsu G, Sakata N, Kawakami R, Tanaka T, Yamada T, et al. Inhibition of NLRP3 inflammasome by MCC950 improves the metabolic outcome of islet transplantation by suppressing IL-1β and islet cellular death. *Sci Rep* (2020) 10(1):17920. doi: 10.1038/s41598-020-74786-3

17. Abecassis A, Schuster R, Shahaf G, Ozeri E, Green R, Ochayon DE, et al. α1-antitrypsin increases interleukin-1 receptor antagonist production during pancreatic islet graft transplantation. *Cell Mol Immunol* (2014) 11(4):377–86. doi: 10.1038/cmi.2014.17

18. Gou W, Wang J, Song L, Kim DS, Cui W, Strange C, et al. Alpha-1 antitrypsin suppresses macrophage activation and promotes islet graft survival after intrahepatic islet transplantation. *Am J Transplant* (2021) 21(5):1713–24. doi: 10.1111/ajt.16342

19. Infante M, Ricordi C, Padilla N, Alvarez A, Linetsky E, Lanzoni G, et al. The role of vitamin D and omega-3 PUFAs in islet transplantation. *Nutrients* (2019) 11(12):2937. doi: 10.3390/nu11122937

20. Jiao ZZ, Li Y, Fan P, Guo J, Xue WJ, Ding XM, et al. 1,25(OH)2D3 prolongs islet graft survival by inflammatory inhibition. *Transplant Proc* (2014) 46(5):1615–20. doi: 10.1016/j.transproceed.2014.02.012

21. Zhang X, Zhou M, Guo Y, Song Z, Liu B. 1,25-dihydroxyvitamin D_3 Promotes high glucose-induced M1 macrophage switching to M2 via the VDR-PPARγ Signaling pathway. *BioMed Res Int* (2015) 2015:157834. doi: 10.1155/2015/157834

22. Kuraya D, Watanabe M, Koshizuka Y, Ogura M, Yoshida T, Asahi Y, et al. Efficacy of DHMEQ, a NF-κB inhibitor, in islet transplantation: I. HMGB1 suppression by DHMEQ prevents early islet graft damage. *Transplantation* (2013) 96(5):445–53. doi: 10.1097/TP.0b013e31829b0744

23. Watanabe M, Lundgren T, Saito Y, Cerami A, Brines M, Östenson CG, et al. A nonhematopoietic erythropoietin analogue, ARA 290, inhibits macrophage activation and prevents damage to transplanted islets. *Transplantation* (2016) 100(3):554–62. doi: 10.1097/TP.0000000000001026

24. Langlois A, Dal S, Vivot K, Mura C, Seyfritz E, Bietiger W, et al. Improvement of islet graft function using liraglutide is correlated with its anti-inflammatory properties. *Br J Pharmacol* (2016) 173(24):3443–53. doi: 10.1111/bph.13575

25. Chen J, Mei A, Wei Y, Li C, Qian H, Min X, et al. GLP-1 receptor agonist as a modulator of innate immunity. *Front Immunol* (2022) 13:997578. doi: 10.3389/fimmu.2022.997578

26. He W, Rebello OD, Henne A, Nikolka F, Klein T, Maedler K. GLP-2 is locally produced from human islets and balances inflammation through an inter-islet-immune cell crosstalk. *Front endocrinol* (2021) 12:697120. doi: 10.3389/fendo.2021.697120

27. Lingwal N, Padmasekar M, Samikannu B, Bretzel RG, Preissner KT, Linn T. Inhibition of gelatinase B (matrix metalloprotease-9) activity reduces cellular inflammation and restores function of transplanted pancreatic islets. *Diabetes* (2012) 61(8):2045–53. doi: 10.2337/db11-1143

28. Cheng EY, Sharma VK, Chang C, Ding R, Allison AC, Leeser DB, et al. Diannexin decreases inflammatory cell infiltration into the islet graft, reduces β-cell apoptosis, and improves early graft function. *Transplantation* (2010) 90(7):709–16. doi: 10.1097/TP.0b013e3181ed55d8

29. Song L, Sun Z, Kim DS, Gou W, Strange C, Dong H, et al. Adipose stem cells from chronic pancreatitis patients improve mouse and human islet survival and function. *Stem Cell Res Ther* (2017) 8(1):192. doi: 10.1186/s13287-017-0627-x

30. Wang H, Strange C, Nietert PJ, Wang J, Turnbull TL, Cloud C, et al. Autologous mesenchymal stem cell and islet cotransplantation: safety and efficacy. *Stem Cells Trans Med* (2018) 7(1):11–9. doi: 10.1002/sctm.17-0139

31. Mohammadi MR, Rodriguez SM, Luong JC, Li S, Cao R, Alshetaiwi H, et al. Exosome loaded immunomodulatory biomaterials alleviate local immune response in immunocompetent diabetic mice post islet xenotransplantation. *Commun Biol* (2021) 4 (1):685. doi: 10.1038/s42003-021-02229-4

32. Mita A, Ricordi C, Messinger S, Miki A, Misawa R, Barker S, et al. Antiproinflammatory effects of iodixanol (OptiPrep)-based density gradient purification on human islet preparations. *Cell transplantation* (2010) 19(12):1537–46. doi: 10.3727/096368910X516600

33. Xiao F, Ma L, Zhao M, Smith RA, Huang G, Jones PM, et al. APT070 (mirococept), a membrane-localizing C3 convertase inhibitor, attenuates early human islet allograft damage in *vitro* and in *vivo* in a humanized mouse model. *Br J Pharmacol* (2016) 173(3):575–87. doi: 10.1111/bph.13388

34. Park YJ, Warnock GL, Ao Z, Safikhan N, Meloche M, Asadi A, et al. Dual role of interleukin-1β in islet amyloid formation and its β-cell toxicity: Implications for type 2 diabetes and islet transplantation. *Diabetes Obes Metab* (2017) 19(5):682–94. doi: 10.1111/dom.12873

35. Zmuda EJ, Viapiano M, Grey ST, Hadley G, Garcia-Ocaña A, Hai T. Deficiency of Atf3, an adaptive-response gene, protects islets and ameliorates inflammation in a syngeneic mouse transplantation model. *Diabetologia* (2010) 53(7):1438–50. doi: 10.1007/s00125-010-1696-x

36. Kourtzelis I, Kotlabova K, Lim JH, Mitroulis I, Ferreira A, Chen LS, et al. Developmental endothelial locus-1 modulates platelet-monocyte interactions and instant blood-mediated inflammatory reaction in islet transplantation. *Thromb haemostasis* (2016) 115(4):781–8. doi: 10.1160/TH15-05-0429

37. Lee HS, Lee JG, Yeom HJ, Chung YS, Kang B, Hurh S, et al. The introduction of human heme oxygenase-1 and soluble tumor necrosis factor-α Receptor type I with human igG1 fc in porcine islets prolongs islet xenograft survival in humanized mice. *Am J Transplant* (2016) 16(1):44–57. doi: 10.1111/ajt.13467

38. Hu X, Gattis C, Olroyd AG, Friera AM, White K, Young C, et al. Human hypoimmune primary pancreatic islets avoid rejection and autoimmunity and alleviate diabetes in allogeneic humanized mice. *Sci Trans Med* (2023) 15(691):eadg5794. doi: 10.1126/scitranslmed.adg5794

39. Wang K, Hou WD, Wang X, Han C, Vuletic I, Su N, et al. Overcoming foreign-body reaction through nanotopography: Biocompatibility and immunoisolation properties of a nanofibrous membrane. *Biomaterials* (2016) 102:249–58. doi: 10.1016/j.biomaterials.2016.06.028

40. Kim MJ, Park HS, Kim JW, Lee EY, Rhee M, You YH, et al. Suppression of fibrotic reactions of chitosan-alginate microcapsules containing porcine islets by dexamethasone surface coating. *Endocrinol Metab (Seoul Korea)* (2021) 36(1):146–56. doi: 10.3803/EnM.2021.879

41. Zhao YZ, Huang ZW, Zhai YY, Shi Y, Du CC, Zhai J, et al. Polylysine-bilirubin conjugates maintain functional islets and promote M2 macrophage polarization. *Acta biomaterialia* (2021) 122:172–85. doi: 10.1016/j.actbio.2020.12.047

42. Poormasjedi-Meibod MS, Jalili RB, Hosseini-Tabatabaei A, Hartwell R, Ghahary A. Immuno-regulatory function of indoleamine 2,3 dioxygenase through modulation of innate immune responses. *PloS One* (2013) 8(8):e71044. doi: 10.1371/journal.pone.0071044

43. Pham-Hua D, Padgett LE, Xue B, Anderson B, Zeiger M, Barra JM, et al. Islet encapsulation with polyphenol coatings decreases proinflammatory chemokine synthesis and T cell trafficking. *Biomaterials* (2017) 128:19–32. doi: 10.1016/j.biomaterials.2017.03.002

44. Barra JM, Kozlovskaya V, Kharlampieva E, Tse HM. Localized immunosuppression with tannic acid encapsulation delays islet allograft and autoimmune-mediated rejection. *Diabetes* (2020) 69(9):1948–60. doi: 10.2337/db20-0248

45. Barra JM, Kozlovskaya V, Burnette KS, Banerjee RR, Fraker CA, Kharlampieva E, et al. Localized cytotoxic T cell-associated antigen 4 and antioxidant islet encapsulation alters macrophage signaling and induces regulatory and anergic T cells to enhance allograft survival. *Am J Transplant* (2023) 23(4):498–511. doi: 10.1016/j.ajt.2023.01.007

46. Delaune V, Berney T, Lacotte S, Toso C. Intraportal islet transplantation: the impact of the liver microenvironment. *Transplant Int* (2017) 30(3):227–38. doi: 10.1111/tri.12919

47. Moberg L, Olsson A, Berne C, Felldin M, Foss A, Källen R, et al. Nicotinamide inhibits tissue factor expression in isolated human pancreatic islets: implications for clinical islet transplantation. *Transplantation* (2003) 76(9):1285–8. doi: 10.1097/01.TP.0000098905.86445.0F

48. Purdy M, Obi A, Myers D, Wakefield T–. and E- selectin in venous thrombosis and non-venous pathologies. *J Thromb haemostasis: JTH* (2022) 20(5):1056–66. doi: 10.1111/jth.15689

49. Langer HF, Chavakis T. Leukocyte-endothelial interactions in inflammation. *J Cell Mol Med* (2009) 13(7):1211–20. doi: 10.1111/j.1582-4934.2009.00811.x

50. Yan LL, Ye LP, Chen YH, He SQ, Zhang CY, Mao XL, et al. The influence of microenvironment on survival of intraportal transplanted islets. *Front Immunol* (2022) 13:849580. doi: 10.3389/fimmu.2022.849580

51. Vivot K, Benahmed MA, Seyfritz E, Bietiger W, Elbayed K, Ruhland E, et al. A metabolomic approach ((1)H HRMAS NMR spectroscopy) supported by histology to study early posttransplantation responses in islet-transplanted livers. *Int J Biol Sci* (2016) 12(10):1168–80. doi: 10.7150/ijbs.15189

52. Sironi JJ, Ouchi T. STAT1-induced apoptosis is mediated by caspases 2, 3, and 7. *J Biol Chem* (2004) 279(6):4066–74. doi: 10.1074/jbc.M307774200

53. Li F, Mahato RI. RNA interference for improving the outcome of islet transplantation. *Advanced Drug delivery Rev* (2011) 63(1-2):47–68. doi: 10.1016/j.addr.2010.11.003

54. Westwell-Roper CY, Ehses JA, Verchere CB. Resident macrophages mediate islet amyloid polypeptide-induced islet IL-1β production and β-cell dysfunction. *Diabetes* (2014) 63(5):1698–711. doi: 10.2337/db13-0863

55. Chittezhath M, Gunaseelan D, Zheng X, Hasan R, Tay VSY, Lim ST, et al. Islet macrophages are associated with islet vascular remodeling and compensatory hyperinsulinemia during diabetes. *Am J Physiol Endocrinol Metab* (2019) 317(6): E1108–e20. doi: 10.1152/ajpendo.00248.2019

56. Zakharov PN, Hu H, Wan X, Unanue ER. Single-cell RNA sequencing of murine islets shows high cellular complexity at all stages of autoimmune diabetes. *J Exp Med* (2020) 217(6):e20192362. doi: 10.1084/jem.20192362

57. MacParland SA, Liu JC, Ma XZ, Innes BT, Bartczak AM, Gage BK, et al. Single cell RNA sequencing of human liver reveals distinct intrahepatic macrophage populations. *Nat Commun* (2018) 9(1):4383. doi: 10.1038/s41467-018-06318-7

58. Kinoshita M, Uchida T, Sato A, Nakashima M, Nakashima H, Shono S, et al. Characterization of two F4/80-positive Kupffer cell subsets by their function and phenotype in mice. *J hepatol* (2010) 53(5):903–10. doi: 10.1016/j.jhep.2010.04.037

59. Bennett H, Troutman TD, Sakai M, Glass CK. Epigenetic regulation of kupffer cell function in health and disease. *Front Immunol* (2020) 11:609618. doi: 10.3389/fimmu.2020.609618

60. Auer VJ, Janas E, Ninichuk V, Eppler E, Weiss TS, Kirchner S, et al. Extracellular factors and immunosuppressive drugs influencing insulin secretion of murine islets. *Clin Exp Immunol* (2012) 170(2):238–47. doi: 10.1111/j.1365-2249.2012.04645.x

61. Bottino R, Fernandez LA, Ricordi C, Lehmann R, Tsan MF, Oliver R, et al. Transplantation of allogeneic islets of Langerhans in the rat liver: effects of macrophage depletion on graft survival and microenvironment activation. *Diabetes* (1998) 47 (3):316–23. doi: 10.2337/diabetes.47.3.316

62. Yang J, Zhang L, Yu C, Yang XF, Wang H. Monocyte and macrophage differentiation: circulation inflammatory monocyte as biomarker for inflammatory diseases. *biomark Res* (2014) 2(1):1. doi: 10.1186/2050-7771-2-1

63. Cossarizza A, Chang HD, Radbruch A, Abrignani S, Addo R, Akdis M, et al. Guidelines for the use of flow cytometry and cell sorting in immunological studies (third edition). *Eur J Immunol* (2021) 51(12):2708–3145. doi: 10.1002/eji.202170126

64. Almubarak A, Tanagala KKK, Papapanou PN, Lalla E, Momen-Heravi F. Disruption of monocyte and macrophage homeostasis in periodontitis. *Front Immunol* (2020) 11:330. doi: 10.3389/fimmu.2020.00330

65. Chow KV, Carrington EM, Zhan Y, Lew AM, Sutherland RM. Monocyte-derived dendritic cells impair early graft function following allogeneic islet transplantation. *Cell transplantation* (2017) 26(2):319–26. doi: 10.3727/096368916X693482

66. Oh BJ, Jin SM, Choi JM, Oh SH, Shim W, Lee MS, et al. Improved revascularization of islet grafts using an angiogenic monocyte subpopulation derived from spheroid culture of bone marrow mononuclear cells. *Am J Transplant* (2015) 15 (6):1543–54. doi: 10.1111/ajt.13157

67. Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat Immunol* (2001) 2(8):675–80. doi: 10.1038/90609

68. Fitzgerald KA, Kagan JC. Toll-like receptors and the control of immunity. *Cell* (2020) 180(6):1044–66. doi: 10.1016/j.cell.2020.02.041

69. Chittezhath M, Wai CMM, Tay VSY, Chua M, Langley SR, Ali Y. TLR4 signals through islet macrophages to alter cytokine secretion during diabetes. *J endocrinol* (2020) 247(1):87. doi: 10.1530/JOE-20-0131

70. Li M, Song L, Gao X, Chang W, Qin X. Toll-like receptor 4 on islet β cells senses expression changes in high-mobility group box 1 and contributes to the initiation of type 1 diabetes. *Exp Mol Med* (2012) 44(4):260–7. doi: 10.3858/emm.2012.44.4.021

71. Vives-Pi M, Somoza N, Fernández-Alvarez J, Vargas F, Caro P, Alba A, et al. Evidence of expression of endotoxin receptors CD14, toll-like receptors TLR4 and TLR2 and associated molecule MD-2 and of sensitivity to endotoxin (LPS) in islet beta cells. *Clin Exp Immunol* (2003) 133(2):208–18. doi: 10.1046/j.1365-2249.2003.02211.x

72. Zammit NW, Walters SN, Seeberger KL, O'Connell PJ, Korbutt GS, Grey ST. A20 as an immune tolerance factor can determine islet transplant outcomes. *JCI Insight* (2019) 4(21):e131028. doi: 10.1172/jci.insight.131028

73. Berney T, Andres A, Toso C, Majno P, Squifflet JP. mTOR inhibition and clinical transplantation: pancreas and islet. *Transplantation* (2018) 102(2S Suppl 1):S30–s1. doi: 10.1097/TP.0000000000001700

74. Liu B, Jiang Q, Chen R, Gao S, Xia Q, Zhu J, et al. Tacrolimus ameliorates bleomycin-induced pulmonary fibrosis by inhibiting M2 macrophage polarization via JAK2/STAT3 signaling. *Int Immunopharmacol* (2022) 113(Pt A):109424. doi: 10.1016/j.intimp.2022.109424

75. Dai C, Walker JT, Shostak A, Padgett A, Spears E, Wisniewski S, et al. Tacrolimus- and sirolimus-induced human β cell dysfunction is reversible and preventable. *JCI Insight* (2020) 5(1):e130770. doi: 10.1172/jci.insight.130770

76. van den Bosch TP, Kannegieter NM, Hesselink DA, Baan CC, Rowshani AT. Targeting the monocyte-macrophage lineage in solid organ transplantation. *Front Immunol* (2017) 8:153. doi: 10.3389/fimmu.2017.00153

77. Guo J, Fu W. Immune regulation of islet homeostasis and adaptation. *J Mol Cell Biol* (2020) 12(10):764–74. doi: 10.1093/jmcb/mjaa009

78. Christoffersson G, Vågesjö E, Vandooren J, Lidén M, Massena S, Reinert RB, et al. VEGF-A recruits a proangiogenic MMP-9-delivering neutrophil subset that induces angiogenesis in transplanted hypoxic tissue. *Blood* (2012) 120(23):4653–62. doi: 10.1182/blood-2012-04-421040

79. Hematti P, Kim J, Stein AP, Kaufman D. Potential role of mesenchymal stromal cells in pancreatic islet transplantation. *Transplant Rev (Orlando Fla)* (2013) 27(1):21–9. doi: 10.1016/j.trre.2012.11.003

80. Galipeau J, Sensébé L. Mesenchymal stromal cells: clinical challenges and therapeutic opportunities. *Cell Stem Cell* (2018) 22(6):824–33. doi: 10.1016/j.stem.2018.05.004

81. Barachini S, Biso L, Kolachalam S, Petrini I, Maggio R, Scarselli M, et al. Mesenchymal stem cell in pancreatic islet transplantation. *Biomedicines* (2023) 11(5):1426. doi: 10.3390/biomedicines11051426

82. Shrestha M, Nguyen TT, Park J, Choi JU, Yook S, Jeong JH. Immunomodulation effect of mesenchymal stem cells in islet transplantation. *Biomedicine pharmacother = Biomed pharmacotherapie* (2021) 142:112042. doi: 10.1016/j.biopha.2021.112042

83. Romano IR, D'Angeli F, Vicario N, Russo C, Genovese C, Lo Furno D, et al. Adipose-derived mesenchymal stromal cells: A tool for bone and cartilage repair. *Biomedicines* (2023) 11(7):1781. doi: 10.3390/biomedicines11071781

84. Szűcs D, Miklós V, Monostori T, Guba M, Kun-Varga A, Póliska S, et al. Effect of inflammatory microenvironment on the regenerative capacity of adipose-derived mesenchymal stem cells. *Cells* (2023) 12(15):1966. doi: 10.3390/cells12151966

85. Ankrum JA, Ong JF, Karp JM. Mesenchymal stem cells: immune evasive, not immune privileged. *Nat Biotechnol* (2014) 32(3):252–60. doi: 10.1038/nbt.2816

86. Gou W, Hua W, Swaby L, Cui W, Green E, Morgan KA, et al. Stem cell therapy improves human islet graft survival in mice via regulation of macrophages. *Diabetes* (2022) 71(12):2642–55. doi: 10.2337/db22-0117

87. Castro-Manrreza ME, Montesinos JJ. Immunoregulation by mesenchymal stem cells: biological aspects and clinical applications. *J Immunol Res* (2015) 2015:394917. doi: 10.1155/2015/394917

88. Oh BJ, Jin SM, Hwang Y, Choi JM, Lee HS, Kim G, et al. Highly angiogenic, nonthrombogenic bone marrow mononuclear cell-derived spheroids in intraportal islet transplantation. *Diabetes* (2018) 67(3):473–85. doi: 10.2337/db17-0705

89. Su Z, Xia J, Shao W, Cui Y, Tai S, Ekberg H, et al. Small islets are essential for successful intraportal transplantation in a diabetes mouse model. *Scandinavian J Immunol* (2010) 72(6):504–10. doi: 10.1111/j.1365-3083.2010.02466.x

90. Maillard E, Juszczak MT, Clark A, Hughes SJ, Gray DR, Johnson PR. Perfluorodecalin-enriched fibrin matrix for human islet culture. *Biomaterials* (2011) 32(35):9282–9. doi: 10.1016/j.biomaterials.2011.08.044

91. Schaschkow A, Mura C, Bietiger W, Peronet C, Langlois A, Bodin F, et al. Impact of an autologous oxygenating matrix culture system on rat islet transplantation outcome. *Biomaterials* (2015) 52:180–8. doi: 10.1016/j.biomaterials.2015.02.031

92. Kahn SE, D'Alessio DA, Schwartz MW, Fujimoto WY, Ensinck JW, Taborsky GJ Jr., et al. Evidence of cosecretion of islet amyloid polypeptide and insulin by beta-cells. *Diabetes* (1990) 39(5):634–8. doi: 10.2337/diab.39.5.634

93. Hui Q, Asadi A, Park YJ, Kieffer TJ, Ao Z, Warnock GL, et al. Amyloid formation disrupts the balance between interleukin-1β and interleukin-1 receptor antagonist in human islets. *Mol Metab* (2017) 6(8):833–44. doi: 10.1016/j.molmet.2017.05.016

94. Westwell-Roper C, Dai DL, Soukhatcheva G, Potter KJ, van Rooijen N, Ehses JA, et al. IL-1 blockade attenuates islet amyloid polypeptide-induced proinflammatory cytokine release and pancreatic islet graft dysfunction. *J Immunol (Baltimore Md: 1950)* (2011) 187(5):2755–65. doi: 10.4049/jimmunol.1002854

95. Alehashemi S, Dasari S, de Jesus AA, Cowen EW, Lee CR, Goldbach-Mansky R, et al. Anakinra-associated amyloidosis. *JAMA Dermatol* (2022) 158(12):1454–7. doi: 10.1001/jamadermatol.2022.2124

96. Triolo TM, Bellin MD. Lessons from human islet transplantation inform stem cell-based approaches in the treatment of diabetes. *Front endocrinol* (2021) 12:636824. doi: 10.3389/fendo.2021.636824

97. Bohman S, Westermark GT. Extensive amyloid formation in transplanted microencapsulated mouse and human islets. *Amyloid: Int J Exp Clin Invest* (2012) 19(2):87–93. doi: 10.3109/13506129.2012.679988

98. Dusseault J, Tam SK, Ménard M, Polizu S, Jourdan G, Yahia L, et al. Evaluation of alginate purification methods: effect on polyphenol, endotoxin, and protein contamination. *J Biomed materials Res Part A.* (2006) 76(2):243–51. doi: 10.1002/jbm.a.30541

99. Liu Q, Wang X, Chiu A, Liu W, Fuchs S, Wang B, et al. A zwitterionic polyurethane nanoporous device with low foreign-body response for islet encapsulation. *Advanced materials (Deerfield Beach Fla)* (2021) 33(39):e2102852. doi: 10.1002/adma.202102852

100. Desai T, Shea LD. Advances in islet encapsulation technologies. *Nat Rev Drug discovery* (2017) 16(5):338–50. doi: 10.1038/nrd.2016.232

101. Aljabali AA, Obeid MA, Bashatwah RM, Serrano-Aroca Á, Mishra V, Mishra Y, et al. Nano-materials and their impact on the immune system. *Int J Mol Sci* (2023) 24(3):2008. doi: 10.3390/ijms24032008

102. Jiang K, Weaver JD, Li Y, Chen X, Liang J, Stabler CL. Local release of dexamethasone from macroporous scaffolds accelerates islet transplant engraftment by promotion of anti-inflammatory M2 macrophages. *Biomaterials* (2017) 114:71–81. doi: 10.1016/j.biomaterials.2016.11.004

103. Zhu H, Wang J, Jiang H, Ma Y, Pan S, Reddy S, et al. Bilirubin protects grafts against nonspecific inflammation-induced injury in syngeneic intraportal islet transplantation. *Exp Mol Med* (2010) 42(11):739–48. doi: 10.3858/emm.2010.42.11.075

104. Vlahos AE, Cober N, Sefton MV. Modular tissue engineering for the vascularization of subcutaneously transplanted pancreatic islets. *Proc Natl Acad Sci United States America* (2017) 114(35):9337–42. doi: 10.1073/pnas.1619216114

105. Huen SC, Cantley LG. Macrophages in renal injury and repair. *Annu Rev Physiol* (2017) 79:449–69. doi: 10.1146/annurev-physiol-022516-034219

106. Tang PM, Nikolic-Paterson DJ, Lan HY. Macrophages: versatile players in renal inflammation and fibrosis. *Nat Rev Nephrol* (2019) 15(3):144–58. doi: 10.1038/s41581-019-0110-2

107. Wang Y, Zhang L, Wu GR, Zhou Q, Yue H, Rao LZ, et al. MBD2 serves as a viable target against pulmonary fibrosis by inhibiting macrophage M2 program. *Sci Adv* (2021) 7(1):eabb6075. doi: 10.1126/sciadv.abb6075

108. Yao W, Chen Y, Li Z, Ji J, You A, Jin S, et al. Single cell RNA sequencing identifies a unique inflammatory macrophage subset as a druggable target for alleviating acute kidney injury. *Advanced Sci (Weinheim Baden-Wurttemberg Germany)* (2022) 9(12):e2103675. doi: 10.1002/advs.202103675

109. Pomposelli T, Schuetz C, Wang P, Yamada K. A strategy to simultaneously cure type 1 diabetes and diabetic nephropathy by transplant of composite islet-kidney grafts. *Front endocrinol* (2021) 12:632605. doi: 10.3389/fendo.2021.632605

110. Kepple JD, Barra JM, Young ME, Hunter CS, Tse HM. Islet transplantation into brown adipose tissue can delay immune rejection. *JCI Insight* (2022) 7(4):e152800. doi: 10.1172/jci.insight.152800

111. Xu K, Xie R, Lin X, Jia J, Zeng N, Li W, et al. Brown adipose tissue: A potential site for islet transplantation. *Transplantation* (2020) 104(10):2059–64. doi: 10.1097/TP.0000000000003322

112. Cantarelli E, Melzi R, Mercalli A, Sordi V, Ferrari G, Lederer CW, et al. Bone marrow as an alternative site for islet transplantation. *Blood* (2009) 114(20):4566–74. doi: 10.1182/blood-2009-03-209973

113. Sakata N, Yoshimatsu G, Kodama S. The spleen as an optimal site for islet transplantation and a source of mesenchymal stem cells. *Int J Mol Sci* (2018) 19(5):1391. doi: 10.3390/ijms19051391

114. Lei J, Zhang A, Deng H, Yang Z, Peters CW, Lee KM, et al. Intrapleural transplantation of allogeneic pancreatic islets achieves glycemic control in a diabetic non-human primate. *Am J Transplant* (2022) 22(3):966–72. doi: 10.1111/ajt.16875

115. Vaithilingam V, Fung C, Ratnapala S, Foster J, Vaghjiani V, Manuelpillai U, et al. Characterisation of the xenogeneic immune response to microencapsulated fetal pig islet-like cell clusters transplanted into immunocompetent C57BL/6 mice. *PloS One* (2013) 8(3):e59120. doi: 10.1371/journal.pone.0059120

116. Wang J, Mao K, Cong X, Tan H, Wu C, Hu Z, et al. Nanoparticle delivery of CD40 siRNA suppresses alloimmune responses by inhibiting activation and differentiation of DCs and macrophages. *Sci Adv* (2022) 8(51):eabq3699. doi: 10.1126/sciadv.abq3699

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A brief review of the current status of pig islet xenotransplantation

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An estimated 1.5 million Americans suffer from Type I diabetes mellitus, and its incidence is increasing worldwide. Islet allotransplantation offers a treatment, but the availability of deceased human donor pancreases is limited. The transplantation of islets from gene-edited pigs, if successful, would resolve this problem. Pigs are now available in which the expression of the three known xenoantigens against which humans have natural (preformed) antibodies has been deleted, and in which several human 'protective' genes have been introduced. The transplantation of neonatal pig islets has some advantages over that of adult pig islets. Transplantation into the portal vein of the recipient results in loss of many islets from the instant blood-mediated inflammatory reaction (IBMIR) and so the search for an alternative site continues. The adaptive immune response can be largely suppressed by an immunosuppressive regimen based on blockade of the CD40/CD154 T cell co-stimulation pathway, whereas conventional therapy (e.g., based on tacrolimus) is less successful. We suggest that, despite the need for effective immunosuppressive therapy, the transplantation of 'free' islets will prove more successful than that of encapsulated islets. There are data to suggest that, in the absence of rejection, the function of pig islets, though less efficient than human islets, will be sufficient to maintain normoglycemia in diabetic recipients. Pig islets transplanted into immunosuppressed nonhuman primates have maintained normoglycemia for periods extending more than two years, illustrating the potential of this novel form of therapy.

KEYWORDS

diabetes, islets, pancreatic, nonhuman primates, pig, genetically-engineered, xenotransplantation

Introduction

Type 1 diabetes (T1D) is an autoimmune disease characterized by insulin-secreting β cell destruction by $CD4^+$ and $CD8^+$ T cells, resulting in insulin deficiency and hyperglycemia. Genetic susceptibility plays a role in the development of T1D, which is associated in part with certain human leukocyte antigens (HLA) (1). Conventional treatment of T1D includes exogenous insulin therapy, which helps reduce hyperglycemia. However, in patients with unstable ('brittle') diabetes, it is difficult to prevent life-threatening hypoglycemia or hyperglycemia, as well as late complications, e.g., retinopathy, nephropathy, vascular disease (2). Islet allotransplantation is viewed as an efficient therapy for T1D.

Studies have demonstrated that islet transplantation can significantly reduce, or eliminate, the need for daily insulin injections, marking a pivotal shift in T1D management (3). Furthermore, the enhanced quality of life, coupled with a notable reduction in diabetes-related complications, underscores the transformative potential of islet transplantation (4). By integrating detailed outcomes from relevant research, this introduction aims to illustrate the broader implications of islet transplantation, not only as a mechanism for blood sugar regulation but also to provide new solutions for the treatment of patients with T1D.

However, the shortage of pancreases from deceased human donors poses a problem of increasing need for another source of islets, which may be met by gene-edited pigs (5–7).

Indeed, xenotransplantation has immense potential for the treatment of numerous disorders and will prove to be the next great medical revolution (8). Pancreatic islet transplantation will benefit greatly from an unlimited number of gene-edited pigs. With the potential advantages of neonatal islets (see below), the transplantation of neonatal islet-like cell clusters (NICC), which will never be available in sufficient numbers from deceased human neonates, will become possible.

As there are an estimated 1.5 million patients with T1D and perhaps 30 million with type 2 diabetes in the USA alone, the number of islet transplants carried out worldwide will increase exponentially. The islet grafts will control the patient's blood glucose for long periods of time (if not permanently) without the need for daily insulin injections. Because of the ready availability of the islet-source pigs, islet re-transplantation will be possible whenever required and will be a relatively simple procedure.

History of islet xenotransplantation

Insulin deficiency can be overcome by transplanting pancreatic allo-islets (9). Early attempts, none of which succeeded, were reported in the late 19th and early 20th centuries (6). Novel insights in pancreatic islet cell biology, the development of improved methods of islet isolation (10), and the introduction of an automated approach for isolating islets from human pancreases were major steps forward (11).

In regard to islet xenotransplantation, the pig represents the most probable source of islets for various reasons (Table 1) (5). The sequence of porcine insulin differs by only a single amino acid from that of human insulin and, moreover, porcine insulin was administered to treat diabetes successfully for nearly a century before the introduction of recombinant human insulin (12).

In the realm of islet xenotransplantation, porcine C-peptide measurements serve as a critical marker for evaluating the survival and functionality of transplanted pig islets in human recipients. This test, measuring the level of C-peptide, a byproduct of insulin production, provides insights into the pancreatic beta cells' ability to produce insulin post-transplantation. Notable studies include Groth et al. (13), which marked the first human islet xenotransplantation attempt, though without significant

TABLE 1 Advantages and disadvantages of the pig as a potential source of organs and cells for humans, in contrast to the baboon in this role.

	Pig	Baboon
Availability	Unlimited	Limited
Breeding potential	Good	Poor
Period to reproductive maturity	4-8 months	3-5 years
Length of pregnancy	114 + 2 days	173-193 days
Number of offspring	5-12	1-2
Growth	Rapid (adult human size by 6 months) [a]	Slow (9 years to reach maximum size)
Size of adult organs	Adequate	Inadequate [b]
Cost of maintenance	Significantly lower	High
Anatomical similarity to humans	Close	Close
Physiological similarity to humans	Moderately close	Close
Immune system in relation to humans	Distant	Close
Knowledge of tissue typing	Considerable (in selected herds)	Limited
Necessity for blood type compatibility with humans	Probably unimportant	Important
Experience with genetic engineering	Considerable	None
Risk of transfer of infection (xenozoonosis)	Low	High
Availability of designated pathogen-free animals	Yes	No
Public opinion	More in favor	Mixed

[a]Breeds of miniature swine vary greatly in size.
[b]The size of certain organs, e.g., the heart, would be inadequate for transplantation into adult humans.

Abbreviations: HLA, human leucocyte antigen; IAPP, islet amyloid polypeptide; IBMIR, instant blood-mediated inflammatory reaction; mAb, monoclonal antibody; NICC, neonatal islet-like cell clusters; NHP, nonhuman primate; SLA, swine leukocyte antigen; T1D, type 1 diabetes; WT, wild-type (i.e., genetically-unmodified).

improvement in glycemic control. The study by Elliott et al. (14) demonstrates the viability of pig islet xenotransplantation through C-peptide tests. The transplantation of neonatal pig islets into diabetic subjects showed a reduction in insulin dosage and an increase in serum pig C-peptide for up to two years, indicating sustained graft function. This evidence supports the potential of pig islets to survive and function in humans, offering a promising avenue for diabetes treatment by reducing insulin dependency.

Valdes-Gonzalez et al. (15) observed a reduction in insulin needs and improvements in HbA1c over time, indicating sustained functionality of transplanted islets. Wang et al. (16) and subsequent trials (17, 18) further supported these findings, demonstrating the potential of porcine islets to ameliorate diabetes management, despite varying degrees of success and the absence of long-term insulin independence in all cases.

The optimal age of the pig as a source of islets

The ideal age of the islet-source pig has been discussed for many years. Pigs can be divided into three age groups – fetal, neonatal (approximately <14 days-old), and adult (>12 weeks-old (Table 2). As fetal pig islets are not currently being considered for xenotransplantation (because of limited β-cell yield and delayed production of insulin), the choice is between adult or neonatal pigs. There are advantages and disadvantages to both (19, 20).

Adult pig pancreases provide more fully-differentiated islets that are thus able to secrete insulin immediately after transplantation (Figure 1) (6). One adult pig pancreas may yield a sufficient number of islets to control diabetes after transplantation into a diabetic patient weighing 60kg (21). However, limitations are (i) the high cost of maintaining the pig until of adequate size (at approximately 6 months of age), (ii) the difficulty and high cost of islet isolation, and (iii) poor proliferation of the islets after transplantation (22) (Table 2). Adult sows (female pigs) that have delivered more than two litters of piglets (i.e., retired breeders, usually >2 years-old and weighing >200kg), may have advantages over young adult pigs as sources of islets by providing a greater yield of high-quality islets (20). However, the cost of maintaining them for two years would be considerable.

TABLE 2 Advantages and disadvantages of neonatal and adult pig islets for clinical xenotransplantation.

	Neonatal	Adult
Isolation procedure	Simple	Difficult
Cost of islet isolation	Low	High
Islet yield/pancreas (IEQs)	25,000-50,000	200,000-500,000
Beta cells (% of islet cells)	25%	>70%
Insulin production	May be delayed	Immediate
Proliferation *in vivo*	Yes	Little/none
Tumorigenicity	Low	None
Risk of pathogen transmission	Low	Low
Cost of housing pig until islets utilized	Low	High

The advantages of neonatal islets (i.e., NICC) include (i) low cost of maintaining the piglets before pancreatectomy (<2 weeks), (ii) much simpler and reproducible NICC isolation, (iii) lower isolation costs compared to adult pig islets (22), and (iv) considerable proliferation of islets after transplantation (Table 2) (23). They may also be less susceptible to anoxic injury post-transplant. However, they have limitations – (i) a greater number is required to provide sufficient islets for a single adult human recipient, and (ii) they must be cultured to mature and re-aggregate before transplantation. Neonatal pigs can yield approximately 25,000-30,000 islets per donor pancreas. However, considering that a patient may require 10,000-20,000 porcine islet equivalents (IE)/kg for effective treatment, a 70kg patient may need as many as 25 or more piglet donors (10,000IE/kg x 70kg) (24). Nevertheless, if diabetes can be efficiently treated, this approach is justified (25).

Neonatal pigs are currently considered by many researchers as the favored age for obtaining islets for clinical use (26). The much greater costs of maintaining the pig until adulthood and of adult islet isolation may eventually prove decisive in favor of neonatal pigs as sources of islets for commercial clinical transplantation.

The optimal site for pig islet xenotransplantation

This is another topic that has been debated for many years. The portal vein/liver is presently the favored location for islet

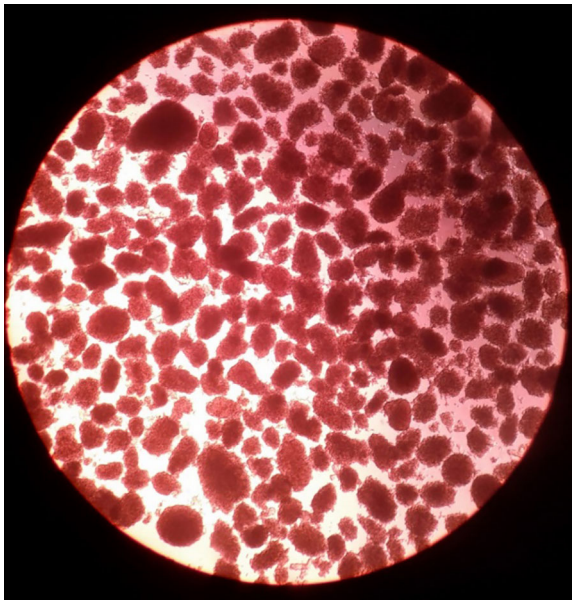

FIGURE 1
Adult pig islets after isolation. Adult pig islets stained in red with dithizone after isolation and purification (magnification 40x). (Reproduced with permission from 6).

allotransplantation (9). Nevertheless, the liver is not an optimal site for islet engraftment (27). Intraportal islet infusion increases the risk of hemorrhage and portal vein thrombosis. Furthermore, oxygen tension in the portal vein is lower than in the pancreas, which may lead to islet cell apoptosis. Most importantly, the instant blood-mediated inflammatory reaction (IBMIR – see below) may reduce the number of surviving islets by 60% within the first few hours or days (28–32). Furthermore, due to the broad distribution, biopsies of the engrafted islets are challenging and graft retrieval impossible. Alternative sites therefore continue to be explored (Table 3) (5, 26, 27). Transplant sites tested include the omental pouch, striated muscle, renal subcapsular space, the gastrointestinal submucosal space, and bone marrow.

Islet transplantation into the renal subcapsular space has shown some success in experimental models, but limited success has been reported in humans, possibly from ischemic injury associated with compression of the islets. Preclinical studies in which pig islets were successfully transplanted either under the kidney capsule of pig littermates or in an autologous setting (thus in the absence of an immune response), demonstrated islet survival and revascularization (33). The established composite islet-kidney was then transplanted into an immunosuppressed allogeneic recipient. In Major Histocompatibility Complex (MHC)-matched pigs, successful engraftment and immediate function of both the islets and kidney was reported. In a similar model, successful engraftment was also reported in an immunosuppressed nonhuman primate (NHP) model (34).

To ensure the clinical relevance of these studies, it would be essential to utilize a xenogeneic model. Now that the rejection of a pig kidney can largely be prevented (35–37; Kinoshita et al. 2024[1]), it is time to explore this approach again. The primary objective is to utilize the combined pig islet-kidney to effectively treat both renal failure and diabetes in individuals suffering from diabetic nephropathy. This would probably best be achieved by implanting pig NICC into identical piglet recipients (possibly littermates), with subsequent transplantation of the islet/kidney into the patient.

Gene editing of the islet-source pig

Quite remarkably, adult wild-type (WT, i.e., genetically-unmodified) pig islets have functioned in anti-CD154mAb-based immunosuppressed diabetic NHPs for up to 965 days (38). However, gene-editing of the pig would almost certainly have been associated with equally good or even better results with less intensive immunosuppressive therapy. Gene editing includes (i) deletion of expression of the 3 known pig carbohydrate xenoantigens (Table 4), and/or (ii) the introduction of one or more human 'protective' transgenes, e.g., complement-regulatory, coagulation-regulatory, and anti-inflammatory (anti-apoptotic) (5, 39).

Knockout of the genes for the 3 glycan xenoantigens (providing triple-knockout, [TKO] pigs) is generally considered the basis of the pigs that will be sources of organs and cells for clinical transplantation (Figure 2) (39). However, while TKO pig organs represent a significant advancement in xenotransplantation, the presence of pre-existing antibodies in Old World NHPs against these cells (Figure 3) (41) presents a complex challenge in pre-clinical studies, necessitating careful consideration and ongoing research to enhance compatibility and reduce immunological rejection.

There is evidence that the expression of protective human proteins adds to survival of pig organs or islets in NHPs. The adverse role of complement in pig islet xenotransplantation is well-known (42). The expression of one or more human complement-regulatory proteins (e.g., CD46, CD55, CD59) on the islets is therefore beneficial (43–45). In 2009, van der Windt et al. achieved insulin-independence in a diabetic monkey for >1 year by transplanting WT pig islets expressing a single human complement-regulatory protein, hCD46 (Figure 4) (43). More recently, Hawthorne and his colleagues achieved consistent long-term function of neonatal islets from GTKO pigs expressing human CD55 and CD59 in immunosuppressed baboons (46).

Expression of one or more human coagulation-regulatory proteins (e.g., thrombomodulin, endothelial cell protein C receptor [EPCR]), contributes resistance to IBMIR (47). The additional expression of a human anti-inflammatory gene (e.g., hemeoxygenase-1 [HO-1] or A20) and/or soluble human tumor necrosis factor receptor I IgG1-Fc provides some protection from the effects of inflammation (39, 48). Our group demonstrated modulation of IBMIR-mediated islet damage by employing multiple human transgenes that included complement and coagulation inhibitors. Despite reduced early islet damage, however, long-term improved outcome was not achieved (44).

There are further specific gene edits that can be made to the pig to modulate the cellular response to the islet graft, e.g., (i) insertion of a mutant (human) MHC class II transactivator gene which down-regulates swine leukocyte antigen (SLA) class II expression, (ii) deletion of expression of SLA class I (SLA class I-KO), or (iii) insertion of a CTLA4-Ig gene to induce local immunosuppression, (iv) expression of PD-L1, and (v) expression of HLA E and G (49–54).

Immunosuppressive therapy

Gene edits designed to protect against innate immunity do not prevent the adaptive immune response (cellular rejection). Exogenous pharmacological immunosuppression is therefore required to modulate the immune response.

Buhler et al. were the first to demonstrate that conventional immunosuppressive therapy, e.g., tacrolimus-based, was inefficient in suppressing the adaptive immune response to a pig xenograft, but that blockade of the CD40/CD154 T cell co-stimulation pathway was much more successful (55). This observation has since been supported by numerous studies including several involving pig islet

1 Kinoshita,K, Maenaka A, Rosales I, Karadagi A, Tomsugi T, Ayares D, et al. Novel factors potentially initiating acute antibody-mediated rejection in pig kidney xenografts despite an efficient immunosuppressive regimen. Xenotransplantation (In press) (2024).

TABLE 3 Comparison of different sites for free (non-encapsulated) islet xenotransplantation[a].

	Liver	Renal capsule	Spleen	Skin	Omentum	Gastric submucosal space	Pancreas	Muscle
Efficacy of clinical trials	Good	Poor	Not reported	Poor	Limited experience	Limited experience	Not reported	Limited experience
Patient safety	Safe	Safe	Safe	Safe	Safe	Safe	Possible pancreatitis	Safe
Oxygen tension	Low	Not reported	High	Low	Not reported	High	Not reported	Not reported
Vasculature	Rich	Poor	Not reported, but probably rich	Poor	Rich	Rich	Not reported	Rich
Site of insulin released by the graft	Liver	Not reported	Portal vein	Systemic circulation	Portal vein	Portal vein	Not reported	Systemic circulation
Surgery	Invasive, some complications	Invasive	Invasive	Non-invasive	Easy	Easy (endoscopy)	Difficult	Easy
IBMIR	Yes	Not reported	Yes	Not reported	Not reported	Not reported	Not reported	Not reported

[a]Table modified from (27).

transplantation in NHPs (38, 43, 44, 56) (Figure 5). Some induction therapy (e.g., anti-thymocyte globulin, an anti-CD20mAb, and possibly transient inhibition of systemic complement activity) appears to be essential (Table 5) (57; Kinoshita et al.[1]). Anti-CD154 mAbs have proved more effective than anti-CD40 mAbs (38, 58), but were originally associated with thrombogenic complications (59), though these were *not* seen after pig islet transplantation (60). However, current modified anti-CD154 mAbs induce no thromboembolic complications in NHPs (36, 37; Kinoshita et al.[1]).

Some immunosuppressive regimens that have proven moderately successful in pig kidney transplantation in NHPs (Table 5) may be considered too intensive for the treatment of diabetic patients receiving a pig islet transplant. A less intensive regimen may need to be developed. Park and his colleagues in South Korea succeeded in rendering diabetic monkeys insulin-independent for approximately 2 years following transplantion of adult WT pig islets using an immunosuppression protocol including anti-CD154mAb (38). When this group substituted anti-CD154mAb treatment with an anti-CD40mAb (58), they were unable to replicate these exceptional findings. Moreover, Park et al. demonstrated that a second islet infusion successfully restored normoglycemia under a clinically applicable maintenance immunosuppressive regimen, without the need for further induction therapy (61). Other co-stimulation-blockade agents, such as CTLA4-Ig, have been less efficient in protecting a xenograft (62). The use of islet transplantation from multi-transgenic pigs combined with anti-CD154 mAb-based therapy seem a promising avenue for successful engraftment.

In summary, genetic modifications in porcine islets aim to enhance insulin production and functionality but introduce complexities such as potential immunogenicity and alterations in islet physiology, impacting their viability and function. Addressing these concerns necessitates precision in gene-editing to minimize unintended effects, thorough preclinical evaluations for safety and efficacy, and adherence to ethical standards in genetic engineering. These measures are critical for advancing porcine islet xenotransplantation as a viable treatment option for diabetes, ensuring both the effectiveness and safety of genetically modified islets.

The problem and prevention of IBMIR

One of the main difficulties in porcine islet xenotransplantation is the initial inflammatory and immune reaction to the transplant – IBMIR (28–30, 32, 63, 64).

IBMIR occurs when pig islets are introduced into the portal vein, which is currently the preferred location for allotransplantation. When blood comes into contact with islets, especially xenogeneic islets, it triggers an inflammatory response that activates the complement and coagulation systems. As a result,

TABLE 4 Known carbohydrate xenoantigens expressed on pig cells.

Carbohydrate (Abbreviation)	Responsible enzyme	Gene-knockout pig
Galactose-α1,3-galactose (Gal)	α1,3-galactosyltransferase	GTKO
N-glycolylneuraminic acid (Neu5Gc)	CMAH	CMAH-KO
Sda	β-1,4N-acetylgalactosaminyltransferase	β4GalNT2-KO

CMAH, Cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMAH).

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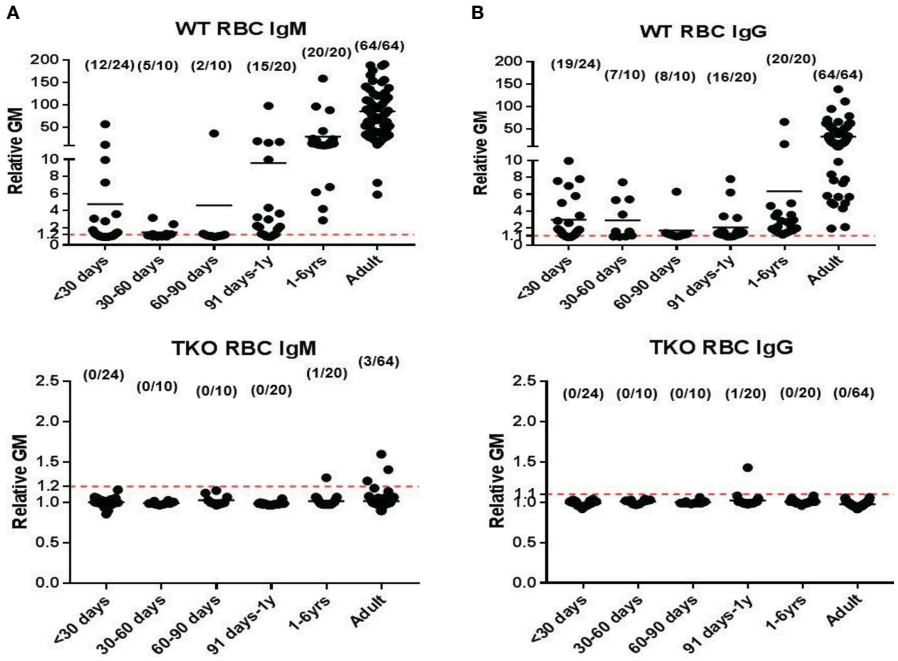

FIGURE 2
Human serum antibody binding to WT and TKO pig red blood cells (pRBCs). Correlation between human serum antibody binding to pig RBCs, by relative geometric mean [rGM]) and age. Human serum **(A)** IgM and **(B)** IgG antibody binding to wild-type (WT) pRBCs (top) and to Triple-knockout (TKO) pRBCs (bottom). The dotted lines indicate no IgM or IgG binding. (Note the great difference in the scale on the Y axis between A and B.) There is almost no anti-TKO pig antibody production during the first year of life and very low levels in adults compared to antibody against WT pig cells. (Reproduced with permission from 40).

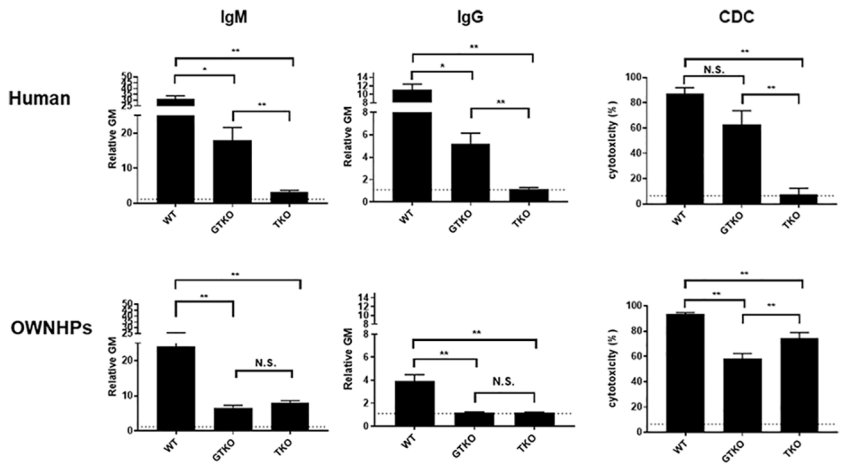

FIGURE 3
Human and Old World monkey serum antibody binding and cytotoxicity to WT, GTKO, and TKO pig peripheral blood mononuclear cells (PBMCs). Human (top) and Old World monkey (OWNHPs) (bottom) IgM (left) and IgG (middle) binding and complement-dependent cytotoxicity (CDC, at 25% serum concentration) (right) to WT, GTKO, and TKO pig PBMCs. Results are expressed as mean ± SEM. (*p<0.05, **p<0.01; N.S. = not significant). On the y axis, the dotted line represents cut-off value of binding (relative geometric mean [GM]: IgM 1.2, IgG 1.1), below which there is no binding. For CDC on the y axis, the dotted line represents cut-off value of cytotoxicity (6.4%), below which there is no cytotoxicity. (Note the difference in scale on the y axis between IgM and IgG.) Although there is reduced antibody binding and cytotoxicity to GTKO PBMCs in both humans and monkeys, there is an increase in antibody binding and cytotoxicity to TKO PBMCs in monkeys. (Reproduced with permission from 40).

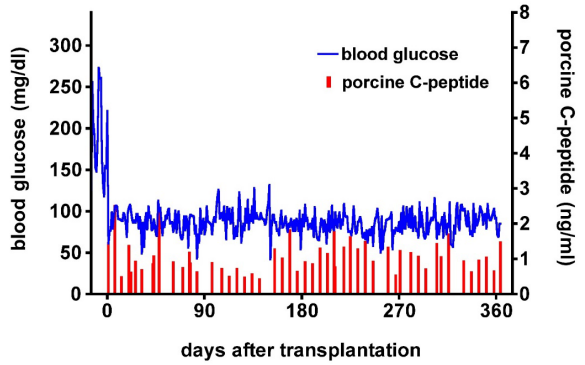

FIGURE 4
Post-transplant course of an immunosuppressed diabetic monkey following hCD46 pig islet transplantation. Blood glucose (blue) and pig C-peptide levels (red) in a streptozotocin-induced diabetic cynomolgus monkey before and after intraportal transplantation of islets from a pig expressing the human complement-regulatory protein, CD46. No exogenous insulin was administered after the transplant. The normoglycemic monkey was electively euthanized after 12 months. Day 0 = day of islet transplantation. (Reproduced with permission from 43).

the islets are quickly destroyed. One of the triggers of IBMIR is the expression of tissue factor on the islets (31, 65, 66), as well as the activation of complement and coagulation (63, 64). In addition, the binding of the host's natural anti-pig antibodies to the islets further exacerbate IBMIR-mediated damage (Figure 6). In line with these mechanistic observations, various complement inhibitors and anti-inflammatory agents have been demonstrated to modulate early islet loss (29, 31, 32), e.g., heparin, thrombin inhibitors, and anti-platelet agents (28, 31, 67, 68).

Targeting IBMIR and immune rejection seems equally important to ensure that pig islet grafts survive and function in the liver (Figure 7) (69).

The destruction of pig islet grafts by IBMIR and rapid antibody-mediated rejection are events that have similarities and differences, but they share common features (63). Many of the genetic modifications that influence IBMIR have a significant impact on reducing antibody-induced rejection (5). Downregulation of pig antigen expression, as well as transgenic expression of human complement- and coagulation-regulatory proteins have all been shown to protect organ and islet grafts (44, 70, 71). With relevance to clinical application, the genetic modifications described do not appear to impair beta cell function *in vivo* —orand *in vitro* (72, 73).

Composite transplantation of porcine islets with mesenchymal stem cells or Sertoli cells demonstrated improved islet engraftment after xenotransplantation (74–81). The mechanisms behind improved islet function are thought to be associated with the anti-inflammatory, regenerative, and immunomodulatory properties of mesenchymal stem cells and Sertoli cells.

Encapsulation

An alternative approach to protect islets from the recipient microenvironment is to physically isolate the islets by 'encapsulation. Ongoing investigations propose micro- and macro-structures that isolate the islet grafts from the host immune system, while also ensuring the provision of oxygen and nutrients to the enclosed cells and tissues (79, 82–86). Encapsulation technology in islet xenotransplantation offers the theoretical advantage of immunoprotection, potentially eliminating the need for systemic immunosuppression. It aims to create a semi-permeable barrier that shields transplanted islets from immune cells while allowing insulin, nutrients, and oxygen to pass through. However, this approach faces challenges, including the risk that the biomaterials may permit cytokine penetration, potentially triggering an immune response, and the possible insufficiency of oxygen and nutrient transport across the encapsulation barrier, which could lead to islet dysfunction or loss. These limitations underscore the need for ongoing research to optimize encapsulation materials and techniques for successful xenotransplantation outcomes.

Sensitization to HLA or to pig xenoantigens

Two important questions have arisen. The first Is whether sensitization to human leukocyte antigens (HLA) harms pig islet xenotransplantation. Blood transfusions, human organ transplants, or pregnancies can trigger the generation of antibodies directed towards HLA antigens. In these instances, if an organ or cell transplant is required, preexisting anti-HLA antibodies can pose challenges in finding a suitable human donor for organ or cell transplantation. There is evidence that anti-HLA antibodies may target some swine leukocyte antigens (SLA), due to cross-reactivity, but cross reactivity is expected to be minimal, thus unlikely negatively affecting xenotransplantation (reviewed in 87).

The second question is whether sensitization to SLA would be detrimental to subsequent human islet allotransplantation. If sensitization to a pig xenograft develops, the existing limited

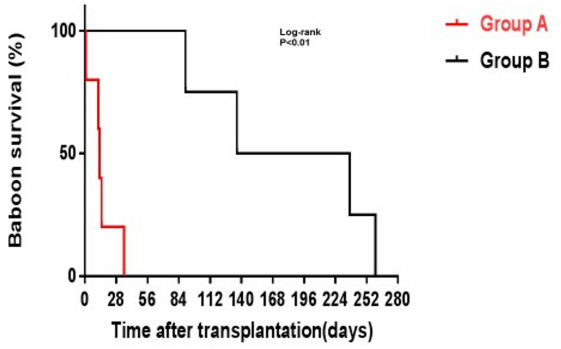

FIGURE 5
GTKO pig kidney survival in baboons receiving US FDA-approved immunosuppressive agents (Group A, in red) was much shorter than in those receiving an anti-CD40mAb-based regimen (Group B, in black). (Reproduced with permission from 56).

TABLE 5 Representative immunosuppressive and adjunctive regimen currently administered in our center to baboons with life-supporting TKO pig kidney grafts (which would be similar for TKO pig islet transplantation).

Agent	Dose (duration)
Induction	
Thymoglobulin (ATG)	5mg/kg i.v. (day -3) (to reduce the lymphocyte count to <500/mm^3)
Anti-CD20mAb (rituximab)	10mg/kg i.v. (day -2)
C1-esterase inhibitor	17.5U/kg i.v. on days 0 and 2.
Maintenance	
Anti-CD154 mAb (Tonix-1500)	30mg/kg (days 0, 2, 7, 10, 14, and weekly)
Rapamycin	0.1-0.2mg/kg i.m./day (target trough 6-12 ng/ml) beginning on day -5.
Methylprednisolone	10mg/kg/d on day 0, tapering to 0.25 mg/kg/d by day 7.
Adjunctive	
Aspirin	40mg p.o. (alternate days), beginning on day 4.
Erythropoietin	2,000 U i.v. x1-2 weekly (if Hct<30),
Anti-CMV and/or antibiotic prophylaxis when considered necessary	

information suggests that the recipient would not be at an immunological disadvantage to subsequently undergo allotransplantation (reviewed in 87).

The induction of immune tolerance

The ultimate goal of organ and cell allo- or xeno-transplantation is to induce a state in which the host immune system recognizes the transplanted pig islets as 'self' and makes no effort to reject them. Discontinuing all immunosuppressive therapy would be possible if immunologic 'tolerance' could be attained. Immune tolerance to allografts has been explored by different approaches, e.g., (i) donor-specific hematopoietic progenitor cell transplantation (chimerism) or (ii) concomitant donor-specific thymus transplantation (88). The role of regulatory cells, however, in immune tolerance remains uncertain (89).

In contrast to allotransplantation with deceased donor organs, xenotransplantation offers the advantage of elective timing of the transplant, which provide a time window for the manipulation of the host's immune system towards immune tolerance. In light of this potential advantage, if the early inflammatory events causing IBMIR, can be successfully modulated, immune tolerance might be achievable to control cellular rejection.

Improving function of porcine islets

Compared to humans, the porcine islet response to stimuli presents some differences requiring further investigation. Pigs use less insulin, need lower levels of C-peptide, and sustain higher blood glucose levels in comparison to NHPs (Table 6) (72, 73, 92). When stimulated with glucose *in vitro*, isolated porcine islets secrete 3 to 6 times less insulin than human islets (86, 93, 94). Genetic modifications aimed at enhancing islet function and

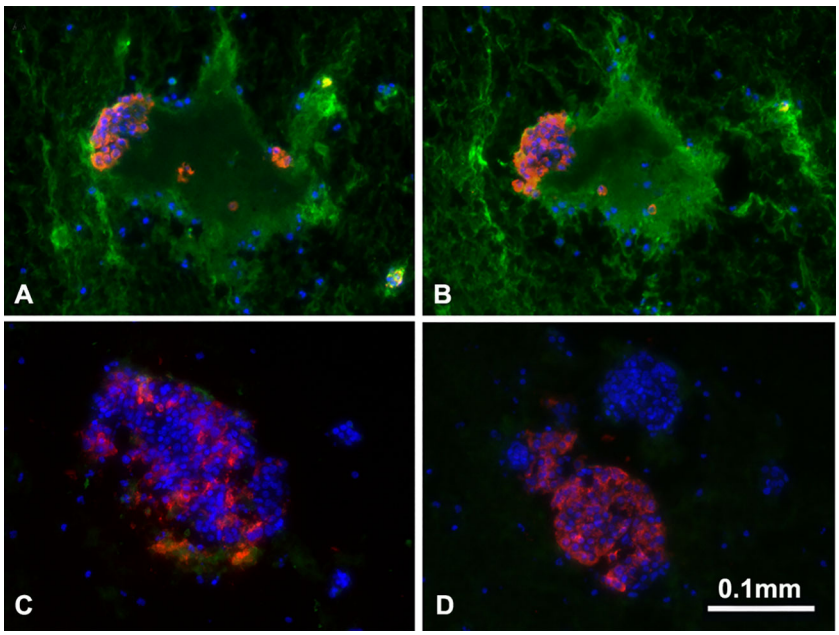

FIGURE 6
Binding of human IgM and IgG antibody to pig islets (xenogeneic) **(A, B)** and to human islets (allogeneic) **(C, D)**. IgM (green, **A, C**), IgG (green, **B, D**), insulin (red), nucleus (DAPI/blue). Yellow indicates colocalization of insulin and IgM/IgG. The greatly increased binding of human IgM and IgG to pig islets (compared to human islets) is obvious. (Reproduced with permission from 63).

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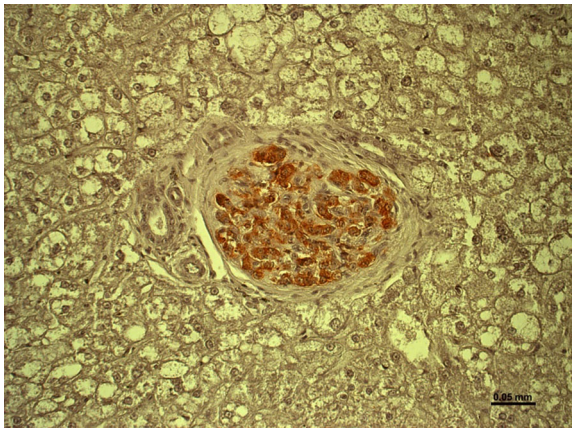

FIGURE 7
Healthy islet in the liver of an immunosuppressed cynomolgus monkey 12 months after hCD46-transgenic pig islet transplantation. (Reproduced with permission from 69).

insulin production in pig islets have been explored (95, 96). However, there is some concern that forcing insulin secretion might result in islet metabolic imbalance, and 'exhaustion' (86). To overcome this potential problem, transplantation of a greater number of islets may provide a solution.

Metabolic aspects and glucose 'counter-regulation'

The ability to control blood glucose levels within a normal range is dependent on the interaction of several factors. Endocrine hormones of the pancreas, paracrine effects, the release of neurotransmitters and neuropeptides, gluconeogenesis and glycogenolysis all play roles in maintaining blood glucose levels. These parameters differ between species, thus raising questions on the potential effects of cross-species metabolic variability in the context of xenotransplantation (92). Understanding the metabolic differences between pigs and humans, and the potential ramifications, is vital for the advancement of clinical xenotransplantation.

Parameters of metabolic control are more similar between pigs and humans than between pigs and NHPs (92). However, pigs are more glucose tolerant and have lower basal insulin levels than humans (97). Thus, metabolic control may be more easily established in pig-to-human than in pig-to-NHP islet transplantation. In response to glucose changes, both isolated neonatal and adult porcine islets demonstrate coherent insulin and glucagon secretion and suppression *in vitro*. A high concentration of glucose increases insulin secretion and inhibits glucagon secretion. Alpha cells may play a more prominent role in the response to glucose changes in pigs than in humans. Glucagon secretion is more pronounced in neonatal compared to adult pig islets (98).

Taken together, these data suggest not only that the metabolic profile of porcine islets may be similar to human islets but also that the highly efficient glucagon response to hypoglycemia may represent a clinically relevant factor predictive of timely glucose counter-regulation.

One aspect of pig metabolism that has not yet been fully explored has emerged from a genetic study aimed on the "thrifty gene hypothesis" in human populations (99). According to this hypothesis, humans have survived famine and starvation for millennia, thus certain populations may have genes that determine increased fat storage, which would facilitate survival in times of want or famine. Nonetheless, in an environment characterized by easy access to food, as in modern Western cultures, for example, such genes predispose the genetic carrier to develop type 2 diabetes. In contrast to this outlook for humans, domesticated pigs and cows have long been selectively bred for their ability to efficiently accumulate and store energy (for later consumption by humans). Pigs and cows should, therefore, be protected against the toxic effects of a "diabetogenic" environment (i.e., one that favors inactivity and energy abundance).

The mechanisms that determine this resistance to diabetes are not fully understood. However, it is known that pigs do not accumulate amyloids (100) and are, therefore, resistant to amyloidosis, which is one of the pathological hallmarks of diabetes (101). Porcine islets transplanted into mice do not accumulate amyloids, in contrast to human islets (100). Similar observations were reported when porcine islets were transplanted into NHPs (44). Sequencing of porcine islet amyloid polypeptide (IAPP, or amylin, the peptide responsible for formation of fibrils of amyloids) and comparison with human IAPP demonstrated 10 substitutions that differentiated the porcine form from the human form and contributed to reduced amyloidogenesis. Reduced toxicity of porcine IAPP was, indeed, demonstrated *in vitro* in rat (INS) cells (100).

Moreover, genetic engineering of pig donor tissues, including the introduction of human transgenes expressed under an insulin promotor, do not appear to affect glucose metabolism (72, 73, 102).

TABLE 6 Fasting blood glucose, C-peptide, insulin, and glucagon levels in cynomolgus monkeys (*Macaca fascicularis*), pigs, and humans [a].

	Cynomolgus monkeys[222a]	Pigs[222a]	Humans
Blood glucose ($mmol \cdot L^{-1}$)	2.2 – 4.1 (3.2)	4.0 – 5.2 (4.8)	3.9 – 5.6[222b]
C-peptide ($nmol \cdot L^{-1}$)	0.47 – 3.14 (1.39)	0.11 – 0.32 (0.16)	0.17 – 0.66[222c]
Insulin ($pmol \cdot L^{-1}$)	15 – 201 (109)	7 – 12 (9)	34 – 138[222c]
Glucagon ($pmol \cdot L^{-1}$)	18.7 – 179.4 (54.3)	11.3 – 13.8 (12.5)	5.7 – 28.7[222c]

Data are presented as ranges (mean). C-peptide ($p<0.001$), insulin ($p=0.021$) and glucagon ($p<0.001$) levels were significantly higher in monkeys than in pigs, while blood glucose levels were significantly ($p<0.001$) lower in monkeys. Human data are obtained from the literature and were measured in venous plasma (90, 91).
[a]Table based on Casu et al. (92).

Clinical trials of pig islet xenotransplantation

With the exception of studies by Groth et al. (13), and Wang et al. (16, 103), free islet xenotransplantation has not undergone clinical testing, though there have been several clinical experiments or trials involving encapsulated islets in the absence of immunosuppressive therapy (6). None has been totally successful. In some of these experiments it was unclear whether improved glycemic control was associated with meticulous medical management (i.e., attention to diet, glucose monitoring, and expert medical attention) rather than to insulin production by the pig islets. However, Matsumoto et al. demonstrated a substantial reduction of HbA1c levels for >600 days in recipients of encapsulated porcine islets in the absence of immunosuppressive therapy (17, 18). Minimal adverse events were reported, but improved and more consistent efficacy is still required.

Islet-source pigs will be housed in biosecure 'designated pathogen-free' facilities that eliminate most potentially-pathogenic microorganisms. By implementing Good Manufacturing Practices and established Standard Operating Procedures, the risk of transfer of a pathogenic microorganism is considered small (104–106). Although there were initial worries that porcine endogenous retroviruses (PERV) could become activated in humans, the risk, although hitherto unknown, is also thought to be small (14, 107, 108). Furthermore, if necessary, PERV-KO is possible (37, 109, 110).

Because the risk to the recipient is considered to be low, clinical trials of pig islet transplantation should possibly not be held to the high standards expected of pig organ xenotransplantation. This particularly relates to trials of encapsulated islets in which *no* immunosuppressive therapy is administered (105, 106).

According to the regulations of the U.S. Food and Drug Administration (FDA), it is required to prioritize the selection of patients who (i) suffer from a life-threatening disease with no access to effective alternative treatment, and (ii) have the potential to experience a noteworthy enhancement in their quality of life following the procedure (111). Individuals suffering from diabetes who are facing repeated and intense unawareness of hypoglycemia even after receiving the best possible medical treatment may be the most appropriate individuals to consider as potential candidates. Those with diabetic nephropathy would benefit from the successful transplantation of both a pig kidney and pig islets. The low risk in pig islet xenotransplantation trials is attributed to rigorous safety protocols, including genetic engineering of pigs to reduce human immune reactions and meticulous screening for pathogens. This approach minimizes potential zoonotic infections and immunogenic complications. Compliance with FDA regulations is ensured through adherence to established guidelines for xenotransplantation, encompassing product safety, ethical standards, and clinical trial conduct. Detailing these aspects can enhance the research's credibility, demonstrating a commitment to safety, regulatory compliance, and ethical considerations in advancing xenotransplantation as a therapeutic option. Furthermore, if the islets are rejected, this is unlikely to be life-threatening for the patient.

Potential insights from single-cell RNA sequencing

The advent of scRNA-seq has inaugurated a new era in the molecular dissection of biological processes. This technique, distinguished by its capacity to unravel the complexities of gene expression at an individual cell level, may prove pivotal in demystifying the heterogeneity inherent within cellular populations (112). This granularity of data may prove valuable in elucidating the nuanced interplays that govern both physiological and pathological states in complex biological systems.

In the realm of xenotransplantation, scRNA-seq may facilitate resolution in characterizing diverse cell types within a xenograft, encompassing the spectrum from immune cells to specialized graft cells (113). This advanced molecular profiling may afford insight into the intricacies of immune rejection mechanisms, graft tolerance phenomena, and the overarching molecular orchestration of transplantation (114). The ability of scRNA-seq to pinpoint cellular stress responses and pathophysiological transformations within xenografts may help refine transplantation strategies and prolong graft viability.

In the specific context of islet transplantation, scRNA-seq has already begun to demonstrate its potential. By dissecting the molecular heterogeneity of islet cells and delineating the complex immune interactions post-transplantation, scRNA-seq may help reshape our comprehension of graft dynamics (115). This molecular clarity may optimize immunomodulatory approaches post-transplantation and enhance overall graft efficacy.

The integration of scRNA-seq into pig islet xenotransplantation research may not only improve our understanding of transplanted islet cell biology but also pioneer novel therapeutic avenues for Type 1 diabetes.

Comment and conclusions

We anticipate that eventually pig free islet transplantation will offer a clinically-applicable therapy for patients with T1D. We suggest that this will be a preferable approach to any form of implantation of encapsulated islets, and that the intensity of immunosuppressive therapy that is required will not be prohibitive.

Porcine islets appear to be metabolically compatible with human islets, with potential advantages in glucose counter-regulation, resistance to beta cell damage, and resistance to a diabetogenic lifestyle.

Author contributions

DC: Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing. LM: Writing – original draft, Writing – review & editing, Funding acquisition. RB: Writing – original draft, Writing – review & editing.

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Conflict of interest

DKCC is a consultant to eGenesis Bio of Cambridge, MA, but the opinions expressed in this article are those of the authors, and do not necessarily reflect those of eGenesis. RB is a full-time employee of Imagine Pharma, Pittsburgh, PA, and reports no COI.

The remaining author declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. *Lancet* (2014) 383:69–82. doi: 10.1016/S0140-6736(13)60591-7.

2. Salama BF, Korbutt GS. Porcine islet xenografts: a clinical source of ß-cell grafts. *Curr Diabetes Rep* (2017) 17:14. doi: 10.1007/s11892-017-0846-7

3. Rickels MR, Robertson RP. Pancreatic islet transplantation in humans: recent progress and future directions. *Endocr Rev* (2019) 40:631–68. doi: 10.1210/er.2018-00154

4. Reid L, Baxter F, Forbes S. Effects of islet transplantation on microvascular and macrovascular complications in type 1 diabetes. *Diabetes Med* (2021) 38:e14570. doi: 10.1111/dme.14570

5. Liu Z, Hu W, He T, Dai Y, Hara H, Bottino R, et al. Pig-to-primate islet xenotransplantation: past, present, and future. *Cell Transplant* (2017) 26:925–47. doi: 10.3727/096368917X694859

6. Smood B, Bottino R, Cooper DKC. Xenotransplantation of the endocrine pancreas. In: Orlando G, Piemonti L, Ricordi C, Stratta RJ, Gruesner RWG, editors. *Transplantation, bioengineering, and regeneration of the Endocrine Pancreas*, vol. 2. Elsevier/Academic Press, San Diego (2020).

7. Mou L, Shi G, Cooper DKC, Lu Y, Chen J, Zhu S, et al. Current topics of relevance to the xenotransplantation of free pig islets. *Front Immunol* (2022) 13:854883. doi: 10.3389/fimmu.2022.854883

8. Cooper DKC, Cozzi E. The immense potential of xenotransplantation. *Eur J Transplant* (2023) 1:1630166. doi: 10.57603/EJT-272

9. Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* (2000) 343:230–8. doi: 10.1056/NEJM200007273430401.

10. Lacy PE, Kostianovsky M. Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes* (1967) 16:35–9. doi: 10.2337/diab.16.1.35.

11. Ricordi C, Lacy PE, Finke EH, Olack BJ, Scharp DW. Automated method for isolation of human pancreatic islets. *Diabetes* (1988) 3:413–20. doi: 10.2337/diabetes.37.4.413.

12. Karamanou M, Protogerou A, Tsoucalas G, Androutsos G, Poulakou-Rebelakou E. Milestones in the history of diabetes mellitus: The main contributors. *World J Diabetes* (2016) 7:1–7. doi: 10.4239/wjd.v7.i1.1.

13. Groth CG, Korsgren O, Tibell A, Tollemar J, Möller E, Bolinder J, et al. Transplantation of porcine fetal pancreas to diabetic patients. *Lancet* (1994) 344:1402–4. doi: 10.1016/S0140-6736(94)90570-3.

14. Elliott RB, Escobar L, Garkavenko O, Croxson MC, Schroeder BA, McGregor M, et al. No evidence of infection with porcine endogenous retrovirus in recipients of encapsulated porcine islet xenografts. *Cell Transplant* (2000) 9:895–901. doi: 10.1177/096368970000900616.

15. Valdés-González RA, Dorantes LM, Garibay GN, Bracho-Blanchet E, Mendez AJ, Dávila-Pérez R, et al. Xenotransplantation of porcine neonatal islets of Langerhans and Sertoli cells: a 4-year study. *Eur J Endocrinol* (2005) 153:419–27. doi: 10.1530/eje.1.01982.

16. Wang W, Mo Z, Ye B, Hu P, Liu S, Yi S. A clinical trial of xenotransplantation of neonatal pig islets for diabetic patients. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* (2011) 36:1134–40. doi: 10.3969/j.issn.1672-7347.2011.12.002

17. Matsumoto S, Tan P, Baker J, Durbin K, Tomiya M, Azuma K, et al. Clinical porcine islet xenotransplantation under comprehensive regulation. *Transpl Proc* (2014) 46:1992–5. doi: 10.1016/j.transproceed.2014.06.008.

18. Matsumoto S, Abalovich A, Wechsler C, Wynyard S, Elliott RB. Clinical benefit of islet xenotransplantation for the treatment of Type 1 diabetes. *EBioMedicine* (2016) 12:255–62. doi: 10.1016/j.ebiom.2016.08.034.

19. Bottino R, Balamurugan AN, Smetanka C, Bertera S, He J, Rood PP, et al. Isolation outcome and functional characteristics of young and adult pig pancreatic islets for transplantation studies. *Xenotransplantation* (2007) 14:74–82. doi: 10.1111/j.1399-3089.2006.00374.x.

20. Nagaraju S, Bottino R, Wijkstrom M, Trucco M, Cooper DKC. Islet xenotransplantation: what is the optimal age of the islet-source pig? *Xenotransplantation* (2015) 22:7–19. doi: 10.1111/xen.12130.

21. Kwak K, Park JK, Shim J, Ko N, Kim HJ, Lee Y, et al. Comparison of islet isolation result and clinical applicability according to GMP-grade collagenase enzyme blend in adult porcine islet isolation and culture. *Xenotransplantation* (2021) 28:e12703. doi: 10.1111/xen.12703

22. Vanderschelden R, Sathialingam M, Alexander M, Lakey JRT. Cost and scalability analysis of porcine islet isolation for islet transplantation: comparison of juvenile, neonatal and adult pigs. *Cell Transplant* (2019) 28:967–72. doi: 10.1177/0963689719847460

23. Dhanasekaran M, George JJ, Loganathan G, Narayanan S, Hughes MG, Williams SK, et al. Pig islet xenotransplantation. *Curr Opin Organ Transplant* (2017) 22:452–62. doi: 10.1097/MOT.0000000000000455

24. Ekser B, Bottino R, Cooper DKC. Clinical islet xenotransplantation: A step forward. *EBioMedicine* (2016) 12:22–3. doi: 10.1016/j.ebiom.2016.09.023.

25. Beckwith J, Nyman JA, Flanagan B, Schrover R, Schuurman HJ. A health-economic analysis of porcine islet xenotransplantation. *Xenotransplantation* (2010) 17:233–42. doi: 10.1111/xen.2010.17.issue-3.

26. Markmann JF, Bartlett ST, Johnson P, Korsgren O, Hering BJ, Scharp D, et al. Executive summary of IPITA-TTS opinion leaders report on the future of beta-cell replacement. *Transplantation* (2016) 100:e25–31. doi: 10.1097/TP.0000000000001054.

27. van der Windt DJ, Echeverri GJ, Ijzermans JN, Cooper DK. The choice of anatomical site for islet transplantation. *Cell Transplant* (2008) 17:1005–14. doi: 10.3727/096368908786991515.

28. Bennet W, Sundberg B, Groth CG, Brendel MD, Brandhorst D, Brandhorst H, et al. Incompatibility between human blood and isolated islets of Langerhans: a finding with implications for clinical intraportal islet transplantation? *Diabetes* (1999) 48:1907–14. doi: 10.2337/diabetes.48.10.1907.

29. Bennet W, Sundberg B, Lundgren T, Tibell A, Groth CG, Richards A, et al. Damage to porcine islets of Langerhans after exposure to human blood in *vitro*, or after

intraportal transplantation to cynomologus monkeys: protective effects of sCR1 and heparin. *Transplantation* (2000) 69:711–9. doi: 10.1097/00007890-200003150-00007.

30. van der Windt DJ, Bottino R, Casu A, Campanile N, Cooper DKC. Rapid loss of intraportally-transplanted islets: an overview of pathophysiology and preventive strategies. *Xenotransplantation* (2007) 14:288–97. doi: 10.1111/j.1399-3089.2007.00419.x.

31. Goto M, Tjernberg J, Dufrane D, lgue G, Brandhorst D, Ekdahl KN, et al. Dissecting the instant blood-mediated inflammatory reaction in islet xenotransplantation. *Xenotransplantation* (2008) 15:225–34. doi: 10.1111/j.1399-3089.2008.00482.x.

32. Liuwantara D, Chew YV, Favaloro EJ, Hawkes JM, Burns HL, O'Connell PJ, et al. Characterizing the mechanistic pathways of the instant blood-mediated inflammatory reaction in xenogeneic neonatal islet cell transplantation. *Transplant Direct* (2016) 2: e77. doi: 10.1097/TXD.0000000000000590.

33. Kumagai N, LaMattina JC, Kamano C, Vagefi PA, Barth RN, O'Neil JJ, et al. Vascularized islet cell transplantation in miniature swine: islet-kidney allografts correct the diabetic hyperglycemia induced by total pancreatectomy. *Diabetes* (2002) 51:3220–8. doi: 10.2337/diabetes.51.11.3220.

34. Yamada K, Hirakata A, Tchipashvili V, Shimizu A, Iwaki H, Griesemer A, et al. Composite islet-kidneys from single baboon donors cure diabetes across fully allogenic barriers. *Am J Transplant* (2011) 11:2603–12. doi: 10.1111/j.1600-6143.2011.03733.x.

35. Adams AB, Lovasik BP, Faber DA, Burlak C, Breeden C, Estrada JL, et al. Anti-C5 antibody tesidolumab reduces early antibody-mediated rejection and prolongs survival in renal xenotransplantation. *Ann Surg* (2021) 274:473–80. doi: 10.1097/SLA.0000000000004996.

36. Ma D, Hirose T, Lassiter G, Sasaki H, Rosales I, Coe TM, et al. Kidney transplantation from triple-knockout pigs expressing multiple human proteins in cynomolgus macaques. *Am J Transplant* (2021) 31:46–57. doi: 10.1111/ajt.16780

37. Anand RP, Layer JV, Heja D, Hirose T, Lassiter G, Firl DJ, et al. Design and testing of a humanized porcine donor for xenotransplantation. *Nature* (2023) 622:393–401. doi: 10.1038/s41586-023-06594-4

38. Shin JS, Kim JM, Kim JS, Min BH, Kim YH, Kim HJ, et al. Long-term control of diabetes in immunosuppressed nonhuman primates (NHP) by the transplantation of adult porcine islets. *Am J Transplant* (2015) 15:2837–50. doi: 10.1111/ajt.13345

39. Cooper DKC, Hara H, Iwase H, Yamamoto T, Li Q, Ezzelarab M, et al. Justification of specific genetic modifications in pigs for clinical kidney or heart xenotransplantation. *Xenotransplantation* (2019) 15:e12516. doi: 10.1111/xen.12516

40. Li Q, Hara H, Banks CA, Yamamoto T, Ayares D, Mauchley DC, et al. Anti-pig antibody levels in infants: can a genetically-engineered pig heart bridge to allotransplantation? *Ann Thorac Surg* (2020) 109:1268–73. doi: 10.1016/j.athoracsur.2019.08.061.

41. Yamamoto T, Hara H, Iwase H, Jagdale A, Bikhet MH, Morsi MA, et al. The final obstacle to successful preclinical xenotransplantation? *Xenotransplantation* (2020) 27: e12596. doi: 10.1111/xen.12596.

42. Zhou H, Hara H, Cooper DKC. The complex functioning of the complement system in xenotransplantation. *Xenotransplantation* (2019) 26:e12517. doi: 10.1111/xen.12517.

43. van der Windt DJ, Bottino R, Casu A, Campanile N, Smetanka C, He J, et al. Long-term controlled normoglycemia in diabetic non-human primates after transplantation with hCD46 transgenic porcine islets. *Am J Transplant* (2009) 9:2716–26. doi: 10.1111/j.1600-6143.2009.02850.x.

44. Bottino R, Wijkstrom M, van der Windt DJ, Hara H, Ezzelarab M, Murase N, et al. Pig-to-monkey islet xenotransplantation using multi-transgenic pigs. *Am J Transplant* (2014) 14:2275–87. doi: 10.1111/ajt.12868.

45. Song M, Fitch ZW, Samy KP, Martin BM, Gao Q, Davis RP, et al. Coagulation, inflammation, and CD46 transgene expression in neonatal porcine islet xenotransplantation. *Xenotransplantation* (2021) 28:e12680. doi: 10.1111/xen.12680

46. Hawthorne WJ, Salvaris EJ, Chew YV, Burns H, Hawkes J, Barlow H, et al. Xenotransplantation of genetically modified neonatal pig islets cures diabetes in baboons. *Front Immunol* (2022) 13:898948. doi: 10.3389/fimmu.2022.898948

47. Gock H, Lee KFE, Murray-Segal L, Mysore TB, d'Apice AJ, Salvaris EJ, et al. Human endothelial protein c receptor overexpression protects intraportal islet grafts in mice. *Transplant Proc* (2016) 48:2200–7. doi: 10.1016/j.transproceed.2016.02.080

48. Kim GA, Lee EM, Cho B, Alam Z, Kim SJ, Lee S, et al. Generation by somatic cell nuclear transfer of GGTA1 knockout pigs expressing soluble human TNFRI-Fc and human HO-1. *Transgenic Res* (2019) 28:91–102. doi: 10.1007/s11248-018-0103-0

49. Phelps CJ, Ball SF, Vaught TD, Vance AM, Mendicino M, Monahan JA, et al. Production and characterization of transgenic pigs expressing porcine CTLA4-Ig. *Xenotransplantation* (2009) 16:477–85. doi: 10.1111/j.1399-3089.2009.00533.x.

50. Hara H, Witt W, Crossley T, Long C, Isse K, Fan L, et al. Human dominant-negative class II transactivator transgenic pigs - effect on the human anti-pig T-cell immune response and immune status. *Immunology* (2013) 140:39–46. doi: 10.1111/imm.12107.

51. Reyes LM, Estrada JL, Wang ZY, Blosser RJ, Smith RF, Sidner RA, et al. Creating class I MHC-null pigs using guide RNA and the Cas9 endonuclease. *J Immunol* (2014) 193:5751–7. doi: 10.4049/jimmunol.1402059.

52. Iwase H, Ekser B, Satyananda V, Zhou H, Hara H, Bajona P, et al. Initial in *vivo* experience of pig artery patch transplantation in baboons using mutant MHC (CIITA-DN) pigs. *Transpl Immunol* (2015) 32:99–108. doi: 10.1016/j.trim.2015.02.003.

53. Martens GR, Reyes LM, Li P, Butler JR, Ladowski JM, Estrada JL, et al. Humoral reactivity of renal transplant-waitlisted patients to cells from GGTA1/CMAH/B4GalNT2, and SLA Class I knockout pigs. *Transplantation* (2017) 101:e86–92. doi: 10.1097/TP.0000000000001646.

54. Cross-Najafi AA, Farag K, Isidan A, Li W, Zhang W, Smith LJ, et al. Co-expression of HLA-E and HLA-G on porcone endothelial cells attenuates human NK cell-mediated cytotoxicity. *Front Immunol* (2023) 14:1217809. doi: 10.3389/fimmu.2023.1217809

55. Buhler L, Awwad M, Basker M, Gojo S, Watts A, Treter S, et al. High-dose porcine hematopoietic cell transplantation combined with CD40 ligand blockade in baboons prevents an induced anti-pig humoral response. *Transplantation* (2000) 69:2296–304. doi: 10.1097/00007890-200006150-00013.

56. Yamamoto T, Hara H, Foote J, Wang L, Li Q, Klein EC, et al. Life-supporting kidney xenotransplantation from genetically-engineered pigs in baboons: a comparison of two immunosuppressive regimens. *Transplantation* (2019) 103:2090–104. doi: 10.1097/TP.0000000000002796.

57. Bikhet MH, Iwase H, Yamamoto T, Jagdale A, Foote JB, Ezzelarab M, et al. What therapeutic regimen will be optimal for initial clinical trials of pig organ transplantation? *Transplantation* (2021) 105:1143–55. doi: 10.1097/TP0000000000003622

58. Shin JS, Kim JM, Min BH, Yoon IH, Kim HJ, Kim JS, et al. Pre-clinical results in pig-to-non-human primate islet xenotransplantation using anti-CD40 antibody (2C10R4)-based immunosuppression. *Xenotransplantation* (2018) 25:e12356. doi: 10.1111/xen.12356

59. Kawai T, Andrews D, Colvin RB, Sachs DH, Cosimi AB. Thromboembolic complications after treatment with monoclonal antibody against CD40 ligand. *Nat Med* (2000) 6:114. doi: 10.1038/72162.

60. Bottino R, Knoll MF, Graeme-Wilson J, Klein EC, Ayares D, Trucco M, et al. Safe use of anti-CD154 monoclonal antibody in pig islet xenotransplantation in monkeys. *Xenotransplantation* (2017) 24:e12283. doi: 10.1111/xen.12283

61. Kim JM, Hong SH, Shin JS, Min BH, Kim HJ, Chung H, et al. Long-term control of diabetes in a nonhuman primate by two separate transplantations of porcine adult islets under immunosuppression. *Am J Transplant* (2021) 21:3561–72. doi: 10.1111/ajt.16704.

62. Iwase H, Ekser B, Satyananda V, Bhama JK, Hara H, Ezzelarab M, et al. Pig-to-baboon heterotopic heart transplantation – exploratory preliminary experience with pigs transgenic for human thrombomodulin and comparison of three costimulation blockade-based regimens. *Xenotransplantation* (2015) 22:211–20. doi: 10.1111/xen.12167.

63. van der Windt DJ, Marigliano M, He J, Votyakova TV, Echeverri GJ, Ekser B, et al. Early islet damage after direct exposure of pig islets to blood: has humoral immunity been underestimated? *Cell Transplant* (2012) 21:1791–802. doi: 10.3727/096368912X653011.

64. Nagaraju S, Bertera S, Tanaka T, Hara H, Rayat GR, Wijkstrom M, et al. *In vitro* exposure of pig neonatal islet-like cell clusters to human blood. *Xenotransplantation* (2015) 22:317–24. doi: 10.1111/xen.12178.

65. Moberg L, Johansson H, Lukinius A, Berne C, Foss A, Källen R, et al. Production of tissue factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation. *Lancet* (2002) 360:2039–45. doi: 10.1016/S0140-6736(02)12020-4.

66. Ji M, Yi S, Smith-Hurst H, Phillips P, Wu J, Hawthorne W, et al. The importance of tissue factor expression by porcine NICC in triggering IBMIR in the xenograft setting. *Transplantation* (2011) 91:841–6. doi: 10.1097/TP.0b013e3182106091.

67. Johansson H, Goto M, Dufrane D, Siegbahn A, Elgue G, Gianello P, et al. Low molecular weight dextran sulfate: a strong candidate drug to block IBMIR in clinical islet transplantation. *Am J Transplant* (2006) 6:305–12. doi: 10.1111/j.1600-6143.2005.01186.x.

68. Cabric S, Sanchez J, Lundgren T, Foss A, Felldin M, Källen R, et al. Islet surface heparinization prevents the instant blood-mediated inflammatory reaction in islet transplantation. *Diabetes* (2007) 56:2008–15. doi: 10.2337/db07-0358.

69. Cooper DK, Ayares D. Potential benefits and risks of clinical xenotransplantation. *Transplant Res Risk Manage* (2012) 4:7–17. doi: 10.2147/TRRM.

70. Cooper DK, Ezzelarab MB, Hara H, Iwase H, Lee W, Wijkstrom M, et al. The pathobiology of pig-to-primate xenotransplantation: a historical review. *Xenotransplantation* (2016) 23:83–105. doi: 10.1111/xen.12219.

71. Cooper DKC, Pierson RN III. Milestones on the path to clinical pig organ xenotransplantation. *Am J Transplant* (2023) 23:326–35. doi: 10.1016/j.ajt.2022.12.023

72. Casu A, Echeverri GJ, Bottino R, van der Windt DJ, He J, Ekser B, et al. Insulin secretion and glucose metabolism in alpha 1,3-galactosyltransferase knock-out pigs compared to wild-type pigs. *Xenotransplantation* (2010) 17:131–9. doi: 10.1111/j.1399-3089.2010.00572.x.

73. Wijkstrom M, Bottino R, Iwase H, Hara H, Ekser B, van der Windt D, et al. Glucose metabolism in pigs expressing human genes under an insulin promoter. *Xenotransplantation* (2015) 22:70–9. doi: 10.1111/xen.12145.

74. Selawry HP, Kotb M, Herrod HG, Lu ZN. Production of a factor, or factors, suppressing IL-2 production and T cell proliferation by Sertoli cell-enriched preparations. A potential role for islet transplantation in an immunologically privileged site. *Transplantation* (1991) 52:846–50. doi: 10.1097/00007890-199111000-00018.

75. Dufour JM, Rajotte RV, Korbutt GS, Emerich DF. Harnessing the immunomodulatory properties of Sertoli cells to enable xenotransplantation in type I diabetes. *Immunol Invest* (2003) 32:275–97. doi: 10.1081/IMM-120025106.

76. Isaac JR, Skinner S, Elliot R, Salto-Tellez M, Garkavenko O, Khoo A, et al. Transplantation of neonatal porcine islets and sertoli cells into nonimmunosuppressed nonhuman primates. *Transplant Proc* (2005) 37:487–8. doi: 10.1016/j.transproceed.2004.11.062.

77. Mancuso F, Calvitti M, Luca G, Nastruzzi C, Baroni T, Mazzitelli S, et al. Acceleration of functional maturation and differentiation of neonatal porcine islet cell monolayers shortly in *vitro* cocultured with microencapsulated Sertoli cells. *Stem Cells Int* (2010) 2010:587213. doi: 10.4061/2010/587213

78. Li Y, Xue W, Liu H, Fan P, Wang X, Ding X, et al. Combined strategy of endothelial cells coating, Sertoli cells coculture and infusion improves vascularization and rejection protection of islet graft. *PloS One* (2013) 8:e56696. doi: 10.1371/journal.pone.0056696.

79. Veriter S, Gianello P, Igarashi Y, Beaurin G, Ghyselinck A, Aouassar N, et al. Improvement of subcutaneous bioartificial pancreas vascularization and function by coencapsulation of pig islets and mesenchymal stem cells in primates. *Cell Transplant* (2014) 23:1349–64. doi: 10.3727/096368913X663550.

80. Montanari E, Meier RPH, Mahou R, Seebach JD, Wandrey C, Gerber-Lemaire S, et al. Multipotent mesenchymal stromal cells enhance insulin secretion from human islets via N-cadherin interaction and prolong function of transplanted encapsulated islets in mice. *Stem Cell Res Ther* (2017) 8:199. doi: 10.1186/s13287-017-0646-7.

81. Bertera S, Knoll MF, Knoll C, Hara H, Kimbrel EA, Kouris NA, et al. Human hemangioblast-derived mesenchymal stem cells promote islet engraftment in a minimal islet mass transplantation model in mice. *Front Med (Lausanne)* (2021) 8:660877. doi: 10.3389/fmed.2021.660877

82. Elliott RB, Escobar L, Tan PL, Garkavenko O, Calafiore R, Basta P, et al. Intraperitoneal alginate-encapsulated neonatal porcine islets in a placebo-controlled study with 16 diabetic cynomolgus primates. *Transplant Proc* (2005) 37:3505–8. doi: 10.1016/j.transproceed.2005.09.038.

83. Dufrane D, Goebbels R-M, Saliez A, Guiot Y, Gianello P. Six-month survival of microencapsulated pig islets and alginate biocompatibility in primates: proof of concept. *Transplantation* (2006) 81:1345–53. doi: 10.1097/01.tp.0000208610.75997.20.

84. Dufrane D, Goebbels RM, Gianello P. Alginate macroencapsulation of pig islets allows correction of streptozotocin-induced diabetes in primates up to 6 months without immunosuppression. *Transplantation* (2010) 90:1054–62. doi: 10.1097/TP.0b013e3181f6e267.

85. Zhu HT, Lu L, Liu XY, Yu L, Lyu Y, Wang B. Treatment of diabetes with encapsulated pig islets: an update on current developments. *J Zhejiang Univ Sci B* (2015) 16:329–43. doi: 10.1631/jzus.B1400310.

86. Cooper DKC, Matsumoto S, Abalovich A, Itoh T, Mourad N, Gianello PR, et al. Progress in clinical encapsulated islet xenotransplantation. *Transplantation* (2016) 100:2301–8. doi: 10.1097/TP.0000000000001371.

87. Cooper DKC, Habibabady Z, Kinoshita K, Hara H, Pierson RN III. The respective relevance of sensitization to alloantigens and xenoantigens in pig organ xenotransplantation. *Hum Immunol* (2022) 84:18–26. doi: 10.1016/j.humimm.2022.06.003

88. Yamada K, Sykes M, Sachs DH. Tolerance in xenotransplantation. *Curr Opin Organ Transplant* (2017) 22:522–8. doi: 10.1097/MOT.0000000000000466.

89. Shin JS, Min BH, Kim JM, Kim JS, Yoon IH, Kim HJ, et al. Failure of transplantation tolerance induction by autologous regulatory T cells in the pig-to-non-human primate islet xenotransplantation model. *Xenotransplantation* (2016) 23:300–9. doi: 10.1111/xen.12246

90. Genuth S, Alberti KG, Bennett P, Buse J, Defronzo R, Kahn R, et al. Follow-up report on the diagnosis of diabetes mellitus. *Diabetes Care* (2003) 26:3160–7. doi: 10.2337/diacare.26.11.3160.

91. Greenspan FS, Gardner DG. *Normal hormone reference ranges. 7th ed.* Greenspan FS, Gardner DG, editors. New York: McGraw-Hill Companies (2006) 920–38.

92. Casu A, Bottino R, Balamurugan AN, Hara H, van der Windt DJ, Campanile N, et al. Metabolic aspects of pig-to-monkey (Macaca fascicularis) islet transplantation: implications for translation into clinical practice. *Diabetologia* (2008) 51:120–9. doi: 10.1007/s00125-007-0844-4.

93. Krickhahn M, Meyer T, Buhler C, Thiede A, Ulrichs K. Highly efficient isolation of porcine islets of Langerhans for xenotransplantation: numbers, purity, yield and in *vitro* function. *Ann Transplant* (2001) 6:48–54.

94. Mueller KR, Balamurugan AN, Cline GW, Pongratz RL, Hooper RL, Weegman BP, et al. Differences in glucose-stimulated insulin secretion in *vitro* of islets from human, nonhuman primate, and porcine origin. *Xenotransplantation* (2013) 20:75–81. doi: 10.1111/xen.12022.

95. Mourad NI, Nenquin M, Henquin JC. cAMP-mediated and metabolic amplification of insulin secretion are distinct pathways sharing independence of beta-cell microfilaments. *Endocrinology* (2012) 153:4644–54. doi: 10.1210/en.2012-1450.

96. Mourad NI, Nenquin M, Henquin JC. Amplification of insulin secretion by acetylcholine or phorbol ester is independent of beta-cell microfilaments and distinct from metabolic amplification. *Mol Cell Endocrinol* (2013) 367:11–20. doi: 10.1016/j.mce.2012.12.002.

97. Manell E, Hedenqvist P, Svensson A, Jensen-Waern M. Establishment of a refined oral glucose tolerance test in pigs, and assessment of insulin, glucagon and glucagon-like peptide-1 responses. *PloS One* (2016) 11:e0148896. doi: 10.1371/journal.pone.0148896

98. Mourad NI, Xhema D, Gianello P. *In vitro* assessment of pancreatic hormone secretion from isolated porcine islets. *Front Endocrinol (Lausanne)* (2022) 13:935060. doi: 10.3389/fendo.2022.935060

99. Gerstein HC, Waltman L. Why don't pigs get diabetes? Explanations for variations in diabetes susceptibility in human populations living in a diabetogenic environment. *CMAJ* (2006) 174:25–6. doi: 10.1503/cmaj.050649

100. Potter KJ, Abedini A, Marek P, Klimek AM, Butterworth S, Driscoll M, et al. Islet amyloid deposition limits the viability of human islet grafts but not porcine islet grafts. *Proc Natl Acad Sci U S A* (2010) 107:4305–10. doi: 10.1073/pnas.0909024107.

101. Hull RL, Westermark GT, Westermark P, Kahn SE. Islet amyloid: a critical entity in the pathogenesis of type 2 diabetes. *J Clin Endocrinol Metab* (2004) 89:3629–43. doi: 10.1210/jc.2004-0405

102. Salama A, Mosser M, Lévêque X, Perota A, Judor JP, Danna C, et al. Neu5Gc and α1-3 Gal xenoantigen knockout does not affect glycemia homeostasis and insulin secretion in pigs. *Diabetes* (2017) 66:987–93. doi: 10.2337/db16-1060

103. clinicaltrials.gov. Available online at: https://clinicaltrials.gov/study/NCT03162237.

104. Spizzo T, Denner J, Gazda L, Martin M, Nathu D, Scobie L, et al. First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes–Chapter 2a: Source pigs–preventing xenozoonoses. *Xenotransplantation* (2016) 23:25–31. doi: 10.1111/xen.12223.

105. Hering BJ, Cozzi E, Spizzo T, Cowan PJ, Rayat GR, Cooper DK, et al. First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes–Executive summary. *Xenotransplantation* (2016) 23:3–13. doi: 10.1111/xen.12231.

106. Hering BJ, O'Connell PJ. First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes–Chapter 6: patient selection for pilot clinical trials of islet xenotransplantation. *Xenotransplantation* (2016) 23:60–76. doi: 10.1111/xen.12228.

107. Paradis K, Langford G, Long Z, Heneine W, Sandstrom P, Switzer WM, et al. Search for cross-species transmission of porcine endogenous retrovirus in patients treated with living pig tissue. The XEN 111 Study Group. *Science* (1999) 285:1236–41. doi: 10.1126/science.285.5431.1236.

108. Denner J, Tonjes RR, Takeuchi Y, Fishman J, Scobie L. First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes–Chapter 5: recipient monitoring and response plan for preventing disease transmission. *Xenotransplantation* (2016) 23:53–9. doi: 10.1111/xen.12227.

109. Niu D, Wei HJ, Lin L, George H, Wang T, Lee IH, et al. Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9. *Science* (2017) 357:1303–7. doi: 10.1126/science.aan4187.

110. Yue Y, Xu W, Kan Y, Zhao HY, Zhou Y, Song X, et al. Extensive germline genome engineering in pigs. *Nat BioMed Eng* (2021) 5:134–43. doi: 10.1038/s41551-020-00613-9

111. US Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research (CBER). *Guidance for Industry: Source Animal, Product, Preclinical, and Clinical Issues Concerning the Use of Xenotransplantation Products in Humans* (2016). Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/source-animal-product-preclinical-and-clinical-issues-concerning-use-xenotransplantation-products.

112. Baysoy A, Bai Z, Satija R, Fan R. The technological landscape and applications of single-cell multi-omics. *Nat Rev Mol Cell Biol* (2023) 24:695–713. doi: 10.1038/s41580-023-00615-w

113. Mohiuddin MM, Singh AK, Scobie L, Goerlich CE, Grazioli A, Saharia K, et al. Graft dysfunction in compassionate use of genetically engineered pig-to-human cardiac xenotransplantation: a case report. *Lancet* (2023) 402:397–410. doi: 10.1016/S0140-6736(23)00775-4

114. Peereboom ETM, Matern BM, Spierings E, Geneugelijk K. The value of single-cell technologies in solid organ transplantation studies. *Transplantation* (2022) 106:2325–37. doi: 10.1097/TP.0000000000004237

115. Chen P, Yao F, Lu Y, Peng Y, Zhu S, Deng J, et al. Single-cell landscape of mouse islet allograft and syngeneic graft. *Front Immunol* (2022) 13:853349. doi: 10.3389/fimmu.2022.853349

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Advancing diabetes treatment: the role of mesenchymal stem cells in islet transplantation

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Diabetes mellitus, a prevalent global health challenge, significantly impacts societal and economic well-being. Islet transplantation is increasingly recognized as a viable treatment for type 1 diabetes that aims to restore endogenous insulin production and mitigate complications associated with exogenous insulin dependence. We review the role of mesenchymal stem cells (MSCs) in enhancing the efficacy of islet transplantation. MSCs, characterized by their immunomodulatory properties and differentiation potential, are increasingly seen as valuable in enhancing islet graft survival, reducing immune-mediated rejection, and supporting angiogenesis and tissue repair. The utilization of MSC-derived extracellular vesicles further exemplifies innovative approaches to improve transplantation outcomes. However, challenges such as MSC heterogeneity and the optimization of therapeutic applications persist. Advanced methodologies, including artificial intelligence (AI) and single-cell RNA sequencing (scRNA-seq), are highlighted as potential technologies for addressing these challenges, potentially steering MSC therapy toward more effective, personalized treatment modalities for diabetes. This review revealed that MSCs are important for advancing diabetes treatment strategies, particularly through islet transplantation. This highlights the importance of MSCs in the field of regenerative medicine, acknowledging both their potential and the challenges that must be navigated to fully realize their therapeutic promise.

KEYWORDS

diabetes, islets, islet transplantation, MSC, immunomodulation, single-cell RNA sequencing, artificial intelligence, regenerative medicine

Abbreviations: ScRNA-seq, single-cell RNA sequencing; MSCs, mesenchymal stem cells; T1D, type 1 diabetes; AI, artificial intelligence; EVs, Extracellular vesicles; iPSCs, induced pluripotent stem cells; ESCs, embryonic stem cells.

1 Introduction

Diabetes mellitus has emerged as a significant chronic disease worldwide that imposes substantial social and economic burdens. In 2021, around 537 million adults aged 20-79 were reported to have diabetes (1), including approximately 8.4 million with type 1 diabetes (T1D) (2). T1D, a chronic autoimmune disease, necessitates lifelong management via daily insulin injections or continuous infusion through a pump (3). Despite advancements, precise insulin dosage control remains challenging for some patients, leading to the risk of hypoglycemia and further complicating treatment (4). The FDA approval of Lantidra, the initial allogeneic pancreatic islet cell therapy product derived from deceased donors, marked a significant innovation in islet transplantation, offering new hope for T1D patients struggling with severe hypoglycemia despite intensive diabetes management (5, 6). This development not only signifies a breakthrough in transplantation technology but also provides an effective alternative treatment for adult T1D patients who cannot reach the desired glycated hemoglobin levels.

Islet transplantation, as an innovative treatment, offers T1D patients the potential to restore endogenous insulin production, reducing reliance on exogenous insulin and preventing long-term complications (7). This minimally invasive method has shown effectiveness beyond traditional treatments (8), with some centers reporting over 50% insulin independence after five years (9, 10). However, challenges remain, including donor cell scarcity, substantial cell loss post-transplantation due to immediate blood-mediated inflammatory reactions, hypoxia, and ischemia-reperfusion injury, as well as complications from immunosuppressants (11). Consequently, achieving optimal glucose control often necessitates multiple transplants.

Recent strides in regenerative medicine have identified mesenchymal stem cells (MSCs) as key players in overcoming these obstacles (12). Due to their multipotency, immunomodulatory properties, and low immunogenicity, MSCs derived from bone marrow, adipose tissue, and umbilical cord blood have demonstrated potential for enhancing islet graft survival, modulating immune responses, and promoting tissue repair and angiogenesis (13). This has been evidenced by their capacity to differentiate into different cell types, such as cells that produce insulin, and their secretion of a vast array of regenerative factors, making them promising candidates for co-transplantation with pancreatic islets (14). The immunomodulatory properties of MSCs, which are pivotal for modulating immune responses and fostering tissue repair, have been extensively documented, suggesting a significant reduction in transplant rejection risk (15, 16). Additionally, the therapeutic versatility of MSCs has been explored in various disease contexts, including cardiovascular diseases and liver injury, further supporting their broad potential in regenerative medicine.

Moreover, the exploration of MSC-derived exosomes represents an advancement in therapeutic strategies for pancreatic islet transplantation, highlighting a new direction in MSC-based treatments (17). These exosomes, as carriers of regenerative and immunomodulatory factors, present a novel approach to enhancing the microenvironment of transplanted islets, potentially reducing immunological rejection and supporting islet cell survival and function. This advancement in MSC-derived exosome research illustrates the ongoing innovation in treatment methodologies, aiming to address the complex challenges of islet transplantation in diabetes management.

The comparison between MSCs, induced pluripotent stem cells (iPSCs), and embryonic stem cells (ESCs) highlights their distinct roles in diabetes treatment. MSCs are favored for their immunomodulatory properties and minimal tumorigenic risk, presenting a safer option for clinical applications. Unlike MSCs, iPSCs and ESCs possess greater pluripotency, enabling the generation of a broader range of cell types. However, this approach is associated with increased ethical concerns for ESCs and increased tumorigenic potential for both iPSCs and ESCs (18, 19). Compared to their broader differentiation capabilities, the unique advantages of MSCs in regenerative medicine, particularly in mitigating autoimmune responses in diabetes, are the heightened risks associated with iPSCs and ESCs.

To enhance our understanding of MSC therapies, particularly in the realms of diabetes treatment and islet transplantation, advanced technologies such as artificial intelligence (AI) and single-cell RNA sequencing (scRNA-seq) offer groundbreaking approaches (20, 21). These innovations allow for the precise characterization of MSCs and in-depth analysis of their molecular behaviors, which is crucial for developing personalized, effective therapies. By incorporating findings from a recent study that utilized scRNA-seq to analyze immune heterogeneity in mouse models of islet transplantation, we obtained valuable insights into the immune mechanisms that may affect graft survival (22). The insights of this study into the transcriptomics of islet grafts underscore the potential of scRNA-seq for identifying key factors influencing the success of MSC therapies, highlighting the importance of these advanced methodologies in advancing personalized medicine and improving diabetes care strategies.

This review not only highlights the potential of MSCs in enhancing diabetes treatment through islet transplantation but also addresses persistent challenges such as MSC heterogeneity and the need for therapeutic optimization. This finding underscores the promise of advanced methodologies such as AI and scRNA-seq for overcoming these hurdles, suggesting that MSC therapy could offer more effective, personalized diabetes treatment solutions in the future.

2 Background on MSCs

2.1 Therapeutic molecules and mechanisms of MSCs

MSCs, which are derived from bone marrow, adipose tissue, the umbilical cord, and the gingiva, play a crucial role in regenerative medicine due to their unique ability to differentiate into multiple cell types essential for tissue repair and regeneration (23). The significant immunomodulatory effects of MSCs, which impact both the innate and adaptive immune systems through the secretion of bioactive factors with immunosuppressive and anti-inflammatory properties, underscore their importance (24). These cells can

differentiate into a variety of different cell types is also important, especially when addressing T1D and enhancing pancreatic islet transplantation outcomes by supporting graft survival, modulating immune responses, and promoting tissue repair and angiogenesis.

2.2 MSC-derived extracellular vesicles (EVs): broadening therapeutic horizons

The potential of MSC-derived EVs extends beyond diabetes to conditions such as ischemic stroke and osteoarthritis, demonstrating the wide range of applications of MSCs (25). Clinical studies have confirmed the ability of MSCs to evolve into insulin-producing cells and secrete healing factors, positioning them as key players in T1D treatment (26). Furthermore, engineered MSC-derived EVs are being developed to enhance regenerative efficacy, overcoming natural limitations (27). Proteomic analyses of MSC exosomes from various sources have revealed that shared mechanisms, notably extracellular matrix interactions, are crucial for their regenerative impact (28). The clinical application of umbilical cord MSCs highlights the ongoing promise of MSC-based therapies in diabetes management and beyond (26).

3 MSCs in improving islet transplantation outcomes

In islet transplantation for diabetes treatment, MSCs play a vital role by safeguarding islet cells and improving the outcomes of both allo- and xenotransplantation (29–31). The broad array of secreted molecules, including growth factors and immunomodulatory agents, contributes to enhancing transplantation efficacy and survival (32). During regeneration, these secretory factors facilitate tissue remodeling and promote cellular homeostasis. This multifaceted action of MSCs addresses key challenges in islet transplantation, including reducing graft rejection and improving graft performance, thus enhancing the efficacy of diabetes treatments (33, 34). Moreover, the potential of MSCs for novel therapeutic strategies marks an advancement in diabetes management, suggesting that MSCs are important elements in both regenerative medicine and autoimmune therapy. The role of MSCs in enhancing outcomes in diabetes patients through islet transplantation is critical, as they emphasize the broad therapeutic potential of these cells and open new paths for diabetes care advancements.

The exploration of the use of MSCs in diabetes treatment, particularly through islet transplantation, highlights their potential for advancing therapeutic strategies. MSCs are at the forefront of regenerative medicine and autoimmune therapy, offering innovative approaches to improve patient outcomes in diabetes care. Their broad therapeutic capabilities and potential for new pathways in diabetes management underscore their role in the field.

The integration of MSCs into pancreatic islet transplantation protocols has been the focus of numerous studies, revealing substantial improvements in transplantation outcomes (34). These cells have demonstrated the capacity to enhance both the engraftment and survival rates of transplanted islets as well as their long-term functionality, which is important for successful transplantation. MSCs exert beneficial effects through immunomodulatory effects, mitigating immune-mediated rejection and autoimmune attacks. Additionally, MSCs promote a supportive microenvironment for islets by stimulating angiogenesis and tissue repair, addressing the challenges of islet transplantation and advancing diabetes treatment modalities.

3.1 The protective role of MSCs during islet isolation and culture

Its ability to protect islet vitality during isolation and culture is crucial because it can mitigate hypoxia and inflammatory stress, which are key factors in islet impairment. Coculturing islets with MSCs not only preserves islet functionality but also improves transplantation results by maintaining insulin secretion and cell vitality. The role of MSCs in improving the internal microenvironment for T1D treatment has been well studied. MSCs contribute to protecting islet vitality during isolation and culture and in mitigating hypoxia and inflammatory stress, which are key factors in islet impairment (29, 35). Coculturing islets with MSCs not only preserves islet functionality but also improves transplantation results by maintaining insulin secretion and cell vitality (29, 35).

3.2 Enhancing islet transplantation through MSC-driven immunomodulation and angiogenesis

MSCs ameliorate the internal microenvironment post-islet transplantation, reducing inflammation and improving patient outcomes (29, 35). The capacity of these cells to alleviate blood-mediated inflammatory responses post-transplant is important for T1D patients to progress toward insulin independence. By mitigating transplant-related stress and curtailing β-cell damage, MSCs play a critical role. Furthermore, the secretion of soluble immunomodulatory factors crucially suppresses immune rejection, fostering graft tolerance.

The synergistic effect of MSCs in islet transplantation is multifaceted, emphasizing not only their anti-inflammatory effects but also their critical contribution to angiogenesis (36). After isolation, islets undergo vascularization loss, increasing susceptibility to stressors such as instant blood-mediated immune reactions, hypoxia, and ischemia-reperfusion injury, which can lead to apoptosis and necrosis. MSCs facilitate rapid revascularization; secrete factors such as VEGF (37), Ang-1 (38), bFGF (32), KGF (39), IGF-1, IGF-2, and HGF; and are important for angiogenesis and enhancing islet graft survival and functionality. MSCs can promote angiogenesis by activating AKT/MAPK signaling and upregulating VEGFR signaling (40). MSCs can also perform angiogenic modulation through complex interactions between bioactive molecules carried by EVs, such as microRNAs (40). Despite the inherent challenges of MSC use, such as limited proliferative capacity and heterogeneity, ongoing studies highlight

the capacity of MSCs to support new blood vessel formation, ensuring that transplanted islets receive necessary nourishment and oxygen. Along with the ability of MSCs to promote tissue repair, this angiogenesis supports the role of MSCs in improving the outcomes of diabetes treatments through islet transplantation (41).

The multifaceted role of MSCs in islet transplantation reflects their capacity for immune modulation and support for angiogenesis and tissue repair. While their use presents challenges such as limited growth and variability, ongoing research into the origins and functionalities of MSCs continues to enhance their application in diabetes treatment. This exploration of the diverse origins of MSCs, such as bone marrow and adipose tissue, and their capacities for angiogenesis and immunosuppression contributes to understanding and leveraging their therapeutic impact in islet transplantation.

4 Clinical applications and insights of MSCs

The integration of MSC therapy into clinical practice presents scientific and ethical challenges, including complexities and potential risks such as immunogenicity and tumorigenicity. Examining these obstacles and emphasizing the importance of rigorous selection, ethical sourcing practices, and adherence to regulatory guidelines will ensure safety and efficacy. This highlights the necessity of continuous research, ethical deliberation, and regulatory updates to optimize MSC therapies for diverse conditions, particularly diabetes treatment through islet transplantation.

Despite the promising potential of MSCs in regenerative medicine and diabetes treatment, MSCs face significant challenges that hinder their clinical translation. These include their inherent heterogeneity, which complicates the consistency of therapeutic outcomes, and concerns about immunogenicity that may elicit immune responses in recipients (42). Additionally, the potential for MSCs to contribute to tumorigenesis remains a critical area of investigation (43). It is important to critically evaluate these challenges, drawing upon data from past clinical trials and current research, to provide a comprehensive understanding of the limitations and barriers facing the use of MSCs in islet transplantation. This underscores the complexity of translating MSC therapy from bench to bedside and emphasizes the need for continued research to overcome these obstacles.

The ethical implications of MSC sourcing involve considerations such as donor consent, particularly for MSCs derived from human tissues (44). Ethical sourcing ensures that donors are fully informed and agree to the use of their cells for research or therapeutic purposes. Regulatory policies for MSC therapies, governed by agencies such as the FDA and the European Medicines Agency, focus on ensuring the safety, efficacy, and quality of MSC-based products (45). These regulations necessitate rigorous clinical trials and manufacturing standards to prevent risks such as immunogenicity and tumorigenicity, aiming to safeguard patient health while fostering innovation in MSC therapy development.

5 Overcoming challenges and charting the future of MSC therapy in clinical applications

The progression of MSCs from early preclinical studies to clinical applications illustrates both their potential and the complexities involved in MSC therapy. Despite advancements, challenges affect their clinical success (42). In diabetes treatment, especially islet transplantation, the variability and adaptability of MSCs require careful clinical evaluation. The variability of MSCs due to their different sources, preparation methods, and delivery techniques poses significant challenges to their standardization and consistency in therapies (46, 47). To minimize heterogeneity and improve the predictability of outcomes, it is important to explore strategies and potential guidelines, such as stringent cell characterization, uniform culture conditions, and standardized delivery methods (48). Emphasizing the need for comprehensive quality control and clinical protocol standardization will offer insights into advancing MSC therapies toward more reliable and effective clinical applications, thus enhancing their utility in regenerative medicine and beyond. Innovations such as AI (49) and scRNA-seq (50) are promising methods for addressing these obstacles, indicating that MSC therapy might become a standard, personalized treatment option for diabetes in the future (Figure 1).

5.1 AI in MSC clinical treatment

The integration of AI into MSC therapies for diabetes treatment is an evolving field that combines two cutting-edge scientific advancements. Although direct applications in diabetes are nascent, the synergy between the predictive capabilities of AI and the therapeutic potential of MSCs offers promising directions for more precise and customized treatments. This interdisciplinary approach aims to enhance diabetes care by leveraging AI to optimize MSC therapy outcomes, indicating a move toward more individualized and effective treatment strategies in regenerative medicine.

5.1.1 Accelerating drug discovery with AI in MSC therapies

Digital technology and AI significantly influence healthcare innovation, particularly in drug research and development (51, 52). The capacity of AI for the *de novo* design of biologically active molecules has the potential to enhance therapeutic efficacy (53). These technological advancements aim to refine MSC therapies by improving the identification of critical molecular components and drug targets, thus increasing the precision of drug development (54, 55). The collaboration of the pharmaceutical industry with AI firms to develop advanced platforms underscores the role of AI in simplifying the drug discovery process, necessitating rigorous clinical validation to ensure the efficacy and safety of these innovations (56).

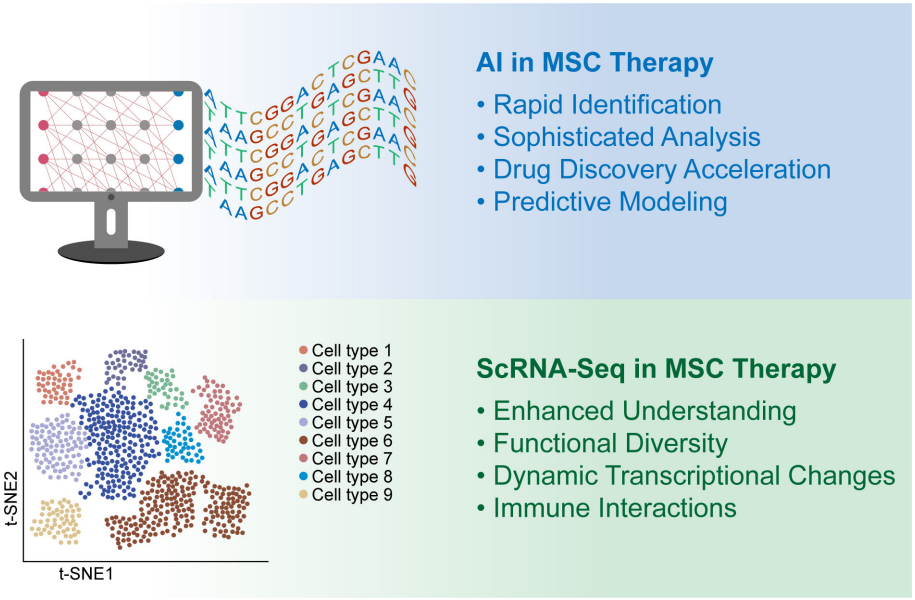

FIGURE 1
Artificial intelligence (AI) and single-cell RNA sequencing (scRNA-seq) for enhancing the clinical application of mesenchymal stem cells (MSCs) (1). The upper panel shows the AI used in MSC clinical treatment. Rapid Identification: AI has drastically accelerated the discovery of novel molecular compounds and drug targets, enhancing the development of MSC-based treatments. Sophisticated Analysis: This study utilized complex biological datasets to optimize graft longevity and functionality within islet transplantation settings. Drug Discovery Acceleration: AI contributes to drug research and development, improving the precision and efficiency of therapeutic discovery and development processes. Predictive Modeling: This method employs dynamic molecular traits for MSC therapy, including protein sequences and molecular interactions, to refine therapeutic strategies (2). The lower panel shows the results of scRNA-seq analysis of MSC therapies. Enhanced Understanding: scRNA-seq offers in-depth insights into MSC heterogeneity, enabling precise characterization and biomarker identification. Functional Diversity: This study reveals the complex roles of MSCs in development, regeneration, and pathology, facilitating the development of targeted therapies. Dynamic Transcriptional Changes: This review sheds light on MSC differentiation and the regulatory pathways involved, supporting the refinement of clinical applications. Immune Interactions: This paper describes how MSCs modulate immune cells, providing valuable information for developing MSC-based therapies for immune modulation and tissue repair.

5.1.2 Leveraging AI for enhanced MSC therapy development

Integrating AI into MSC therapy research is a methodical strategy for exploring the detailed landscape of regenerative medicine. The ability of AI to analyze extensive data enhances the precision of identifying the molecular attributes of MSCs, which is fundamental in regenerative therapies aimed at repairing or regenerating tissues affected by conditions such as diabetes. The role of AI in automating the creation of therapeutic compounds represents a meaningful advancement toward improving regenerative treatment efficacy (57, 58). This synergy between AI and regenerative medicine research is poised to propel the development of new therapeutic strategies, potentially bringing significant benefits to patients with various chronic ailments.

5.2 ScRNA-seq in MSC therapies

The advent of scRNA-seq has enhanced the understanding and application of MSCs (59, 60). Despite the promising potential of MSCs in regenerative medicine and their success in preclinical models, clinical trials have often not met expectations, partly due to the heterogeneity of MSCs and inconsistent identification criteria. ScRNA-seq has bridged crucial gaps by enabling precise MSC characterization and biomarker identification and revealing gene expression heterogeneity within MSC subclusters (61). Such insights are invaluable for comprehending the functional diversity of MSCs and their roles in development, regeneration, and pathology. Furthermore, scRNA-seq helps to elucidate the dynamic transcriptional changes in MSCs during differentiation and the intricate signaling pathways regulating their key functions (20). This refined understanding, facilitated by evolving analytical methods and integration with histological research, promises more targeted MSC-based therapies, particularly in complex treatments such as islet transplantation for diabetes, contributing to personalized and effective interventions.

5.2.1 Unveiling heterogeneity and potentials through ScRNA-seq

ScRNA-seq has improved MSC research, offering unprecedented insights into the isolation, identification, and classification of MSCs based on heterogeneity and subclusters (62). This technology allows for more precise characterization of MSCs, revealing the diversity of MSC subclusters and their specific molecular expression and functions (63). By revealing dynamic transcriptional changes and complex signaling pathways, scRNA-seq facilitates a deeper understanding of the roles of MSCs in development, regeneration, and pathology (64, 65). This in-depth knowledge is crucial for developing targeted MSC-based therapies, particularly for applications such as islet transplantation in diabetes

treatment, by identifying MSC subpopulations with optimal therapeutic properties and guiding the refinement of clinical applications.

5.2.2 Unveiling the biological function of MSCs through ScRNA-seq

The role of scRNA-seq extends beyond MSC differentiation to uncovering MSC-immune interactions. ScRNA-seq has illuminated the diverse ways in which MSCs modulate immune cells, from T cells to macrophages, through various cytokines and chemokines (66). This understanding is pivotal in diseases such as acute lung injury, where MSCs reduce proinflammatory immune cell infiltration and cytokine expression (67). Studies have also shown that MSCs influence macrophage behavior in lung fibrosis, indicating the potential of MSCs in immune modulation and tissue repair (68). This detailed understanding is crucial for the development of MSC-based treatments, providing fresh perspectives for personalized and effective diabetes care strategies.

6 Conclusion

This review underscores the significant impact of MSCs on islet transplantation for diabetes treatment. This finding highlights the role of MSCs in enhancing islet graft survival, modulating immune responses, and promoting angiogenesis and tissue repair, indicating their potential for use in diabetes management. Challenges such as MSC heterogeneity and the need for optimization in therapeutic applications are acknowledged, with advanced technologies such as AI and scRNA-seq offering promising solutions. The synergy between MSCs and islet transplantation is emphasized as a forward-looking approach to personalized, MSC-based interventions, setting a new direction in therapeutic strategies against diabetes.

Author contributions

LM: Funding acquisition, Writing – original draft, Writing – review & editing. TW: Writing – review & editing. XW: Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing. ZP: Conceptualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Ogurtsova K, Guariguata L, Barengo NC, Ruiz PL-D, Sacre JW, Karuranga S, et al. IDF diabetes Atlas: Global estimates of undiagnosed diabetes in adults for 2021. *Diabetes Res Clin Pract.* (2022) 183:109118. doi: 10.1016/j.diabres.2021.109118

2. Gregory GA, Robinson TIG, Linklater SE, Wang F, Colagiuri S, de Beaufort C, et al. Global incidence, prevalence, and mortality of type 1 diabetes in 2021 with projection to 2040: a modelling study. *Lancet Diabetes Endocrinol.* (2022) 10:741–60. doi: 10.1016/S2213-8587(22)00218-2

3. Martens P-J, Mathieu C. Type 1 diabetes mellitus: a brave new world. *Nat Rev Endocrinol.* (2024) 20:71–2. doi: 10.1038/s41574-023-00936-y

4. Tatovic D, Narendran P, Dayan CM. A perspective on treating type 1 diabetes mellitus before insulin is needed. *Nat Rev Endocrinol.* (2023) 19:361–70. doi: 10.1038/s41574-023-00816-5

5. Mullard A. FDA approves first cell therapy for type 1 diabetes. *Nat Rev Drug Discovery.* (2023) 22:611. doi: 10.1038/d41573-023-00113-w

6. Commissioner O of the. FDA approves first cellular therapy to treat patients with type 1 diabetes (2023). FDA. Available at: https://www.fda.gov/news-events/press-announcements/fda-approves-first-cellular-therapy-treat-patients-type-1-diabetes (Accessed February 5, 2024).

7. Bornstein SR, Ludwig B, Steenblock C. Progress in islet transplantation is more important than ever. *Nat Rev Endocrinol.* (2022) 18:389–90. doi: 10.1038/s41574-022-00689-0

8. Piemonti L. Islet transplantation (2000). South Dartmouth (MA). Available at: http://www.ncbi.nlm.nih.gov/books/NBK278966/ (Accessed February 5, 2024).

9. Rickels MR, Eggerman TL, Bayman L, Qidwai JC, Alejandro R, Bridges ND, et al. Long-term outcomes with islet-alone and islet-after-kidney transplantation for type 1 diabetes in the clinical islet transplantation consortium: The CIT-08 study. *Diabetes Care.* (2022) 45:2967–75. doi: 10.2337/dc21-2688

10. Marfil-Garza BA, Imes S, Verhoeff K, Hefler J, Lam A, Dajani K, et al. Pancreatic islet transplantation in type 1 diabetes: 20-year experience from a single-centre cohort in Canada. *Lancet Diabetes Endocrinol.* (2022) 10:519–32. doi: 10.1016/S2213-8587(22)00114-0

11. Cayabyab F, Nih LR, Yoshihara E. Advances in pancreatic islet transplantation sites for the treatment of diabetes. *Front Endocrinol (Lausanne).* (2021) 12:732431. doi: 10.3389/fendo.2021.732431

12. Guo Y, Yang Y, Hu S, Chen Y, Shen Z. The therapeutic potential of mesenchymal stem cells for cardiovascular diseases. *Cell Death Dis.* (2020) 11:349. doi: 10.1038/s41419-020-2542-9

13. Zepeng Q, Zhaofeng J, Xi H, Zhiming C, Lisha M. Application and research progress on mesenchymal stem cell in organ transplantation. *Organ Transplant.* (2018) 9:348. doi: 10.3969/j.issn.1674-7445.2018.05.005

14. Shufang Z, Lisha M. Application progress of mesenchymal stem cells in islet transplantation for type 1 diabetes mellitus. *Organ Transplant.* (2024) 15:214. doi: 10.3969/j.issn.1674-7445.2023194

15. Huang Y, Wu Q, Tam PKH. Immunomodulatory mechanisms of mesenchymal stem cells and their potential clinical applications. *Int J Mol Sci*. (2022) 23(17):10023. doi: 10.3390/ijms231710023

16. Shang L, Shao J, Ge S. Immunomodulatory functions of oral mesenchymal stem cells: novel force for tissue regeneration and disease therapy. *J Leukocyte Biol*. (2021) 110(3):539–52. doi: 10.1002/jlb.3mr0321-766r

17. Mattke J, Vasu S, Darden CM, Kumano K, Lawrence MC, Naziruddin B. Role of exosomes in islet transplantation. *Front Endocrinol*. (2021). doi: 10.3389/fendo.2021.681600

18. Yamatani Y, Nakai K. Comprehensive comparison of gene expression diversity among a variety of human stem cells. *NAR Genom Bioinform*. (2022) 4:lqac087. doi: 10.1093/nargab/lqac087

19. Thanaskody K, Jusop AS, Tye GJ, Wan Kamarul Zaman WS, Dass SA, Nordin F. MSCs vs. iPSCs: Potential in therapeutic applications. *Front Cell Dev Biol*. (2022) 10:1005926. doi: 10.3389/fcell.2022.1005926

20. Li H, Wang Y, Zhu G, Ma Q, Huang S, Guo G, et al. Application progress of single-cell sequencing technology in mesenchymal stem cells research. *Front Cell Dev Biol*. (2024) 11:1336482. doi: 10.3389/fcell.2023.1336482

21. Mak K-K, Pichika MR. Artificial intelligence in drug development: present status and future prospects. *Drug Discovery Today*. (2019) 24:773–80. doi: 10.1016/j.drudis.2018.11.014

22. Chen P, Yao F, Lu Y, Peng Y, Zhu S, Deng J, et al. Single-cell landscape of mouse islet allograft and syngeneic graft. *Front Immunol*. (2022) 13:853349. doi: 10.3389/fimmu.2022.853349

23. Hoang DM, Pham PT, Bach TQ, Ngo ATL, Nguyen QT, Phan TTK, et al. Stem cell-based therapy for human diseases. *Signal Transduct Target Ther*. (2022) 7:272. doi: 10.1038/s41392-022-01134-4

24. Wang Y, Fang J, Liu B, Shao C, Shi Y. Reciprocal regulation of mesenchymal stem cells and immune responses. *Cell Stem Cell*. (2022) 29:1515–30. doi: 10.1016/j.stem.2022.10.001

25. Tan F, Li X, Wang Z, Li J, Shahzad K, Zheng J. Clinical applications of stem cell-derived exosomes. *Signal Transduct Target Ther*. (2024) 9:17. doi: 10.1038/s41392-023-01704-0

26. Al Madhoun A, Koti L, Carrió N, Atari M, Al-Mulla F. Clinical application of umbilical cord mesenchymal stem cells preserves B-cells in type 1 diabetes. *Stem Cells Trans Med*. (2023) 13(2):101–6. doi: 10.1093/stcltm/szad077

27. Sun F, Sun Y, Wu F, Xu W, Qian H. Mesenchymal stem cell-derived extracellular vesicles: A potential therapy for diabetes mellitus and diabetic complications. *Pharmaceutics*. (2022) 14(10):2208. doi: 10.3390/pharmaceutics14102208

28. Wang Z, He Z, Liang S, Yang Q, Cheng P, Chen A, et al. Comprehensive proteomic analysis of exosomes derived from human bone marrow, adipose tissue, and umbilical cord mesenchymal stem cells. *Stem Cell Res Ther*. (2020) 11:511. doi: 10.1186/s13287-020-02032-8

29. Wei L, Zhang L, Yang L, Wang X, Chun-hua Z, Zhao D. Protective effect of mesenchymal stem cells on isolated islets survival and against hypoxia associated with the HIF-1α/PFKFB3 pathway. *Cell Transplant*. (2022), 31. doi: 10.1177/09636897211073127

30. Nguyen TT, Pham D-V, Park J, Phung CD, Nepal MR, Pandit M, et al. Engineering of hybrid spheroids of mesenchymal stem cells and drug depots for immunomodulating effect in islet xenotransplantation. *Sci Adv*. (2022) 8:eabn8614. doi: 10.1126/sciadv.abn8614

31. Shufang Z, Zepeng Q, Ying L, Dengke P, Lisha M. Isolation of bone marrow mesenchymal stem cells in transgenic pigs and co-culture with porcine islets. *Organ Transplant*. (2024) 15:55. doi: 10.3969/j.issn.1674-7445.2023205

32. Han Y, Yang J, Fang J, Zhou Y, Candi E, Wang J, et al. The secretion profile of mesenchymal stem cells and potential applications in treating human diseases. *Signal Transduct Target Ther*. (2022) 7:92. doi: 10.1038/s41392-022-00932-0

33. Naqvi RA, Naqvi A. Co-transplantation with mesenchymal stem cells and endothelial cells improvise islet engraftment and survival in STZ treated hyperglycemic mice. *bioRxiv*. (2023) 2023:525444. doi: 10.1101/2023.01.24.525444

34. Qu Z, Lou Q, Cooper DKC, Pu Z, Lu Y, Chen J, et al. Potential roles of mesenchymal stromal cells in islet allo- and xenotransplantation for type 1 diabetes mellitus. *Xenotransplantation*. (2021) 28:e12678. doi: 10.1111/xen.12678

35. Teratani T, Kasahara N, Fujimoto Y, Sakuma Y, Miki A, Goto M, et al. Mesenchymal stem cells secretions enhanced ATP generation on isolated islets during transplantation. *Islets*. (2022) 14(1):69–81. doi: 10.1080/19382014.2021.2022423

36. Maacha S, Sidahmed H, Jacob S, Gentilcore G, Calzone R, Grivel J-C, et al. Paracrine mechanisms of mesenchymal stromal cells in angiogenesis. *Stem Cells Int*. (2020) 2020:4356359. doi: 10.1155/2020/4356359

37. Beckermann BM, Kallifatidis G, Groth A, Frommhold D, Apel A, Mattern J, et al. VEGF expression by mesenchymal stem cells contributes to angiogenesis in pancreatic carcinoma. *Br J Cancer*. (2008) 99:622–31. doi: 10.1038/sj.bjc.6604508

38. Hu S, Park J, Liu A, Lee J, Zhang X, Hao Q, et al. Mesenchymal stem cell microvesicles restore protein permeability across primary cultures of injured human lung microvascular endothelial cells. *Stem Cells Transl Med*. (2018) 7:615–24. doi: 10.1002/sctm.17-0278

39. Guan Y, Xie Y, Li D, Zhu Y, Zhang X, Feng Y, et al. Comparison of biological characteristics of mesenchymal stem cells derived from the human umbilical cord and decidua parietalis. *Mol Med Rep*. (2019) 20(1):633–9. doi: 10.3892/mmr.2019.10286

40. Huang C, Luo W, Wang Q, Ye Y, Fan J, Lin L, et al. Human mesenchymal stem cells promote ischemic repairment and angiogenesis of diabetic foot through exosome miRNA-21-5p. *Stem Cell Res*. (2021) 52:102235. doi: 10.1016/j.scr.2021.102235

41. Zhang W, Ling Q, Wang B, Wang K, Pang J, Lü J, et al. Comparison of therapeutic effects of mesenchymal stem cells from umbilical cord and bone marrow in the treatment of type 1 diabetes. *Stem Cell Res Ther*. (2022) 13(406). doi: 10.1186/s13287-022-02974-1

42. Zhou T, Yuan Z, Weng J, Pei D, Du X, He C, et al. Challenges and advances in clinical applications of mesenchymal stromal cells. *J Hematol Oncol*. (2021) 14:24. doi: 10.1186/s13045-021-01037-x

43. Li C, Zhao H, Wang B. Mesenchymal stem/stromal cells: Developmental origin, tumorigenesis and translational cancer therapeutics. *Transl Oncol*. (2021) 14:100948. doi: 10.1016/j.tranon.2020.100948

44. Feier AM, Portan D, Manu DR, Kostopoulos V, Kotrotsos A, Strnad G, et al. Primary MSCs for personalized medicine: ethical challenges, isolation and biocompatibility evaluation of 3D electrospun and printed scaffolds. *Biomedicines*. (2022) 10:1563. doi: 10.3390/biomedicines10071563

45. Fernández-Garza LE, Barrera-Barrera SA, Barrera-Saldaña HA. Mesenchymal stem cell therapies approved by regulatory agencies around the world. *Pharm (Basel)*. (2023) 16:1334. doi: 10.3390/ph16091334

46. Hass R, Kasper C, Böhm S, Jacobs R. Different populations and sources of human mesenchymal stem cells (MSC): A comparison of adult and neonatal tissue-derived MSC. *Cell Commun Signal*. (2011) 9:12. doi: 10.1186/1478-811X-9-12

47. Kean TJ, Lin P, Caplan AI, Dennis JE. MSCs: delivery routes and engraftment, cell-targeting strategies, and immune modulation. *Stem Cells Int*. (2013) 2013:732742. doi: 10.1155/2013/732742

48. Wilson AJ, Brown N, Rand E, Genever PG. Attitudes towards standardization of mesenchymal stromal cells-A qualitative exploration of expert views. *Stem Cells Transl Med*. (2023) 12:745–57. doi: 10.1093/stcltm/szad056

49. Wang H, Fu T, Du Y, Gao W, Huang K, Liu Z, et al. Scientific discovery in the age of artificial intelligence. *Nature*. (2023) 620:47–60. doi: 10.1038/s41586-023-06221-2

50. Wen L, Li G, Huang T, Geng W, Pei H, Yang J, et al. Single-cell technologies: From research to application. *Innovation (Camb)*. (2022) 3:100342. doi: 10.1016/j.xinn.2022.100342

51. Tropsha A, Isayev O, Varnek A, Schneider G, Cherkasov A. Integrating QSAR modelling and deep learning in drug discovery: the emergence of deep QSAR. *Nat Rev Drug Discovery*. (2024) 23:141–55. doi: 10.1038/s41573-023-00832-0

52. Mullowney MW, Duncan KR, Elsayed SS, Garg N, van der Hooft JJJ, Martin NI, et al. Artificial intelligence for natural product drug discovery. *Nat Rev Drug Discovery*. (2023) 22:895–916. doi: 10.1038/s41573-023-00774-7

53. Yang Z, Zeng X, Zhao Y, Chen R. AlphaFold2 and its applications in the fields of biology and medicine. *Signal Transduct Target Ther*. (2023) 8:115. doi: 10.1038/s41392-023-01381-z

54. McCradden MD, Stephenson EA, Anderson JA. Clinical research underlies ethical integration of healthcare artificial intelligence. *Nat Med*. (2020) 26:1325–6. doi: 10.1038/s41591-020-1035-9

55. Schneider P, Walters WP, Plowright AT, Sieroka N, Listgarten J, Goodnow RA, et al. Rethinking drug design in the artificial intelligence era. *Nat Rev Drug Discovery*. (2020) 19:353–64. doi: 10.1038/s41573-019-0050-3

56. Nagra NS, van der Veken L, Stanzl E, Champagne D, Devereson A, Macak M. The company landscape for artificial intelligence in large-molecule drug discovery. *Nat Rev Drug Discovery*. (2023) 22:949–50. doi: 10.1038/d41573-023-00139-0

57. Qureshi R, Irfan M, Gondal TM, Khan S, Wu J, Hadi MU, et al. AI in drug discovery and its clinical relevance. *Heliyon*. (2023) 9:e17575. doi: 10.1016/j.heliyon.2023.e17575

58. Bédard A-C, Adamo A, Aroh KC, Russell MG, Bedermann AA, Torosian J, et al. Reconfigurable system for automated optimization of diverse chemical reactions. *Science*. (2018) 361:1220–5. doi: 10.1126/science.aat0650

59. Lin K, Yang Y, Cao Y, Liang J, Qian J, Wang X, et al. Combining single-cell transcriptomics and CellTagging to identify differentiation trajectories of human adipose-derived mesenchymal stem cells. *Stem Cell Res Ther*. (2023) 14:14. doi: 10.1186/s13287-023-03237-3

60. Zhang C, Han X, Liu J, Chen L, Lei Y, Chen K, et al. Single-cell transcriptomic analysis reveals the cellular heterogeneity of mesenchymal stem cells. *Genomics Proteomics Bioinf*. (2022) 20:70–86. doi: 10.1016/j.gpb.2022.01.005

61. Chen P, Tang S, Li M, Wang D, Chen C, Qiu Y, et al. Single-cell and spatial transcriptomics decodes wharton's jelly-derived mesenchymal stem cells heterogeneity and a subpopulation with wound repair signatures. *Adv Sci (Weinh)*. (2023) 10:e2204786. doi: 10.1002/advs.202204786

62. Xie Z, Yu W, Ye G, Li J, Zheng G, Liu W, et al. Single-cell RNA sequencing analysis of human bone-marrow-derived mesenchymal stem cells and functional subpopulation identification. *Exp Mol Med*. (2022) 54:483–92. doi: 10.1038/s12276-022-00749-5

63. Zhang S, Wang JY, Li B, Yin F, Liu H. Single-cell transcriptome analysis of uncultured human umbilical cord mesenchymal stem cells. *Stem Cell Res Ther*. (2021) 12:25. doi: 10.1186/s13287-020-02055-1

64. Rauch A, Mandrup S. Transcriptional networks controlling stromal cell differentiation. *Nat Rev Mol Cell Biol*. (2021) 22:465–82. doi: 10.1038/s41580-021-00357-7

65. Soliman H, Theret M, Scott W, Hill L, Underhill TM, Hinz B, et al. Multipotent stromal cells: One name, multiple identities. *Cell Stem Cell*. (2021) 28:1690–707. doi: 10.1016/j.stem.2021.09.001

66. Tang Z, Gao J, Wu J, Zeng G, Liao Y, Song Z, et al. Human umbilical cord mesenchymal stromal cells attenuate pulmonary fibrosis *via* regulatory T cell through interaction with macrophage. *Stem Cell Res Ther*. (2021) 12:397. doi: 10.1186/s13287-021-02469-5

67. Zhu J, Feng B, Xu Y, Chen W, Sheng X, Feng X, et al. Mesenchymal stem cells alleviate LPS-induced acute lung injury by inhibiting the proinflammatory function of Ly6C+ CD8+ T cells. *Cell Death Dis*. (2020) 11:829. doi: 10.1038/s41419-020-03036-1

68. Rahman M, Wang Z-Y, Li J-X, Xu H-W, Wang R, Wu Q. Single-cell RNA sequencing reveals the interaction of injected ADSCs with lung-originated cells in mouse pulmonary fibrosis. *Stem Cells Int*. (2022) 2022:9483166. doi: 10.1155/2022/9483166

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Innovations in bio-engineering and cell-based approaches to address immunological challenges in islet transplantation

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Human allogeneic pancreatic islet transplantation is a life-changing treatment for patients with severe Type 1 Diabetes (T1D) who suffer from hypoglycemia unawareness and high risk of severe hypoglycemia. However, intensive immunosuppression is required to prevent immune rejection of the graft, that may in turn lead to undesirable side effects such as toxicity to the islet cells, kidney toxicity, occurrence of opportunistic infections, and malignancies. The shortage of cadaveric human islet donors further limits islet transplantation as a treatment option for widespread adoption. Alternatively, porcine islets have been considered as another source of insulin-secreting cells for transplantation in T1D patients, though xeno-transplants raise concerns over the risk of endogenous retrovirus transmission and immunological incompatibility. As a result, technological advancements have been made to protect transplanted islets from immune rejection and inflammation, ideally in the absence of chronic immunosuppression, to improve the outcomes and accessibility of allogeneic islet cell replacement therapies. These include the use of microencapsulation or macroencapsulation devices designed to provide an immunoprotective environment using a cell-impermeable layer, preventing immune cell attack of the transplanted cells. Other up and coming advancements are based on the use of stem cells as the starting source material for generating islet cells 'on-demand'. These starting stem cell sources include human induced pluripotent stem cells (hiPSCs) that have been genetically engineered to avoid the host immune response, curated HLA-selected donor hiPSCs that can be matched with recipients within a given population, and multipotent stem cells with natural immune privilege properties. These strategies are developed to provide an immune-evasive cell resource for allogeneic cell therapy. This review will summarize the immunological challenges facing islet transplantation and highlight recent bio-engineering and cell-based approaches aimed at avoiding immune rejection, to improve the accessibility of islet cell therapy and enhance treatment outcomes. Better understanding of the different approaches and their

limitations can guide future research endeavors towards developing more comprehensive and targeted strategies for creating a more tolerogenic microenvironment, and improve the effectiveness and sustainability of islet transplantation to benefit more patients.

KEYWORDS

stem cells, regenerative medicine, diabetes, islet cells, beta cells, islet transplantation, HLA, hypoimmune

1 Introduction

Diabetes is a chronic metabolic disorder that affects 537 million adults aged between 20 to 79 years old. Disease prevalence is increasing year on year and is predicted to reach 643 million by 2030 (1). Individuals with poorly controlled diabetes face increased risk of heart disease, kidney disease, nerve complications and eye disorders. Type 1 diabetes (T1D) constitutes 5% to 10% of all diabetes cases, whereas the more common Type 2 diabetes (T2D) accounts for majority of the remaining 90% to 95% of diagnosed cases (2). T1D is an autoimmune disease resulting from the body's immune system attacking the insulin-producing β cells of the pancreatic islets (3). This irreversible loss of β cells results in insulin deficiency, impaired glucose uptake in the peripheral tissues, and consequently hyperglycemia. The early onset of T1D, often during adolescence, results in the need for life-long insulin therapy and intensive blood glucose monitoring to prevent both hyperglycemia and hypoglycemic episodes, which can severely impact the quality of life of patients (4). Approximately 25% of T1D patients additionally suffer from impaired awareness of hypoglycemia (IAH) (defined as the diminished ability to perceive the onset of low blood glucose levels), which is associated with elevated risk of severe hypoglycemic events (SHEs) and consequently higher risk of morbidity and mortality (5). Furthermore, the risk of hypoglycemic events increases with the duration of T1D.

On the other hand, T2D is a common chronic condition caused primarily by defective insulin secretion from the pancreatic β cells and/or insulin resistance (6). It has a multitude of risk factors including obesity, genetic predisposition, physical inactivity, diet contributions and ageing, and therefore has a wide range of treatment options from lifestyle intervention to oral medications and insulin therapy. A vicious cycle exists in which persistent hyperglycemia leads to progressive decline in β cell compensation and eventual onset of β cell dysfunction (7–9). As a result, subjects progress from normal glucose tolerance to impaired glucose tolerance, and ultimately develop full-fledged T2D.

Replacement of β cell function through pancreatic islet transplantation is an established standard of care procedure (akin to organ tissue transplant) to treat T1D patients with impaired hypoglycemia awareness and who experience multiple SHEs in several countries, such as Canada, Australia, parts of Europe and Asia (10). Human islets for allogeneic use are isolated from deceased donor pancreases, following a series of tissue digestion, isolation, purification, and qualification steps. As prescribed in the Edmonton Protocol, which played a key role in revolutionizing islet transplantation since the 2000s, human islets are transplanted by infusion into the hepatic portal vein of the recipient based on the required islet equivalents (IEQ) per kilogram of the recipient's body weight, alongside a steroid-free immunosuppression regimen (11). Patients may need to be dosed with multiple islet infusions from different donor pancreases to achieve euglycemia successfully. The procedure has since remained the standard protocol for islet transplantation and was seen as a promising step towards a T1D cure. Long term follow ups of patients for up to 20 years after transplant at a single centre showed that those with sustained graft survival no longer suffered from SHEs, displayed better insulin independence, and long term safety despite chronic immunosuppression (12). Another 5 year follow up of over 1200 patients across multiple centres similarly established the overall safety and efficacy of islet transplantation (13). Patients with T1D benefit from allogeneic islet transplantation through substantial improvement in glycemic control, almost complete abrogation of SHEs, reduction in insulin doses, and ultimately improvement in quality of life.

While islet transplantation may be life-changing for T1D patients, patients need to be willing and able to undergo long term, intensive immunosuppression. As with other solid organ transplantation, the side effects and complications that result require careful consideration of the risk to benefit ratio. The treatment can result in serious side effects such as increased risk of infections, malignancies, kidney damage, vomiting, nausea and diarrhoea (14–16). Immediate complications associated with intrahepatic islet transplantation includes instant blood-mediated inflammatory reaction (IBMIR), caused by direct exposure of the islets to the bloodstream, which triggers pro-inflammatory cytokine release followed by complement activation and recruitment of innate immune cells which further exacerbates inflammation and

destruction of islets (17). For these reasons, the overall impact of islet transplantation in its present form remains limited. With regards to T2D, due to the multifactorial nature of diabetes development and the presence of insulin resistance, patients with T2D have yet to be considered for islet cell replacement therapy in the clinical setting. However, it is possible to consider that specific subsets of T2D patients that have severe insulin deficiency with normal insulin sensitivity (18) may benefit from renewable islet cell replacement to reinstate insulin production.

With the increasing prevalence of T1D and T2D globally, patient eligibility issues and complications associated with immunosuppression, coupled with overall shortage of cadaveric human islets will aggravate the socio-economic burden from disease. This situation highlights the need for novel approaches to protect islet allografts and overcome immunological challenges associated with allogeneic islet transplantation. In this review, we will examine the current status of primary human islet transplantation, the key challenges surrounding the need to undergo chronic immunosuppression and the lack of sufficient human donor islets. We also touch on developments in transplantation of islet cells derived from alternative sources, and promising avenues using bio-engineering or cell-based engineering approaches to protect transplanted islets from immune rejection.

2 Human allogeneic primary islet transplantation and its associated challenges

2.1 Current status of pancreatic islet transplantation

Human pancreatic islet transplantation offers a functional source of β cells for the treatment of diabetes, especially in a subset of patients with T1D who are prone to hypoglycemic unawareness and experience severe hypoglycemia despite optimal management of glycemic levels (11, 12, 19, 20). These have far-reaching benefits beyond physiological changes such as improvement in patients' mental health, relief for caregivers, resumption of work productivity and reduced ambulance conveyance and emergency care needed. The success of the treatment was made possible due to the seminal research by Shapiro and team, who developed the Edmonton Protocol, building on previous achievements by others (for a detailed review on the history of clinical islet transplantation please see (21)). The procedure recommends transplantation of a cumulative islet mass of at least 10,000 IEQ per kilogram of the recipient's body weight. This typically required at least two infusions from different donor material, unless insulin independence was achieved with a single infusion (11, 20). Islets are transplanted into the hepatic portal vein, the current clinical gold standard route, avoiding the need for surgery. The procedure allows the islet cells to access the circulatory system and facilitate glucose sensing and insulin release into the bloodstream, effectively restoring glycemic control in patients. Repeated islet infusions do carry the risk of procedure-related bleeding arising from elevated intraportal vein pressure and portal vein thrombosis (22). The protocol had set a standard for the infusion of an adequate islet mass combined with a glucocorticoid-free immunosuppressive regimen (e.g tacrolimus and sirolimus) (20). In recent practices, daclizumab (non-depleting monoclonal anti-interleukin-2 receptor antibody) and/or anti-thymocyte globulin is administered as pre-procedural induction immunosuppression, whereas low-dose tacrolimus (calcineurin inhibitor) in combination with mycophenolate mofetil or sirolimus is prescribed for maintenance immunosuppression (23). Sirolimus (mTOR inhibitor) has been found to be more poorly tolerated by patients with adverse side effects, hence its exclusion may result in improved longer-term outcomes. Likewise, although tacrolimus-based immunosuppression is effective against allo- and auto-immune rejection, its side effects include nephrotoxicity and diabetogenicity due to effects on the islet cells. Additionally, other anti-inflammatory agents are needed in the peri-transplant periods to counter proinflammatory cytokines and preserve islet function, such as etanercept (TNFα blocker) and anakinra (IL-1 receptor antagonist) which were found to be associated with improved clinical outcomes as compared to regimens without the use of anti-inflammatory agents (12, 24). Thus, ongoing research efforts remain important to define immunosuppressant and anti-inflammatory drug combinations with better safety profiles while remaining effective for preserving islet graft function.

Islet transplantation has been and will continue to be a life-changing therapy as it has resulted in positive outcomes for patients including insulin independence, glycemic control, freedom from SHEs and restoration of hypoglycemia awareness. These outcomes are positively correlated with graft survival and function (fasting C-peptide >0.1 nmol/L post-transplantation) and achievement of HbA1c level of <7.0% (53 mmol/mol) at least 1 year post-transplant (12, 19, 25). Furthermore, despite the long period under an immunosuppressive regimen, sustained islet function in those with sustained graft survival is possible, though the incidence of cancer appeared to be higher (12, 19, 25). In the recently FDA-approved donor-derived pancreatic islet cell therapy for T1D, known as Lantidra or donislecel (manufactured by CellTrans Inc.), the therapy is indicated for adults with T1D where exogenous administration of insulin is insufficient to maintain the HbA1c target and who experience hypoglycemia unawareness (26, 27). FDA approval was based on experiences from Phase I/II clinical trials that demonstrated graft survival in all 10 patients and insulin independence maintained in 60% of patients 5 years post-transplant, as well as in another Phase III clinical trial revealing that all 21 patients were free from hypoglycemic episodes and most maintained HbA1c levels at ≤ 6.5% at a 1-year follow up (28, 29). Importantly, no significant side effects were reported for the cell therapy except for procedural-related bleeding. The approval represents a positive step forward for T1D management in the US, though there remained controversy over the recognition of islets as drugs instead of organs that may place a limitation over patient access.

2.2 Obstacles limiting widespread adoption of human primary allogeneic islet transplantation

Islet transplantation has proven to be a promising curative approach for T1D patients, both saving lives and improving quality of life. However, there remain several challenges hindering its widespread clinical utility, particularly the need for chronic immunosuppressive therapy and limited donor islet availability (Figure 1).

Firstly, as islet transplant patients are required to undergo intensive life-long immunosuppression to prevent graft rejection and loss of islet function, the selection of immunosuppressants used may induce side effects or autoimmunity recurrence, which will influence islet transplantation outcome. For instance, induction of immunosuppression with anti-thymocyte globulin as compared to daclizumab, and maintenance of immunosuppression with tacrolimus as compared to sirolimus, has been shown to increase risk of autoantibody recurrence in islet transplantations (30). This study highlighted the "off-target" effects of immunosuppressants, particularly how immunosuppressants influence the profile of regulatory T cells (Tregs), which are an important subset of immunomodulatory T cells responsible for promoting immune tolerance. Immunosuppressants that foster a richer Tregs environment could drive tolerance and further minimize the need for immunosuppression (31). Previous studies investigating the impact of immune-modulatory drugs on the function of Tregs showed that sirolimus has a Tregs-favoring effect as compared to tacrolimus (32). Greater clarity on the immunological mechanisms mediated by the immunosuppressants would guide future directions in preserving Tregs numbers and function for better success in balancing immunosuppression and transplant outcome.

Chronic immunosuppression has also been associated with other detrimental side effects, such as β cell toxicity, kidney toxicity, higher risk of cancer and opportunistic infections as the protective function of the patient's immune response is jeopardized. Reported symptoms experienced by patients include anaemia, nausea, fatigue, diarrhoea, and abdominal pain, though the incidents varied amongst patients and depended on the number of islet infusions and length of follow-up (33, 34). Common adverse effects occurred in between 20% to 90% of patients from initial infusion up to 1 year following final infusion, that are mostly related to the infusion procedure and immunosuppressive regimen administered (34). In the event of a life-threatening infection or cancer requiring discontinuation of immunosuppressive medications, there was eventual loss of islet cell function and resumption of insulin dependence (34). Additionally, patients who are contraindicated for immunosuppression, such as those with relevant drug allergies or who are highly susceptible to acute or chronic infections, are not eligible for islet transplant. T1D patients often possess pre-existing renal impairment due to longstanding diabetes and their renal dysfunction may be exacerbated by tacrolimus which can result in calcineurin-induced nephrotoxicity (35, 36). In addition to nephrotoxicity, tacrolimus is further associated with gastrointestinal side effects leading to episodic diarrhoea. Sirolimus has also been linked to several side effects including mouth ulcerations, neutropenia, dyslipidemia, small bowel ulceration, peripheral edema, and the development of ovarian cysts in females (37). The lifetime risk of lymphoma is estimated to be 1-2% in transplant recipients undergoing long-term immunosuppression, with the most common malignancies being non-melanomatous skin cancers (38). At supratherapeutic levels, tacrolimus and sirolimus have also been associated with human islet toxicity caused by increased amyloid deposition and disrupted insulin granule formation, though the detrimental effects on the β cells may be reversible upon withdrawal of drug treatment (39). For all these reasons, patients must be screened for endogenous infections or pre-existing medical conditions that can be aggravated following immunosuppressant therapy, and the risks weighed alongside the benefits (non-recurring severe hypoglycemia and achieved target HbA1c) to patients. Hence, islet transplantation has only been considered for T1D patients complicated by IAH and SHEs, when other lines of treatment have failed to prevent life-threatening SHEs, placing a significant limitation on the large pool of diabetes patients who could benefit from islet cell replacement therapy.

A second major factor limiting the accessibility of islet transplantation is the lack of sufficient cadaveric donor islets to meet the global demands for human islet cell replacement. More than 2,000 patients have received allogeneic islet transplantation globally since year 2000 (10). This is only a small fraction of the millions of individuals who have been afflicted with brittle T1D, not

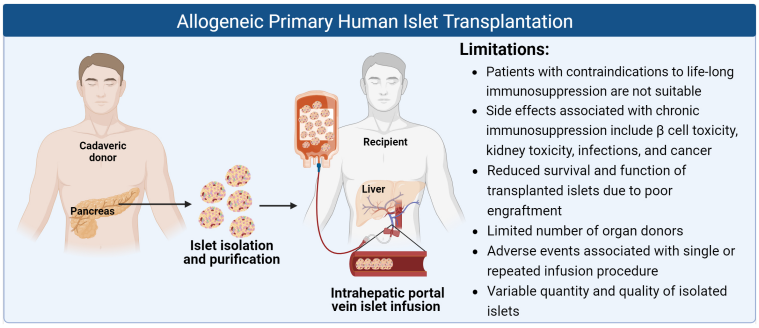

FIGURE 1
Schematic of allogeneic primary human islet transplantation and its limitations (Created with BioRender.com).

to mention an even larger number of patients with insulin-deficient T2D for which such a treatment is currently not an option. A recent report published by National Health Service (NHS) England stated that the waiting time for islet transplant was 631 days and the number of patients on the active islet transplant list in the UK is 29 by the end of September 2021 (40). In addition to the lack of suitable deceased donor pancreases, the quality of islet products is highly variable depending on the circumstances under which the donor organ is obtained, donor characteristics, the complex islet isolation and culturing process and the preservation conditions before transplant (41). The method of isolating islets by enzymatic digestion and mechanical separation can lead to potential damage of the endocrine cells. Majority of islet transplant recipients receive islets from multiple donors (2 to 4) as up to 60% of islet mass is lost within the first few days following islet infusion. While transplantation of larger islet mass (>11,000 IEQ/kg of recipient weight) over multiple islet infusions contributes to a larger mass of surviving β cells, this limits the number of patients that can receive the islet allografts (42). Efforts have been made across several islet isolation facilities to harmonize the donor selection criteria, manufacturing procedures, and lot release attributes, but this remains a huge undertaking to be controlled at all phases and implemented at a larger scale (43, 44).

2.3 Xenogeneic islets as an alternative primary cell source for transplantation

Xenogeneic islets, in particular porcine islets, have been explored as an alternative primary cell source to supplement the supply of primary human islets for transplantation. Porcine islets are more readily available and possess functional characteristics that make them a suitable substitute for human islets. They have weaker immunogenicity and porcine insulin is structurally similar to human insulin (with one amino acid difference that is alanine in pigs and threonine in humans) (45). Major hurdles need to be overcome for xenografts to be a feasible alternative in the clinic. These include physiological incompatibility and immunological reaction to non-human donor tissues that trigger both innate and adaptive barriers of the immune system, resulting in rejection. In addition, xenotransplantation presents the potential risk of zoonosis and porcine endogenous retrovirus (PERVs). As such, previous studies have evaluated the feasibility of xenotransplantation of porcine islets into non-human primates (NHPs). One study showed that an anti-CD40 (2C10R4) monoclonal antibody-based immunosuppressive regimen together with tacrolimus was effective in circumventing graft rejection and prolonging porcine islet graft survival in diabetic rhesus monkeys, with median survival (serum porcine C-peptide concentration of >0.15 ng/mL) of 60 days. All monkeys also received anti-thymocyte globulin, cobra venom factor (CVF), adalimumab, and sirolimus (46). In another study, a newly engineered anti-CD40L-specific monoclonal antibody AT-1501 was tested in a cynomolgus macaque model that had undergone intrahepatic islet allotransplantation. The study showed that AT-1501 enabled long-term graft survival with higher C-peptide levels detected compared with conventional immunosuppression (47). AT-1501 was modified to minimize risk of thromboembolic complications that were previously reported for CD40L-based therapies in clinical trials, and therefore appears to be a promising and safe agent for further testing. Another strategy to enhance graft survivability is to utilize genetically-modified pigs with alterations in expression of known xeno-antigens, and modification of the complement and coagulation systems to improve immunological compatibility between pigs and NHPs (48). In one example, cardiac xenografts from genetically-modified pigs with alpha 1-3 galactosyltransferase gene knockout, expression of human complement regulatory protein CD46 and human thrombomodulin, were transplanted into baboons (49). The pre-transplant immunomodulatory induction regimen included anti-thymocyte globulin and 2C10R4 antibody, followed by maintenance with intensively-dosed 2C10R4 antibody and mycophenolate mofetil (49). This combination of genetic modifications and immunosuppressive regimen resulted in sustained survival of the xenografts with median of 298 days up to the longest of 945 days observed (49).

In further attempts to reduce immune rejection after xenogeneic islet transplantation, porcine islets may be encapsulated in a protective layer to avoid immune cell recognition. In one study, neonatal porcine islets were encapsulated in a stable and permeable alginate gel and enclosed in a biocompatible, immunoprotective membrane, and transplanted in the abdominal cavities of immunocompetent diabetic mice. Islet xenograft survival, rapid lowering of blood glucose and long-term glycemic control for >200 days was achieved without any immunosuppressants (50). Furthermore, the devices were shown to retain their integrity after they were retrieved and re-transplanted in new immunocompetent diabetic mice. In a clinical study, alginate-based encapsulation of neonatal porcine islets were transplanted into the peritoneal cavity of eight T1D patients without immunosuppression at up to 20,000 IEQ/kg body weight over two separate transplantations (51). The procedure was shown to be safe with no PERVs infection detected. Some fibrosis of the microcapsules were observed post-transplant, however long-term efficacy was shown with HbA1c <7% over more than 600 days and significant reduction of serious unaware hypoglycemia (51). More encapsulation studies involving porcine islets are discussed in a later section and in Table 1, which lend support to the clinical benefit provided by porcine islet xenotransplantation in T1D patients.

Besides the need to address the genetic and molecular discrepancies between human recipients and xeno-organs, other challenges to note include psychosocial and ethical barriers, tension from religious beliefs, concerns for animal welfare and the use of animals for research. Nonetheless, the careful use of existing or novel immunosuppressive therapies, development of genetically-modified pigs to obtain porcine islets with better immune tolerance, and use of encapsulation to provide immune protection (to be discussed in greater detail in section 3.1) make it possible for porcine islets to be considered as another safe, functional and readily available source of primary cells for T1D patients. This will help to overcome the ongoing shortage of donor human islets.

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TABLE 1 Summary of studies investigating the use of encapsulation (micro/macro) technologies for immune isolation of allogeneic primary islets or stem cell-derived islets for cell transplantation.

Strategy	Encapsulation Material/Device	Cell type	*In vivo* transplantation in humans or animal models (If any)	Outcome of transplanted cells	Ref
Microencapsulation	Chitosan hydrogel	Wistar rat islets	Yes, diabetic C57BL/6J mice	Encapsulated islets secreted insulin in response to glucose stimulation, reduced blood glucose levels for four weeks, and resulted in faster glucose disappearance rate after IPGTT compared to naked islets. Immunostaining confirmed insulin-positive cells in the graft and negative staining for T-cell lineages and monocyte/macrophages.	(52)
	Alginate/ polyaminoacidic-based (patented)	Human islets	Yes, T1D patients (non-immunosuppressed)	Improved HbA1c levels with positive serum C-peptide response for 3 years post-transplant. Absence of immune infiltration observed by negative expression of anti-MHC class I-II and GAD65 antibodies 3 years post-transplant.	(53)
	Alginate/poly-L-lysine/ alginate (APA)	Neonatal porcine islets	Yes, T1D patients (non-immunosuppressed), diabetic C57BL/6J mice	Reduced unaware hypoglycemia events in all patients. HbA1c < 7% achieved in 4 of 14 patients (from 1 of 14 at baseline). Reversal of diabetes and positive porcine C-peptide in mouse study.	(54)
	Alginate/poly-L-lysine/ alginate (APA)	Neonatal porcine islets	Yes, T1D patients (non-immunosuppressed)	Improved HbA1c < 7% for >600 days with reduced frequency of unaware hypoglycemia events.	(51)
	Multiple alginate sphere formulations with chemically modified alginate derivatives	Cynomolgus monkey islets	Yes, non-diabetic macaques (non-immunosuppressed)	Allogeneic islets encapsulated with Z1-Y15 alginate derivative retained high viability, were glucose-responsive 4 months post-implantation in the bursa omentalis. Reduced macrophage infiltration and foreign-body reaction (FBR) and pericapsular fibrotic overgrowth (PFO) score in encapsulated islet grafts.	(55)
	Alginate polymer incorporated with immunomodulatory chemokine CXCL12	hESC-derived β cells	Yes, diabetic C57BL/6J mice	Enhanced insulin secretion of β cells, accelerated normalization of hyperglycemia with glycemic correction lasting >150 days. Limited infiltration of effector T cells, macrophages and increased recruitment of $Foxp3^+$ regulatory T cells to the islet grafts.	(56)
Macroencapsulation	Collagen-covered device	Neonatal porcine islets combined with Sertoli cells	Yes, T1D patients (non-immunosuppressed)	Two of 4 patients had significant reduction in insulin requirement maintained up to 4 years. Porcine insulin following glucose stimulation was detectable up to 4 years. Presence of insulin-positive cells from the explanted grafts were observed in all patients post-transplant.	(57)
	Semi-permeable ethylene-vinyl alcohol copolymer membrane	Mouse pancreatic β cell line MIN6	Yes, diabetic C57BL/6 mice	Lowered blood glucose levels for 30 days in diabetic mice, no host cells within device found, no difference in circulating inflammatory cytokines in mice with and without transplant.	(58)
	TheraCyte™ device	Lewis rat islets	Yes, diabetic Wistar-Furth (WF) rats	Graft function was maintained for 6 months in both immunized and nonimmunized rats. Immunized rats showed high IFN-γ producing $CD8^+$ T cells as compared to control rats transplanted with encapsulated islets.	(59)
	Sernova Cell Pouch	Syngeneic mouse islets	Yes, diabetic BALB/c mice	Restored glycemic control and showed glucose-responsiveness for 40 days. Islets within cell pouch were stained positive for insulin, glucagon, and endothelial cells.	(60)
	TheraCyte™ macroencapsulation device	Wild-type C57BL/6 neonatal pancreatic tissue	Yes, T1D RIP-LCMV.GP mice	Lowered blood glucose and the onset of diabetes was prevented in some recipients. Absence of $CD8^+$ T cells in the vicinity of encapsulated C57BL/6 grafts.	(61)
	VC-01 (PEC-Encap); Physical barrier that protects transplanted grafts from host immune cell infiltration	hESC-derived pancreatic endoderm progenitor cells	Yes, T1D patients (non-immunosuppressed)	Prolonged cell survival for 24-months and positive staining for pancreatic islet cell markers, NKX6.1, insulin and glucagon was observed. No evidence of autoimmune rejection based on a panel of immune function markers.	(62)

(Continued)

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TABLE 1 Continued

Strategy	Encapsulation Material/Device	Cell type	*In vivo* transplantation in humans or animal models (If any)	Outcome of transplanted cells	Ref
	βAir device with two compartments: a refillable oxygen tank and an alginate and polymembrane covered chamber for immune isolation	Allogeneic human pancreatic islets	Yes, T1D patients (non-immunosuppressed)	Islet survival for 3-6 months, however, limited functionality, minute circulating C-peptide levels and no benefit on metabolic control was observed. Fibrotic tissue with immune cells were formed surrounding the capsule.	(63)
	VC-02 (PEC-Direct); non-immunoprotective to allow direct vascularization of implanted cells	hESC-derived pancreatic endoderm progenitor cells	Yes, T1D patients (immunosuppressed)	Engraftment and insulin expression were observed in 63% of subjects. Detectable C-peptide in 35% of subjects from 6 to 24 months post-implantation though with little clinical benefit. Infiltration of host myofibroblasts into devices.	(64)
	Macro device with alginate gel microcapsules enclosed in a semipermeable membrane bag with immuno-isolation	Neonatal porcine islets	Yes, diabetic C57BL/6NCr mice	Improved glycemic control for more than 200 days. Explanted devices exhibited almost no adhesion or fibrosis and showed sustained insulin secretion.	(50)
	VX-264; "channel array" macroencapsulated β cells	Allogeneic hiPSC-derived β cells	Yes, T1D patients	Ongoing clinical trial with no disclosed outcomes yet.	(65)
	Sernova Cell Pouch	Allogeneic human islets	Yes, T1D patients	Insulin independence observed for 6 to 38 months with persistent fasting and stimulated C-peptide levels. Surviving functional islets detected in Cell Pouches excised at >90 days post-transplant.	(66)

3 Innovations in bio-engineering and cell-based approaches for cell replacement therapy to address immunological issues

Various strategies have emerged to specifically address the immunogenicity of transplanted cells in islet cell replacement therapy (Figure 2). These not only aim to improve engraftment and functionality of the islet cells, but also make such a therapy more accessible to a wider diabetes population.

3.1 Development of encapsulation technologies to provide an immunoprotective environment for transplanted islets

To protect from allograft rejection and recurrence of autoimmunity, cell encapsulation is a common strategy that can provide a physical barrier to shield the islets from immune cell recognition and attack. Specifically, encapsulation helps to mediate IBMIR which is caused by islet contact with the blood and is highly detrimental to cell survivability. An ideal encapsulation device or material should be biocompatible but not biodegradable, made of a semi-permeable material to allow entry of nutrients and oxygen, and enable release of hormones and metabolic by-products into the bloodstream. Such a strategy can be based on microencapsulation or macroencapsulation (Table 1). Microencapsulation is a method in which islet cell clusters are individually encapsulated in spherical capsules typically 300 µm to 600 µm in diameter (67), and commonly in alginate-based hydrogels. In contrast, macroencapsulation is based on devices greater than 100 mm with capacity to house a larger mass of islet cells within the membrane. Micro-capsules have an optimal surface-to-volume ratio compared to macro-capsules that require vascularization and sufficient oxygenation to improve islet survival and function. Biomaterials used for microencapsulation are often made of natural polymers such as alginate (55), agarose (68), chitosan (52) but also synthetic polymers such as polyethylene glycol (PEG) that form a hydrogel (50, 54, 56, 69) (Table 1). Microencapsulation encompasses a semi-permeable membrane that has demonstrated some success in providing immune protection and mechanical stability in mice (50, 52), NHP (55) models and human studies (51, 53). However, microencapsulation requires more complex and individualized fabrication processes (70), as opposed to macroencapsulation devices that may be easier to manufacture, are more easily retrievable after implantation, and are more favourable for commercialization. Several devices that have been developed include Theracyte™ from TheraCyte Inc., βAir from BetaO$_2$ Technologies, the Cell Pouch System from Sernova, and PEC-

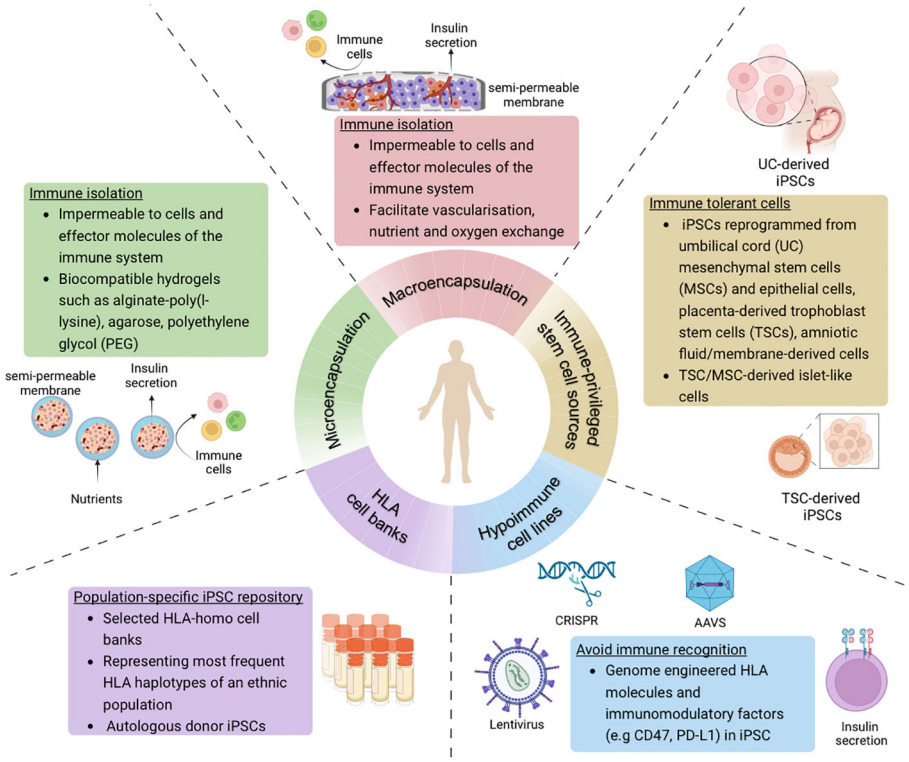

FIGURE 2
Overview of strategies to avoid immune recognition and allograft rejection of stem cell-derived islet cells in the context of cell-based therapy for diabetes treatment (Created with BioRender.com).

Encap (VC-01) and PEC-Direct (VC-02) from ViaCyte (now acquired by Vertex Pharmaceuticals) (Table 1).

The Theracyte™ planar macroencapsulation device consists of an inner hydrogel semi-permeable membrane layer for immune protection and an outer polytetrafluoroethlene membrane layer for neovascularization (71). Rodent islets encapsulated within the Theracyte™ device demonstrated survival and functionality in an immunized rat model for at least 6 months post-transplantation (59). Porcine islets transplanted subcutaneously within Theracyte™ also survived and was able to reverse diabetes up to 8 and 16 weeks in cynomolgus monkeys and NOD mice respectively (72). Another device known as the βAir bioartificial pancreas (BAP) has been tested clinically and consists of two compartments for islets, an oxygenated chamber that maintains physiological oxygen pressure, covered with a porous polytetrafluoroethylene (PTFE) membrane impregnated with alginate to provide the immunoprotective barrier (73–75). Stable graft function and insulin secretion were observed in NHP models of diabetes (73) and human patients (75) who received βAir containing porcine islets and human islets respectively, both in the absence of immunosuppressants, though complete insulin independence was not achieved. In one Phase I clinical study, βAir containing allogeneic human islets were subcutaneously implanted in T1D patients (clinicaltrials.gov: NCT02064309) (63). Although the transplanted islets survived 6 months post-transplantation, limited functionality was observed based on minute levels of circulating C-peptide with no impact on glycemic control in the patients (63). Additionally, fibrosis or inflammation were observed on the surface of the chamber. Another promising technology is the proprietary Cell Pouch system developed by Sernova, which is an implantable device that provides a vascularized tissue matrix for cells in addition to local microencapsulation of cells in polymer spheres. The Cell Pouch is undergoing testing in T1D patients in an ongoing Phase I/II clinical trial (clinicaltrials.gov: NCT03513939). In an interim update, patients with islet transplants in the 8-channel Cell Pouches were found to achieve insulin independence for as long as 3 years as a result of both functional islet grafts in the Cell Pouches supplemented by a modest intraportal islet transplant top-up through the portal vein (66). Additionally, a second version of the Cell Pouch with higher capacity is being evaluated and early patient data so far revealed persistent serum C-peptide levels detected from a single islet transplant in the 10-channel Cell Pouch (66). The company announced a collaboration with Evotec to test out human induced pluripotent stem cell (hiPSC)-derived islet cells in the Cell Pouch system in future clinical trials (66).

In another Phase I/II clinical trial by ViaCyte, human embryonic stem cell (hESC)-derived pancreatic endoderm cells (PECs) were encapsulated in a cell-impermeable device designed to be immunoprotective against recipient immune systems (clinicaltrials.gov: NCT02239354). The macroencapsulation devices containing cells (also known as VC-01 or "PEC-Encap") were implanted subcutaneously in T1D patients in the absence of immunosuppression (62, 76), and evaluated for efficacy, tolerability,

and safety. Formation of neovasculature was observed in the grafts and the PECs were able to mature *in vivo* into insulin-expressing β cells, as shown by immunohistochemical staining for pancreatic islet cell markers (NKX6.1, insulin, and glucagon) (62). VC-01 was found to be safe, well-tolerated and immunoprotective with evidence of prolonged cell survival up to 24 months. However, some inconsistency of cell survival was observed amongst subjects due to varying foreign body responses in the host (62, 76). Furthermore, no evidence of insulin secretion was found due to chronic damage to islets resulting from device fibrosis (62). This suggested that the macroencapsulation device, although well-tolerated in recipients, resulted in poor long-term engraftment and diminished efficacy due to poor oxygenation and nutrient supply to the transplanted cells. In efforts to mitigate cell loss due to device fibrosis, a subsequent version of the combination product (also known as VC-02 or "PEC-Direct") was developed to include engineered portals in the device to enable direct capillary permeability and facilitate better vascularization to the implanted cells (clinicaltrials.gov: NCT03163511) (76). However, this was a non-immunoprotective device and patients still required immunosuppression to limit allo- and autoimmune responses. This time, patients exhibited meal-stimulated C-peptide secretion following maturation of the PECs *in vivo* and achieved the target blood glucose range for longer periods (26 weeks) as compared to VC-01 (76). Subjects in which substantial cell engraftment were observed after evaluating the explants were shown to have higher meal-responsive C-peptide levels during the follow-up period as compared to those with poor cell engraftment (64). While VC-02 is generally safe and well-tolerated, the side effects of immunosuppression accounted for majority of adverse events (AEs). Another study utilizing the same VC-02 device but with an optimized membrane perforation increased the initial implanted cell dose (14 x 10^6 cells per kg body weight) such that it is within the range of that used for intrahepatic primary islet transplants (6-18x 10^6 cells per kg body weight) (20, 77, 78). After 6 months post-transplantation, only 3 of 10 patients achieved C-peptide levels ≥0.1 nmol/L with reduced insulin dependence, and the detectable β cell mass in the retrieved implants was found to be less than 5% of the initial cell mass, indicating high cell loss and limited efficacy from the device-delivered PECs (78). This could be due to insufficient vascularization in the devices to support the metabolically functional β cell mass. Further optimization remains needed to increase the efficacy of the encapsulated PECs to be comparable to that of conventional human primary islet transplantation. These outcomes suggest that the macroencapsulation devices not only need to prevent entry of immune cells, but also facilitate (even encourage) vascularization to enable better cell survival and maturation into functional β cells and reduce infiltration of fibroblasts into the devices.

In another recent effort to evaluate macroencapsulated stem cell-derived islets in the absence of immunosuppressants, Vertex Pharmaceuticals, who has an ongoing first-in-human Phase I/II clinical trial (clinicaltrials.gov: NCT04786262) for their allogeneic stem cell-derived, fully differentiated islet cells (79), revealed the development of their second cell therapy program (clinicaltrials.gov: NCT05791201) investigating the islet cell product encapsulated in a "channel array" device and implanted subcutaneously (65). Though the design of the device used in the trial has not been disclosed, based on publicly-available patent filing information from the company, such a device would have a thickness of at least 300μm, an average pore size ranging from 5 nm to 2500 nm and comprising 1 x 10^6 to 1 x 10^9 (PCT/US2018/053665). The proprietary design also showed deformation of the membrane to a formed configuration instead of a flat configuration, with channels that enable vascularization in and around the device. The pore size had to be fine-tuned to ensure long-term structural integrity while allowing release of insulin and restricting leakage of cells out of the device (PCT/US2018/053665 and PCT/US2018/037637). It remains to be seen whether the device allows sufficient nutrient and oxygen supply, as well as provide immune tolerance in the absence of immunosuppression.

Across the numerous efforts from academic labs and commercial companies to develop and test macroencapsulation devices for islet cell transplant (Table 1), prevention of immune attack is found to be achievable, but a balance needs to be struck to achieve other outcomes including better vascularization and oxygenation, maximising transplanted β cell mass, preservation of β cell viability and function following implantation, and reduction of foreign body reactivity. These devices also require additional unique considerations related to manufacturing and regulatory oversight as medical devices for use in a clinical setting, evaluation of the biocompatibility of the materials, and selection of transplantation site and protocol given the larger size of the devices.

3.2 Combining hiPSC technology and genetic engineering to generate hypoimmune cells

Since the use of hiPSC technologies became widespread, hiPSCs have proven to be highly versatile and amenable to genetic manipulation. The generation of functional hiPSC-derived islets (SC-islets) has also made significant headway in recent years, making it possible for regenerative medicine to be part of a not-so-distant future in diabetes therapy. The journey of developing SC-islets in the lab to be as close to their primary human islet counterparts as possible, and the promise of using these cells as off-the-shelf therapy for islet cell replacement, have been extensively discussed in other recent reviews (80–82). Previously, promising preliminary clinical results had been released from Vertex Pharmaceuticals on their ongoing first-in-human Phase I/II clinical trial of lead candidate VX-880 (clinicaltrials.gov: NCT04786262), which is a hiPSC-derived, fully differentiated islet cell product administered to T1D patients in a similar fashion as primary human islets, in the presence of intensive chronic immunosuppressive therapy (83). Six patients with a history of undetected insulin secretion tolerated the therapy well, demonstrated islet cell engraftment with production of endogenous glucose-stimulated insulin and had improved glycemic control. Patients that were followed up at the 1-year mark also displayed successful elimination of SHEs and reduction

in HbA1c <7.0% (83). This was a landmark shift from ViaCyte's PEC grafts, which required cellular maturation *in vivo* into functional glucose-sensing and insulin-secreting β cells, a process that cannot be monitored and qualified before transplant.

In combining SC-islet differentiation protocols and genome engineering techniques (84–89), several novel approaches have been employed to generate human islet cells that are protected from immune rejection, potentially eliminating, or reducing the need for systemic immune suppression and/or encapsulation. These immune evasive strategies typically work by either artificially elevating immune suppressive proteins (e.g. immune checkpoint manipulation) or removing receptors important for immune cell recognition on the cell surface (Figure 3). An essential component of innate and adaptive immune responses is the major histocompatibility complex (MHC) class I and II molecules which serve to present foreign antigens to the cell surface for recognition by the host immune system. In humans, these MHC molecules, also known as human leukocyte antigens (HLA), are highly polymorphic with almost 10,000 alleles. Immune rejection of hiPSC-derived cells or tissues from an allogeneic donor are mediated through these MHC molecules, limiting the survivability and therapeutic potential of the transplanted cells. Immunological mechanisms governing allograft rejection occurs in two stages: (1) non-specific innate responses predominate in the early phase, and (2) antigen-specific adaptive responses by antigen presenting cells (APCs) and dendritic cells (DCs) that result in recognition of donor antigens by host T cells (90). Both innate and adaptive immunity contribute to acute or chronic graft rejection.

To prevent innate immune rejection and further suppress adaptive immune responses, various groups have developed genetically engineered hypoimmunogenic hiPSCs or hESCs through modification of selected HLA genes and other immunomodulatory factors, and evaluated the ability of these pluripotent stem cells and their derivatives to escape immune recognition (91–93). Table 2 provides a summary of key studies that developed hypoimmunogenic cells and evaluated the ability of the cells to evade the host immune response in both *in vitro* and *in vivo* assays.

The potential application of hypoimmunogenic hPSC-derived islet cells for cell replacement therapy has been demonstrated in several pre-clinical studies in immunocompetent diabetic animal

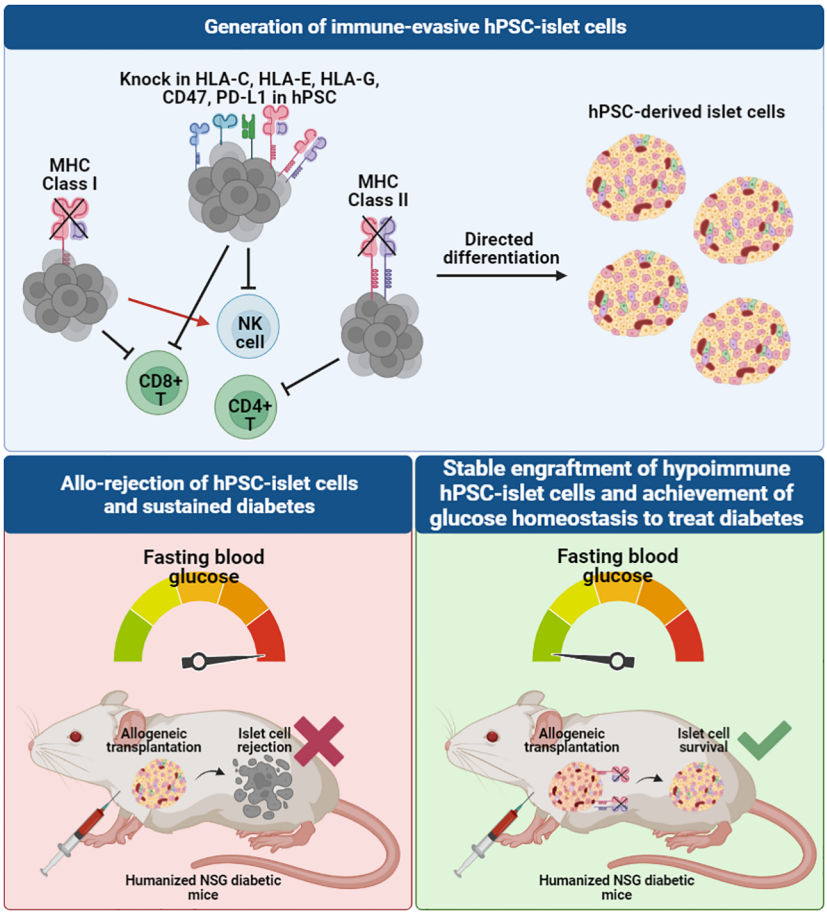

FIGURE 3
Immune-evasive hPSC-derived islet cells can be developed through genome-editing of the hiPSC source to knock out MHC class I and II molecules and knock in other immunomodulatory markers to evade different T cell and NK cell recognition, creating a tolerogenic microenvironment for allogeneic transplantation. When transplanted in humanized diabetic mouse models, unedited allogeneic hiPSC-derived islet cells face graft rejection, whereas hypoimmunogenic allogeneic hiPSC-derived islet cells survive and are able to rescue diabetes to achieve normal blood glucose levels in mice.

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TABLE 2 Strategies for developing and evaluating hypoimmunogenic stem cell-derived islet cells, primary islets, and other cell types.

Experimental strategy	Cell type evaluated	Outcome of *in vitro* validation	Outcome of *in vivo* validation	Reference
Pancreatic islet cells or β cells				
CRISPR/Cas9 knockout of *B2M* and *CIITA*, expression of CD47 by lentiviral transduction	miPSC and miPSC derived endothelial cells (EC), smooth muscle cells (SMC), cardiomyocytes (CM).	Enzyme-linked immunospots assay (Elispots) with splenocytes recovered 5 days after transplantation showed that WT miPSCs had strong IFN-γ and a moderate IL-4 response, as compared to engineered miPSCs that did not induce any antibody response in allogeneic mice.	*In vivo* monitoring of luciferase expression from transplanted cells showed that all three WT miPSC derivatives survived up to 50 days in syngeneic C57BL/6 mice, whereas WT miPSC derivatives were rejected in allogeneic mice, in the absence of immunosuppressants. In contrast, engineered miPSC-derived ECs, SMCs showed 100% long-term survival in both syngeneic and allogeneic mice.	(91)
	hiPSCs and hiPSC derived ECs and CMs	Mice transplanted with WT hiPSCs, and hiPSC-derived ECs and CMs demonstrated strong IFN-γ response and elevated IgM levels, as compared to recipients of engineered hiPSCs that did not mount an IFN-γ response or cellular or humoral immune response. Engineered hiPSC derivatives did not trigger NK cell activation or NK cell killing in killing assays.	WT and engineered hiPSCs were injected into allogeneic humanized NSG-SGM3 mice. *In vivo* monitoring of luciferase expression demonstrated WT hiPSC derivatives were rejected, whereas engineered hiPSC derivatives showed stable luciferase signals over time and long-term graft survival (50 days).	
Expression of PD-L1 by lentiviral transduction in hiPSCs	hiPSC-derived β-like cells	Reduced expression of immune ($CD45^+$) cells in recovered grafts based on *ex vivo* flow cytometric analysis.	Kidney capsule transplantation of PD-L1-expressing β-like cells in C57BL/6J diabetic mice provided sustained control of blood glucose, as compared to those lacking PD-L1 expression. Glycemic control correlated with detectable serum human C-peptide and glucose homeostasis was observed for up to 50 days in immune-competent mice.	(88)
CRISPR/Cas9 knockout of *HLA-A/B/C* and *CIITA* while retaining $HLA\text{-}A2^R$ in hESCs	hESC-derived β-like cells	Retaining expression of HLA-A2 in combination with HLA-E expression reduced NK cell activation in NK cell degranulation assays. Flow cytometric analysis of mice splenocytes and peripheral blood demonstrated absence of T cells (CD45) and NK cells (CD3) 4 weeks post-transplantation. Hence, suggesting resistance to T cell and NK cell cytotoxicity.	Transplantation in the spleen of immunodeficient NSG and $NSG\text{-}MHC^{null}$ mice followed by luciferase monitoring and survival of grafts with $HLA\text{-}A2^R$ cells up to 16 weeks post-transplantation	(87)
CRISPR/Cas9 knockout of *B2M*, overexpression of PD-L1 and HLA-E in hESCs	hESC-derived β-like cells	Measurement of luminescence demonstrated *B2M* knockout SC-islets exhibited significantly improved survival compared to WT SC-islets when IFN-γ treated SC-islets were co-cultured with PBMCs *in vitro*.	No significant difference in blood glucose levels of mice transplanted with WT and *B2M* knockout SC-islets under the kidney capsule of diabetic humanized NSG-MHC class I/II knockout mice. However, upon injection of mismatched HLA-A2 PBMCs, WT SC-islets were rejected within 2 weeks resulting in loss of *in vivo* graft function. In contrast, *B2M* knockout SC-islets showed delayed graft rejection while retaining some *in vivo* graft function as demonstrated by GSIS.	(86)
CRISPR/Cas9 knockout of *B2M* and *CIITA*, expression of CD47 by lentiviral transduction	Primary human pancreatic islets, hiPSC-derived islets	Reduced or lack of NK cell or macrophage killing of engineered hypoimmune primary pseudo-islets and iPSC-derived islets based on in vitro impedence killing assays.	Transplantation in the hindlimb muscles of immunocompetent, diabetic humanized NSG-SGM3 mice, followed by glucose monitoring. WT islet grafts were fully rejected over 7 to 10 days, whereas hypoimmune islets survived, engrafted, and achieved glycemic control for up to 29 days, as shown by *in vivo* luciferase assay.	(85)
CRISPR/Cas9 knockout of *B2M* and *CIITA*, expression of macaque CD47 by lentiviral transduction in hiPSCs	hiPSCs	Serum collected from rhesus macaque transplanted with WT islets demonstrated a peak in total IgM (after 7 days) and IgG (after 13 days) donor specific antibodies (DSA), based on antibody-dependent cellular cytotoxicity (ADCC) and macrophages or complement-dependent cytotoxicity (CDC) assays. In contrast, rhesus macaque transplanted with hypoimmune cells did not induce DSAs and did not undergo ADCC or CDC cytotoxicity.	Hypoimmune hiPSCs injected subcutaneously in the back of immunocompetent allogeneic rhesus macaque demonstrated unrestricted survival for 16 weeks, whereas WT cells were rejected within 6 weeks, as shown by *in vivo* luciferase assay.	(84, 89)

(Continued)

TABLE 2 Continued

Experimental strategy	Cell type evaluated	Outcome of *in vitro* validation	Outcome of *in vivo* validation	Reference
Pancreatic islet cells or β cells				
CRISPR/Cas9 knockout of *B2M* and *CIITA*, expression of macaque CD47 by lentiviral transduction in hiPSCs	hiPSC-derived β-like cells	Histology staining demonstrated the injected sites for WT islets had no evidence of injected cells after 28 days, whereas sites injected with hypoimmune β-like cells had well-formed islets with no apparent inflammation observed.	WT and hypoimmune β-like cells were injected into the thigh muscle of immunocompetent allogeneic humanized NSG-SGM3 mice. Reduction in fasting hyperglycemia, and ameliorated diabetes in mice injected with hypoimmune β-like cells were observed up to 28 days. In contrast, WT islet transplants showed no effect on glucose levels in diabetic mice.	(84)
CRISPR/Cas9 knockout of *B2M* and *CIITA* and expression of rhesus macaque CD47 by lentiviral transduction	Primary rhesus macaque islets	Flow cytometric analysis of WT and hypoimmune rhesis macaque demonstrated nulled expression of HLA Class I, no difference in HLA Class I expression and significant increase in CD47 expression as compared to WT islets.	WT and hypoimmune islets were injected into the quadricep muscle of immunocompetent rhesus macaques. Hypoimmune islets achieved long-term survival up to 40 weeks, whereas WT islets were rejected within 1 week, as demonstrated by *in vivo* luciferase assay.	(84)
Other cells				
AAV-mediated knockout of *HLA-A/B/C* and knock in of *HLA-E* in hESCs	hESC-derived CD45+ hematopoietic derivatives	T cell-mediated cytotoxicity assay demonstrated that $CD8^+$ T cells efficiently lysed $B2M^{+/+}$ $CD45^+$ cells, but did not kill $B2M^{-/Edimer}$, and $B2M^{-/Etrimer}$ cells *in vitro*.	Luciferase-expressing $B2M^{+/+}$ and $B2M^{-/Etrimer}$ ESC-derived teratomas and primed allogeneic $CD8^+$ T cells were subcutaneously injected in immunodeficient NSG-B2M knockout mice. More growth was observed in $B2M^{-/Etrimer}$ teratoma as compared to $B2M^{+/+}$ after $CD8^+$ cell infusion.	(92)
CRISPR/Cas9 knockout of *HLA-A/B/C* and *CIITA*, knock in of PD-L1, *HLA-G* and *CD47* in AAVS1 site in hESCs	hESC-derived endothelial cells (ECs) and vascular smooth muscle cells (VSMCs)	WT and engineered hESCs-derived ECs were pretreated with IFN-γ and co-cultured with carboxyfluorescein succinimidyl ester (CFSE)-labeled allogeneic $CD3^+$ T cells. Flow cytometric analysis demonstrated reduced proliferating T cells ($CD3^+$), reduced activation markers ($CD69^+$ and $CD154^+$) in engineered ECs as compared to WT. Similarly, allogeneic NK cells co-incubated with engineered hESC-VSMCs demonstrated significantly reduced NK cell degranulation compared to WT, as shown by flow cytometric analysis of CD107a.	WT and engineered hPSCs were subcutaneously injected in immunodeficient mice and monitored for teratoma formation over the course of 4 to 6 weeks. WT teratomas displayed a slower increase in volume compared to engineered teratomas. Furthermore, histology staining and qPCR analysis of human effector T cell markers CD8 and IL-2 had demonstrated reduced T cell infiltration in engineered cell lines compared to WT.	(93)
CRISPR/Cas9 knockout of *HLA-A/B* (haploid *HLA-C*) or *B2M* knockout in hiPSCs	hiPSC-derived $CD43^+$ blood cells and cardiomyocytes	^{51}Cr release assays performed with HLA-reactive T cells demonstrated that *HLA-A/B* knockout (haploid *HLA-C*) and *B2M* knockout hiPSC-$CD43^+$ blood cells could evade $CD8^+$ T cell-mediated cytolytic activity, but not in WT cells. Flow cytometric analysis measuring CD107a expression in NK cells co-cultured with hiPSC-$CD43^+$ blood cells, had demonstrated significantly lower NK cell-mediated cytotoxicity in engineered hiPSC-$CD43^+$ blood cells compared to WT.	Luciferase-expressing iPSC-$CD43^+$ blood cells were pre-treated with IFN-γ and injected intraperitoneally into NRG mice. After transplantation of $CD43^+$ blood cells, $CD8^+$ T cells were injected. *In vivo* luciferase monitoring demonstrated significantly higher survival ratio of HLA-A/B knockout (haploid HLA-C) $CD43^+$ blood cells as compared to WT after 7 days. *HLA-A/B* knockout (haploid *HLA-C*) also showed significantly better survival *in vivo* when NK cells were injected after transplantation of hiPSC-$CD43^+$ blood cells, as shown by *in vivo* luciferase expression.	(94)

models (85–89). Simultaneous deletion of beta-2-microglobulin (B2M), a component of MHC class I-encoded HLA-A/B/C molecules, and MHC class II transactivator CIITA in hiPSCs (93) and primary human islets (85) resulted in ablation of cytotoxic $CD8^+$ and $CD4^+$ helper T cell responses. When evaluated for immune tolerance *in vitro*, B2M and CIITA knockout hESC- and hiPSC-derivatives were co-cultured with T cells, NK cells and PBMCs and were found to be resistant to T cell, NK cell, and complement-dependent cytotoxicity and macrophage engulfment (86–88, 93, 94) (Figure 3). The expression of the non-classical MHC molecules, HLA-E and HLA-G, were also found to contribute to establishing an immunosuppressive microenvironment by binding the inhibitory NK cell receptors CD94/NKG2A and facilitating the escape of human tumors from the host immune response (95). A previous study showed that HLA-A/B/C knockout hESCs and their differentiated CD45+ cells and RPE cells that overexpress HLA-E are resistant to CD8+ T cell cytotoxicity and NK cell-mediated lysis in both *in vitro* and *in vivo* models (92).

Another immunomodulatory effector is CD47 which acts as an anti-phagocytic ligand to inhibit activation of the innate immune

system (96). CRISPR/Cas9-mediated knockout of B2M and CIITA together with lentiviral transduction-based overexpression of CD47 in mouse and human iPSCs were effective in generating hypoimmunogenic derivatives that did not trigger NK cell activation *in vitro* (91). Loss of immunogenicity was also recapitulated *in vivo*, as observed by significant improvement in graft survival post-injection of mouse iPSC- and hiPSC-derived smooth muscle cells (SMCs), endothelial cells (ECs) and cardiomyocytes (CMs) into the right thigh muscle of immunocompetent C57BL/6 mice (91). This approach was replicated using hiPSC-derived SMCs, ECs and CMs that were transplanted into humanized NSG-SGM3 mice. Humanized mice have been widely used as a pre-clinical *in vivo* model that recapitulates the human context, in this case the human immune system. NSG-SGM3 mice used in the study supports the stable engraftment of human myeloid lineages, regulatory T cell populations and production of hIL-15, thereby promoting the development and/or function of human NK cells. $B2M^{-/-}$/$CIITA^{-/-}$/$CD47^{+}$ hiPSC-derivatives showed sustained graft survival for more than 50 days, whereas unedited WT derivatives were rejected within 14 days (91).

A similar strategy was also validated in hypoimmune $B2M^{-/-}$/$CIITA^{-/-}$ and CD47-overexpressing primary human islets (85). WT and hypoimmune human islets injected intramuscularly in humanized immunocompetent mice were monitored using bioluminescence imaging. WT islets were fully rejected within 7 to 10 days, exerted no beneficial effect on glucose homeostasis and no detectable C-peptide secretion after 29 days. In contrast, mice injected with hypoimmune islets showed allograft survival and achieved glycemic control, indicating that the function of allogeneic hypoimmune islets was sustained and confirming the ability of $B2M^{-/-}$/$CIITA^{-/-}$/$CD47^{+}$ to modulate immunogenicity and escape immune attack (85). More recently, Hu et al. also reported the successful rescue of an immunocompetent, diabetic cynomolgus monkey with allogeneic, hypoimmune $B2M^{-/-}$/$CIITA^{-/-}$ and CD47-overexpressing primary rhesus macaque islets (89). C-peptide remained detectable in the monkey serum and insulin independence was achieved without immunosuppression for up to 6 months (89). These results show that hypoimmune islets can be protected from immune rejection while maintaining graft function *in vivo*.

Programmed death-ligand (PD-L1) is an immune checkpoint protein that has also been in the spotlight as it plays a role in suppression of adaptive immune response by inducing a co-inhibitory signal in activated T cells and promoting T cell apoptosis (97). Gerace et al. reported genetically engineered B2M-deficient hESCs with PD-L1 overexpression in addition to HLA-E overexpression. The authors found that in response to PBMC injection, WT SC-islets transplanted under the kidney capsule of diabetic humanized NSG-double knockout (hu-NSG-DKO) mice were destroyed within 2 weeks due to PBMC-mediated cytotoxicity, whereas graft rejection was delayed when $B2M^{-/-}$ SC-islets were transplanted. At 7 weeks post-PBMC injection, $B2M^{-/-}$ SC-islets gave rise to positive glucose-stimulated insulin secretion (GSIS) outcomes and were able to reverse diabetes in mice whereas graft function was lost in mice transplanted with WT SC-islets (86). These results suggested that removal of B2M could delay the rejection of the SC-islets, though it is possible that the grafts may eventually be completely rejected in longer term studies. The authors however showed that overexpression of PD-L1 in the $B2M^{-/-}$ SC-islets did not protect the cells from xeno-rejection, and that overexpression of HLA-E did not provide additional protective benefit against NK cell cytotoxicity in their model. Instead, they found that SC-islets engineered to secrete tolerogenic cytokines such as IL-10 and TGF-β are protected against xeno-rejection likely due to recruitment of Tregs to induce a tolerogenic environment. The engineered SC-islets could reverse diabetes in NOD mice up to 8 weeks post-transplantation. Another group however showed that PD-L1 overexpression could create an immune-evasive microenvironment for SC-islets transplanted in immunocompetent diabetic mice, by restricting T cell activation and delaying graft rejection (88). While both SC-islets with and without PD-L1 overexpression had similar efficacy in restoring glycemic control in diabetic mice within a few days, the functionality of the islets lacking PD-L1 was quickly lost. On the other hand, islet cells overexpressing PD-L1 provided sustained blood glucose homeostasis, with human C-peptide levels correlating with glycemic control for more than 50 days (88).

While most of the reported work on the development of hypoimmune cells have been within pre-clinical settings, new efforts are now emerging to evaluate the cells in clinical studies. CRISPR Therapeutics (previously in conjunction with ViaCyte) is conducting first-in-human Phase I clinical trials with an investigational, allogeneic, gene-edited, hypoimmune stem cell-derived PECs for T1D (clinicaltrials.gov: NCT05210530, NCT05565248). The cells are also encapsulated in a device to be implanted in patients without immunosuppressive therapy. Vertex Pharmaceuticals also announced that it will license CRISPR Therapeutics' gene-editing technology to add value to their ongoing efforts in the clinical development of iPSC-derived islet cell therapy for T1D (98). Although details of the partnership were not disclosed, the collaboration is likely to explore the development of hypoimmune, fully-differentiated iPSC-derived islet cells for transplantation into T1D patients without immunosuppression (with or without encapsulation). These studies aim to establish whether generation of universal hypoimmunogenic hPSCs differentiated into insulin-producing islets could provide long-term survival due to evasion of immune-mediated detection and killing. Positive outcomes from the trials will mean maximising the efficacy of the transplanted islet cell mass and providing a longer term, immunosuppression-free curative therapy for allogeneic recipients.

Despite the attractiveness of genome-edited hypoimmune cells as a cell source for allogeneic cell therapy, the long-term safety and efficacy remains to be ascertained as most studies are currently conducted *in vitro* or in animal models. As hypoimmune cells can escape immune detection, this raises concerns on cell malignancy, especially for hPSC derivatives which may give rise to tumour formation in the presence of any residual hPSCs or incompletely differentiated cells in the graft. Furthermore, CRISPR-based genome editing may induce unintended off-target genomic mutations that may contribute to aberrant gene expression that may contribute to malignancy (99–101). Therefore, tumorigenicity tests as well as evaluation of the genomic and epigenomic stability of modified cell lines remain essential to qualify any hPSC-based cell product for clinical applications. New generations of gene-editing

tools have also been developed to improve on the design of nucleases, repair templates and analysis of potential off-target editing to reduce tumorigenicity risk and unintended outcomes (102–104). For instance, expression systems containing suicide gene constructs can potentially eliminate any tumorigenic cells that arise, to safeguard against tumour formation in hPSC-based cell therapies (105). A recent study showed that a combination of immune-cloaked mouse ESCs in which several immunomodulatory transgenes are being expressed, coupled with a genomically integrated FailSafe suicide transgene system, was able to generate various ESC-derived tissues that possess immune privilege. Allogeneic cells transplanted in these 'artificially-created', immune-privileged sites could be protected from rejection for months (106). The FailSafe system is a patented technology which creates a transcriptional link between the suicide herpes simplex virus thymidine kinase gene (*HSV-TK*) and a cell division gene (*CDK1*) to enable killing of any undesired dividing cells using a pro-drug treatment (106–108). Such safeguards help to improve the safety profile of cell therapy products, particularly those that are engineered to be more immune tolerant. For similar safety reasons, having the cells implanted within an encapsulation device also facilitates easy removal of the cells in case there is a need for the graft to be excised.

3.3 Use of immune privileged stem cell sources to generate islet cells

Another strategy for transplanted grafts to be potentially shielded from the immune system involves the use of naturally immune privileged stem cell sources. Several tissues in the body are evolutionarily adapted to be protected from inflammatory immune responses, including extra-embryonic tissues such as the amnion, placenta and umbilical cord. These tissues possess immune privileges so that maternal tolerance toward fetal cells may be maintained. Specifically, these tissues contain stem cells such as umbilical cord lining mesenchymal stromal cells (CL-MSCs), amniotic MSCs and placenta-derived trophoblast stem cells (TSCs). These cells also represent valuable cell sources for the generation of hiPSCs. It is postulated that hiPSCs derived from immune privileged cells may retain some of the same genetic signatures and epigenetic memory. Whether the differentiated cells from these hiPSCs also maintain their 'privileged' status however remains to be tested in a cell type-specific manner. Accumulating evidence suggests that the immunogenicity of hiPSC-derived cells are cell type-dependent, as different cell types exhibit different immunomodulatory mechanisms. Retaining at least some extent of the immune privileges of the original tissue stem cells may help in resisting immune destruction in the event of allogeneic transplantation.

MSCs are multipotent stem cells with high proliferative capacity, low immunogenicity and immune modulation properties due to the expression of tolerogenic factors. Successful reprogramming of placental amniotic membrane MSCs and amniotic fluid stem cells at high efficiency has previously been shown (109–111). The hiPSCs retained the immunomodulatory signatures of the MSCs, such as absence of expression of MHC class I and II proteins, and expression of HLA-G and CD59 (109). Umbilical cord lining epithelial cells (CL-ECs) are another population of cells that do not express MHC class II molecules and co-stimulatory molecules, and express non-classical HLA-E and -G, that function to suppress maternal T cell and NK cell responses (112). Therefore they not only have low immunogenicity but also possess some immunosuppressive capacity (113, 114). In a recent study, CL-ECs differentiated into retinal pigment epithelial (RPE) cells and transplanted into mice and monkey models were found to elicit reduced pro-inflammatory responses and immune cell infiltration compared to transplanted RPE cells differentiated from skin-derived hiPSCs (115). There have been few published reports, if any, exploring differences in immune tolerance of islet cells derived from hiPSCs reprogrammed from different cell sources, representing a gap that warrants further study.

Besides using hiPSCs as the starting source of cells for differentiation into insulin-secreting islet cells, MSCs have also been directly differentiated into islet-like cells by genetic manipulation (116) or step-wise induction using specific medium and small molecules *in vitro* (117–125). Umbilical cord-derived MSCs (UC-MSCs) are attractive as a starting material as they can be obtained through pain-free and non-invasive methods, are available in abundance, and have high proliferation and differentiation capacity (118). Previous studies demonstrated that UC-MSCs do not induce allogeneic PBMC immune responses and can suppress the function of mature dendritic cells *in vitro* (113). UC-MSC-derived islet-like cell clusters also retained their immune privileged properties *in vivo* and were capable of regulating glucose homeostasis (118). Primitive stromal cells isolated from the umbilical cord Wharton's jelly have also been differentiated directly into insulin-secreting islet-like cell clusters that express beta cell markers C-peptide and PDX1, and higher levels of secreted insulin compared to bone marrow-derived MSCs (126). Such an approach is however less widely adopted than hiPSC-based differentiation into islet cells, in part due to the lack of reproducibility in the functionality of the β-like cells derived using these methods, and the fact that MSCs experience replicative senescence, which will limit the ability to continuously generate differentiated cells at larger scale. Another immune privileged cell type of note are TSCs, which are a unique population of stem cells derived from the placenta that are fetal in origin, and that form the interface between the fetus and mother throughout pregnancy (127). They are immune tolerant as they have little to no expression of the classical MHC molecules and may also be differentiated into the different germ layers. Therefore, TSCs are another understudied cell source for allogeneic cell therapy, and attempts to derive islet cells directly from TSCs have yet to be reported.

In conclusion, the use of immune privileged stem cells as alternative cell sources for hiPSC generation or for direct differentiation into islet cells could be another strategy to eliminate or reduce the intensity of immunosuppressive therapy without the need for genome editing. Nonetheless, whether the differentiated cells retain their immune privilege, and to what extent, would be crucial to ascertain in a cell type-specific manner.

3.4 Comprehensive stem cell banks to facilitate HLA donor matching

It is known that the predominant mediator of allograft rejection is HLA mismatch triggering T cell-mediated rejection. HLA molecules found on the surface of most cells have an important role in enabling the immune system to recognise "self" versus "non-self" antigens (128). The MHC system in humans consists of the classical MHC class Ia (HLA-A, -B, -C), non-classical MHC class Ib (such as HLA-E, -F, -G) and MHC class II (HLA-DR, -DQ, -DM, -DP) molecules that are involved in antigen presentation to $CD8^+$ T cells (129), natural killer cells (NK cells) (130), and $CD4^+$ T cells (131). HLA matching is not currently a criterion for primary human islet transplantation, however retrospective studies have showed that matching at selected loci, particularly HLA-DR and HLA-B, could improve long term islet allograft survival (132, 133), which would further lead to more prolonged insulin independence in patients. Another study involving follow-up of pancreas transplants purported that the number of HLA-DR and HLA-B matches correlated with a reduction in acute graft rejection, though there was no evidence to suggest a similar correlation with graft or patient survival rate (134). In other studies featuring other tissue transplantations, HLA matching has been shown to result in reduced allogeneic immunogenicity, increased graft survival, and therefore potential reduction in the intensity of immunosuppression required (135–138). Although HLA typing to match donor and recipient antigens at selected loci is clinically feasible, incorporating HLA haplotype matching in primary islet transplantation remains challenging due to existing pressure from limited cadaveric donors and the need for islet infusions from multiple donors. Thus, an avenue that may be explored relates to the formation of a repository of hiPSCs carrying different HLA haplotypes that may be matched to many recipients within a given population. Such a repository would be made up of clinical-grade HLA homozygous hiPSCs derived from carefully selected donors with homozygous HLA types to enable HLA matching when the need for an allogeneic transplantation arises (139). Based on prior experiences from cord blood and kidney grafting studies, HLA-A, -B, and -DR have been indicated as the most important HLA loci to match for long-term graft survival, with or without immunosuppressive drugs (140–142).

Several groups across different countries have embarked on efforts to derive repositories of HLA-homozygous hiPSCs that capture the high frequency HLA haplotype backgrounds (most typically for HLA-A, -B and -DRB1) in their population. The Center for iPS Cell research and Application (CiRA) of Kyoto University runs an iPSC Stock Project that aims to establish an HLA-homozygous iPSC haplobank for most of the Japanese population (143, 144). They recently reported a clinical-grade iPSC haplobank consisting of 27 iPSC lines from seven HLA-homozygous donors that could cover 40% of the Japanese population (145). Generation of HLA-homozygous iPSC lines for coverage of other geographical populations and ethnic groups have also been shown in Korea, where ten of the most frequent HLA-homozygous lines can match 41% of the population (146, 147), in United Kingdom (139), Spain (148), and China (149). The feasibility of such an endeavour has also been explored in Brazil, where it is estimated that 3.8 million people have to be screened to obtain 559 triple HLA-homozygous cell lines covering 95% of the population (150). In Finland (151), the top ten most frequent haplotypes homozygous for HLA-A to -DQB1 were compatible with 49.5% of the population. In Australia (152), haplotyping frequencies could be estimated from existing national blood banks or cord blood banks. A probabilistic model developed by Gourraud et al. evaluated multiple ancestry backgrounds and estimated that construction of a hiPSC bank representing 20 of the most frequent HLA haplotypes in each of the European American and African American populations would require screening of 26,000 and 110,000 donors respectively (153). This would match with over 50% of the European American and 22% of the African American populations respectively (153). Population-specific hiPSC haplobanks may also be deployed for other populations, especially closely related ones compared to ethnically-diverse populations. For such a biobank to be used for clinical applications, the cell banks have to be manufactured in compliance with Good Manufacturing Practice (GMP) and be qualified as a clinical grade cell bank, which is is highly costly, resource-intensive and not a trivial undertaking. The efficiency of such an effort depends on successfully identifying the desired HLA-homozygous haplotypes in an opportunistic manner within a screened population (potentially a prohibitively large one), unless a population-wide genomic data or blood bank typing data exists that allows for donor recall by genotype. In addition, data have shown that HLA-matching alone may be insufficient for successful allogeneic engraftment, and immunosuppression may still be required (137, 154). Furthermore, tolerability of HLA-matched hiPSCs may be dependent on other non-MHC factors such as the method of reprogramming, the cell type being transplanted (different cell types have different levels of immunogenicity), and the site of transplantation (whether the site is immunologically privileged) (155).

In addition to universal and 'super donor' hiPSC banks that we have discussed so far, personalized donor-specific hiPSC banks can also be generated for autologous use. As protocols to reprogramme easily accessible somatic cells (such as blood) are now standardized, it is possible to generate hiPSCs successfully for many individuals, at scale. There are increasingly more solutions being developed for large scale, high throughput generation of hiPSCs (156–159). These support the potential for autologous transplantation using patients' own iPSCs as starting material to reduce immune-mediated graft rejection and therefore eliminate or reduce the need for immunosuppressive therapy. Guha et al. demonstrated that syngeneic mouse iPSCs differentiated into the three embryonic germ layers had little to no immunogenicity when transplanted into the subcapsular renal space of preclinical models (160). Morizane et al. also demonstrated that autologous transplantation of iPSC-derived dopaminergic neurons in the brains of NHPs elicited minimal immune response (161). These studies evaluated immunogenicity in different transplant sites, and transplantation in immune-privileged sites such as the central nervous system, brain and eyes do not generally trigger an immune response as compared to other sites, hence the site of transplantation must be considered

even in an autologous setting. There are also several other considerations unique to hiPSC-derived cell therapy. The immunogenicity of autologous grafts remains to be validated as potential causes of immunogenicity include immaturity of the hiPSC derivatives, the reprogramming process and extended period of culturing and passaging of hiPSCs leading to genetic and epigenetic changes, and other off-target effects when gene correction is done on the donor cell line (162). To support the notion that stem cell derivatives exhibit variable immunogenic properties, Zhao et al. showed that autologous iPSC-derived SMCs are highly immunogenic to the immune system due to the dysregulated expression of immunogenic proteins, whereas iPSC-derived RPE cells are not immunogenic (163). Therefore, hiPSC-derived cells may not retain their immune privileged properties upon differentiation, resulting in immune attack, possibly due to differing levels of expression and activity of immunomodulatory proteins during the cellular differentiation process (163). Long-term (>4 months) evaluation of graft function and immune responses need to be considered when translating pre-clinical findings to the human context. The creation of donor cell banks for patients in need would be costly and time-consuming, given that reprogramming and qualification of the cell bank, followed by subsequent differentiation into islet cells, could easily take at least a few months. This is in contrast with other currently approved autologous therapies such as chimeric antigen receptor (CAR)-T cell immunotherapy which takes 2 to 3 weeks from apheresis to cell infusion. Nonetheless, autologous hiPSC-based cell therapy remains a useful platform for evaluating the safety and efficacy of regenerative medicine treatments for disease without many of the concerns that allogenic transplantations pose (164).

4 Discussion

Human islet transplantation has demonstrated substantial success in improving the lives of T1D patients who were suffering from SHEs, and restoring their insulin independence. There lies a lot more potential for T1D patients and even selected T2D patients,to benefit from an islet cell replacement therapy. However, here we have discussed major obstacles that need to be overcome including the need for chronic immunosuppression, lack of sufficient organ donors, and immune responses that negatively impact on graft function.

Reduction or removal of immunosuppression is the key to being able to treat diabetes sustainably with a curative therapy, whether through organ/tissue transplant or a regenerative medicine approach. The rise of hPSC-derived islet cells for therapeutic use represents a new paradigm shift in regenerative medicine. Various strategies have been highlighted here, namely: (1) fine-tuning of the immunosuppressive regimen to reduce side effects, (2) exploring alternative primary cell sources such as porcine islets, (3) using immunoprotective encapsulation materials or devices to preserve the long-term function of the transplanted cells, (4) using hiPSC-derived islet cells and genetic engineering approaches to provide a renewable and well-characterized source of cells that can evade the host immune system, (5) harnessing the immune-privileged properties of tissue-derived stem cells to make hiPSCs or perform direct differentiation to islet cells, and (6) manufacturing a repository of HLA-homozygous hiPSCs suitable for clinical applications. In reality, it is likely that a combination of a few of these strategies will be needed. Current and future pre-clinical and clinical work will need to be at the intersection of multiple strategies, such as the use of encapsulated islet cells derived from HLA-selected hiPSC lines that have been genetically engineered to possess more immune-tolerant and safety features. However, adopting multiple strategies will also mean needing to address the shortcomings of each, and adding layers of complexity to eventual clinical translation (Table 3). It is likely that there is no one-size-fits-all strategy.

Immune isolation or encapsulation of islets relies on a physical barrier to protect graft function. There are many gold standard biomaterials used for encapsulation of islets (refer to Table 1) that are straightforward to mass produce. The long-term durability of the biomaterials *in vivo* will need to be tested and optimized in an application specific manner. For translational purposes, production of the encapsulation materials/devices need to conform with good manufacturing practices and ISO standards normally under the regulation of medical devices. Encapsulation has been tested on all of primary human islets, porcine islets and SC-islets (Table 1), and it is feasible for such platform technologies to be developed to suit different cell types and disease applications. Macroencapsulation devices have been shown to be applied to cardiovascular diseases (165–167) and CAR-T cell therapy (168, 169) and shown promising preclinical outcomes as well.

Although more hiPSC-based cell therapeutic products are being tested in the clinic now, indicating that safety testing can meet the regulatory barrier for clinical trial authorizations of specific products, there remains many reservations about product safety that have to be managed for each unique cell type. The creation of universal hiPSC lines that elude immune recognition can offer tremendous promise for regenerative medicine applications, beyond cell therapy for diabetes. Genetic modifications to engineer hypoimmune iPSC-derived endothelial cells and cardiomyocytes have demonstrated efficacy in treating cardiovascular and pulmonary diseases in immunocompetent allogeneic mice (170). However, these hypoimmune cells also present a safety risk after human application and need to be carefully monitored. This is due to the potential for undesirable tumorigenicity arising from residual undifferentiated, pluripotent cells in the final product. Therefore, in the presence of conventional immunosuppressive therapy, or in the case of modified hiPSCs that can escape immune surveillance, the body may not be able to detect and respond to potential malignancies. Additional genetically engineered safeguards for hiPSC-based products are being developed for elimination of aberrant cell growth (106, 171), but it is uncertain how these cells would behave in human patients over time. As for ethnic-specific HLA haplotype cell banks, though established from homozygous HLA haplotypes, they may not provide a complete match and therefore a combination of encapsulation technologies to provide additional immune isolation, and/or some use of immunosuppressants or anti-inflammatory drugs are still needed to prevent graft rejection. There are also other factors not related to MHC compatibility that can trigger immune responses, such as undesirable gene disruptions

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TABLE 3 Comparative analysis of immune evasion strategies for islet cell therapy.

	Microencapsulation Material	Macroencapsulation Device	Immune privileged stem cell sources	Hypoimmune hiPSCs	HLA-selected hiPSC repository
Advantages	Prevents immune cells from recognizing the transplanted cells	Prevents immune cells from recognizing the transplanted cells	Naturally possess low immunogenicity and even immunosuppressive capacity	Genetically engineered in a customizable manner to evade the host immune system	Facilitates HLA matching with large numbers of recipients
	Larger surface-to-volume ratio enables more efficient diffusion of oxygen and nutrients	Longer *in vivo* durability/stability compared to microencapsulation materials	Can be used to derive iPSCs or other cell types that may retain their immune privilege	Potentially a universal stem cell line	Provides a country/population-specific national cell resource
	Facilitates vascularization *in vivo*	Cell chambers may be refillable without device retrieval	Potentially applied without the need for immunosuppressant drugs		Potentially used with reduced intensity of immunosuppressant regimen
Potential for clinical applications	Can be optimized for different cell types	Can be optimized for different cell types but restricted to limited transplantation sites	Multipotent stem cells may be differentiated into a few (but limited) cell types; iPSCs may be differentiated into many different cell types	iPSCs may be differentiated into many different cell types	
	Provides flexibility for transplantation at different sites	Suitable for less invasive transplantation methods or sites (e.g subcutaneous implantation)	May be transplanted at different sites to suit different regenerative medicine applications		
Manufacturability	Requires manufacturing in conjunction with cells	May be mass manufactured independently of cells initially	Stem cells may be scaled up easily but have limited proliferative lifetime	Unlimited quantities of iPSCs may be generated to obtain universal cell bank	Unlimited quantities of iPSCs may be generated to obtain HLA type-specific cell bank
	Many medically-approved, biocompatible biomaterials available	Manufacturing of different layers or components required (such as inner membrane for immune protection, outer membrane for neovascularization) but may be highly tunable	High cost and resource-intensive for manufacturing clinical grade hiPSCs at scale, though with new technological developments the costs are likely to decrease in future		
Safety	Biocompatible materials available	Biocompatible materials available	Presence of partially differentiated cells or residual hiPSCs may pose tumorigenic risk		
	Difficult to retrieve depending on implantation site	Easy to retrieve in case of therapeutic failure or safety concerns	Difficult to retrieve depending on implantation site, especially without accompanying macroencapsulation device		
Other limitations	May be susceptible to enzymatic or hydrolytic breakdown in the body	Smaller surface-to-volume ratio may result in inefficient diffusion of oxygen and nutrients into and within the device	Immune privilege properties may be lost upon reprogramming and/or differentiation	Potential unintended off-target mutations from genome editing procedure	Generation of cell bank requires extensive screening and selection of donors
	Non-refillable and non-reusable	Need for vascularization to improve graft survival	Multipotent stem cells may not generate mature cell types that fully recapitulate the native function	Potential for aberrant malignant cells to escape immune detection	Large number of cell lines needed to cover majority of population
	Weak mechanical strength	Limited device volume requiring use of multiple separate devices	Immunogenicity of stem cell/hiPSC derivatives is cell-type dependent and every cell type generated needs to be evaluated		
		Limited options for transplantation site			

arising from the iPSC reprogramming or gene editing process, components of the culture media, and minor histocompatibility antigens (due to recognition of mutated proteins recognized as foreign antigens) even in the case of HLA-matched transplants.

As SC-islets are expected to be regulated as biologics, similar to the route that the US FDA had taken for donor-derived isolated pancreatic islets, drug manufacturing principles will apply for the regulation of the cell therapy (26). Unlike the limitations for freshly harvested and isolated primary human islets, it is possible for the sterility and potency of SC-islets, among other critical quality attributes, to be verified prior to clinical use (172). Lessons from clinical failures due to MSC product inconsistencies highlighted the need to establish appropriate product quality controls, owing to the variability in cell initiation and differentiation procedures, culture conditions and expansion processes among others (173).

There remain various ongoing efforts for improving the outcomes of islet transplantation. One area of research is on graft vascularization. Previous studies sought to improve graft re-vascularization through various methods such as transfection of tissues with mRNA encoding angiogenic growth factors (e.g vascular endothelial growth factor (VEGF-A)) (174), co-transplantation with vascular fragments (175), and pre-vascularization of the engrafted site (176, 177). Alternatively, re-vascularization of islets was also shown to be improved by resizing the islets into smaller clusters (≈150μm diameter) combined with a biocompatible polycation coating, that resulted in achievement of long-term euglycemia in immunocompetent mice up to 6 months (178). Alternative transplant sites have also been explored. For example, the intramuscular (179), gastric submucosa (180), eye (181), and perihepatic surface (182) are being investigated as alternative engraftment locations that may enhance the viability of grafts. Some immunologically privileged transplant sites enable allografts to survive for extended or even indefinite periods, however not all sites are suitable for islet transplantation in human patients due to site accessibility and potential side effects (more extensive review of alternative transplantation sites are out of the scope of this review).

Another innovative strategy to circumvent immunosuppression include co-transplantation of islets with immunosuppressive cells such as MSCs engineered to express PD-L1 and CTLA-4 (183), which act as accessory cells to induce local immunomodulation. Another study had showed that recipient-derived MSCs co-transplanted with islet allografts and MSCs infused in diabetic cynomolgus monkeys (fully MHC mismatched) after islet transplantation exhibited delayed rejection due to downregulation of memory T cells, reduced anti-donor T cell proliferation and increased Tregs (184). While promising, administration of immunosuppressive drugs and anti-inflammatory drugs albeit at reduced doses is still required, and the sustenance of the immunomodulatory effects exerted by the MSCs in the long run remains to be determined. As allogeneic MSCs provided poorer outcomes than autologous MSCs when used alone in the same study (184), the need to collect and process autologous MSCs will add to the complexity during clinical translation. Additionally, myeloid-derived suppressor cells (MDSCs), a cell population of myeloid origin that can mediate allogeneic immune responses, may potentially be co-transplanted with islet allografts to help prolong graft survival (185). Alternatively, a recent report combined cell and gene therapy by co-transplanting allogeneic islets with streptavidin-FasL-presenting microgels in the omental pouch of diabetic non-human primates (186). Using FasL as an immunomodulatory agent induced local tolerance in the absence of immunosuppression, due to increased number of FoxP3+ cells in the graft site.

Other areas that need to be addressed include reducing cost of manufacturing of the hPSC-derived islet cells, through automation, cryopreservation and better economies of scale when produced in large scale batches. Even if the risk from immunosuppression can be eliminated, there is also the question of where cell therapy falls within the pipeline of standard of care treatments for poorly controlled diabetes, in the face of insulin therapy or insulin pumps which are less invasive. This would also depend on the availability of resources to administer the cell product in the clinic, willingness to attend frequent follow-ups, and availability of insurance reimbursement.

Overall, the different areas in which the immunogenicity of transplanted islet cells can be tackled that we have discussed here, can help to direct current and future research and development work, to better formulate strategies to minimise or circumvent immune recognition and rejection in islet transplantation. These strategies will not only positively impact the lives of patients with complex T1D who tend to develop complications from conventional therapy, but hopefully be accessible by a wider group of diabetes patients in future.

Author contributions

BXH: Conceptualization, Visualization, Writing – original draft, Writing – review & editing. AKKT: Conceptualization, Funding acquisition, Supervision, Writing – review & editing. NHJN: Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

BXH is employed by BetaLife Pte Ltd. AKKT and NHJN are co-founders and shareholders of BetaLife Pte Ltd but are not employed by BetaLife Pte Ltd.

Publisher's note

References

1. Federation ID. *IDF Diabetes Atlas*. Brussels, Belgium: International Diabetes Federation (2021).

2. Mobasseri M, Shirmohammadi M, Amiri T, Vahed N, Hosseini Fard H, Ghojazadeh M. Prevalence and incidence of type 1 diabetes in the world: a systematic review and meta-analysis. *Health Promot Perspect*. (2020) 10:98–115. doi: 10.34172/hpp.2020.18

3. Atkinson MA, Maclaren NK. The pathogenesis of insulin-dependent diabetes mellitus. *N Engl J Med*. (1994) 331:1428–36. doi: 10.1056/NEJM199411243312107

4. Joish VN, Zhou FL, Preblick R, Lin D, Deshpande M, Verma S, et al. Estimation of annual health care costs for adults with type 1 diabetes in the United States. *J Manag Care Spec Pharm*. (2020) 26:311–8. doi: 10.18553/jmcp.2020.26.3.311

5. Lin YK, Fisher SJ, Pop-Busui R. Hypoglycemia unawareness and autonomic dysfunction in diabetes: Lessons learned and roles of diabetes technologies. *J Diabetes Investig*. (2020) 11:1388–402. doi: 10.1111/jdi.13290

6. DeFronzo RA, Ferrannini E, Groop L, Henry RR, Herman WH, Holst JJ, et al. Type 2 diabetes mellitus. *Nat Rev Dis Primers*. (2015) 1:15019. doi: 10.1038/nrdp.2015.19

7. Porte DJr. Clinical importance of insulin secretion and its interaction with insulin resistance in the treatment of type 2 diabetes mellitus and its complications. *Diabetes Metab Res Rev*. (2001) 17:181–8. doi: 10.1002/(ISSN)1520-7560

8. Poitout V, Robertson RP. Minireview: Secondary beta-cell failure in type 2 diabetes–a convergence of glucotoxicity and lipotoxicity. *Endocrinology*. (2002) 143:339–42. doi: 10.1210/en.143.2.339

9. Prentki M, Nolan CJ. Islet beta cell failure in type 2 diabetes. *J Clin Invest*. (2006) 116:1802–12. doi: 10.1172/JCI29103

10. Berney T, Andres A, Bellin MD, de Koning EJP, Johnson PRV, Kay TWH, et al. A worldwide survey of activities and practices in clinical islet of Langerhans transplantation. *Transpl Int*. (2022) 35:10507. doi: 10.3389/ti.2022.10507

11. Shapiro AM, Ricordi C, Hering BJ, Auchincloss H, Lindblad R, Robertson RP, et al. International trial of the Edmonton protocol for islet transplantation. *N Engl J Med*. (2006) 355:1318–30. doi: 10.1056/NEJMoa061267

12. Marfil-Garza BA, Imes S, Verhoeff K, Hefler J, Lam A, Dajani K, et al. Pancreatic islet transplantation in type 1 diabetes: 20-year experience from a single-centre cohort in Canada. *Lancet Diabetes Endocrinol*. (2022) 10:519–32. doi: 10.1016/S2213-8587(22)00114-0

13. Chetboun M, Drumez E, Ballou C, Maanaoui M, Payne E, Barton F, et al. Association between primary graft function and 5-year outcomes of islet allogeneic transplantation in type 1 diabetes: a retrospective, multicentre, observational cohort study in 1210 patients from the Collaborative Islet Transplant Registry. *Lancet Diabetes Endocrinol*. (2023) 11:391–401. doi: 10.1097/01.tp.0000994628.94091.b6

14. Ruiz R Fau - Kirk AD, Kirk AD. Long-term toxicity of immunosuppressive therapy. *Transplant Liver*. (2015), 1354–63. doi: 10.1016/B978-1-4557-0268-8.00097-X

15. Ryan EA, Paty BW, Senior PA, Shapiro AM. Risks and side effects of islet transplantation. *Curr Diabetes Rep*. (2004) 4:304–9. doi: 10.1007/s11892-004-0083-8

16. de Mattos AM, Olyaei AJ, Bennett WM. Nephrotoxicity of immunosuppressive drugs: long-term consequences and challenges for the future. *Am J Kidney Dis*. (2000) 35:333–46. doi: 10.1016/S0272-6386(00)70348-9

17. Naziruddin B, Iwahashi S, Kanak MA, Takita M, Itoh T, Levy MF. Evidence for instant blood-mediated inflammatory reaction in clinical autologous islet transplantation. *Am J Transpl*. (2014) 14:428–37. doi: 10.1111/ajt.12558

18. Ahlqvist E, Prasad RB, Groop L. Subtypes of type 2 diabetes determined from clinical parameters. *Diabetes*. (2020) 69:2086–93. doi: 10.2337/dbi20-0001

19. Brennan DC, Kopetskie HA, Sayre PH, Alejandro R, Cagliero E, Shapiro AM, et al. Long-term follow-up of the Edmonton protocol of islet transplantation in the United States. *Am J Transpl*. (2016) 16:509–17. doi: 10.1111/ajt.13458

20. Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med*. (2000) 343:230–8. doi: 10.1056/NEJM200007273430401

21. Marfil-Garza BA, Shapiro AMJ, Kin T. Clinical islet transplantation: Current progress and new frontiers. *J Hepatobil Pancreat Sci*. (2021) 28:243–54. doi: 10.1002/jhbp.891

22. Villiger P, Ryan EA, Owen R, O'Kelly K, Oberholzer J, Al Saif F, et al. Prevention of bleeding after islet transplantation: lessons learned from a multivariate analysis of 132 cases at a single institution. *Am J Transpl*. (2005) 5:2992–8. doi: 10.1111/j.1600-6143.2005.01108.x

23. Dajani KZ, Shapiro AMJ. Chapter 54 - Treating diabetes with islet cell transplantation: Lessons from the Edmonton experience. In: Orlando G, Piemonti L, Ricordi C, Stratta RJ, Gruessner RWG, editors. *Transplantation, Bioengineering, and Regeneration of the Endocrine Pancreas*. Japan: Japanese Society of Hepato-Biliary-Pancreatic Surgery: Academic Press (2020). p. 671–84.

24. Szempruch KR, Banerjee O, McCall RC, Desai CS. Use of anti-inflammatory agents in clinical islet cell transplants: A qualitative systematic analysis. *Islets*. (2019) 11:65–75. doi: 10.1080/19382014.2019.1601543

25. Hering BJ, Clarke WR, Bridges ND, Eggerman TL, Alejandro R, Bellin MD, et al. Phase 3 trial of transplantation of human islets in type 1 diabetes complicated by severe hypoglycemia. *Diabetes Care*. (2016) 39:1230–40. doi: 10.2337/dc15-1988

26. Affan M, Dar MS. Donislecel-the first approved pancreatic islet cell therapy medication for type 1 diabetes: a letter to the editor. *Ir J Med Sci*. (2023), 231–2. doi: 10.1007/s11845-023-03464-w

27. First cell therapy for diabetes approved. *Nat Biotechnol*. (2023) 41:1036. doi: 10.1038/s41587-023-01912-7

28. Harris E. FDA greenlights first cell therapy for adults with type 1 diabetes. *JAMA*. (2023) 330:402. doi: 10.1001/jama.2023.12542

29. Release FaDAFN. *FDA approves first cellular therapy to treat patients with type 1 diabetes*. United States of America (2023).

30. Piemonti L, Everly MJ, Maffi P, Scavini M, Poli F, Nano R, et al. Alloantibody and autoantibody monitoring predicts islet transplantation outcome in human type 1 diabetes. *Diabetes*. (2013) 62:1656–64. doi: 10.2337/db12-1258

31. Camirand G, Riella LV. Treg-centric view of immunosuppressive drugs in transplantation: A balancing act. *Am J Transpl*. (2017) 17:601–10. doi: 10.1111/ajt.14029

32. Furukawa A, Wisel SA, Tang Q. Impact of immune-modulatory drugs on regulatory T cell. *Transplantation*. (2016) 100:2288–300. doi: 10.1097/TP.0000000000001379

33. Mullard A. FDA approves first cell therapy for type 1 diabetes. *Nat Rev Drug Discovery*. (2023) 22:611. doi: 10.1038/d41573-023-00113-w

34. Medscape. Donislecel (Rx). Available at: https://reference.medscape.com/drug/lantidra-donislecel-4000210#4.

35. Emamaullee JA, Pepper A, Shapiro AMJ. Chapter 56 - islet cell transplantation. In: Atala A, Lanza R, Mikos AG, Nerem R, editors. *Principles of Regenerative Medicine (Third Edition)*. Academic Press, Boston (2019). p. 987–1007.

36. Farouk SS, Rein JL. The many faces of calcineurin inhibitor toxicity-what the FK? *Adv Chronic Kidney Dis*. (2020) 27:56–66. doi: 10.1053/j.ackd.2019.08.006

37. Molinari M, Al-Saif F, Ryan EA, Lakey JR, Senior PA, Paty BW, et al. Sirolimus-induced ulceration of the small bowel in islet transplant recipients: report of two cases. *Am J Transpl*. (2005) 5:2799–804. doi: 10.1111/j.1600-6143.2005.01082.x

38. Ruiz R, Kirk AD. Chapter 97 - long-term toxicity of immunosuppressive therapy. In: Busuttil RW, Klintmalm GBG, editors. *Transplantation of the Liver (Third Edition)*. W.B. Saunders, Philadelphia (2015). p. 1354–63.

39. Dai C, Walker JT, Shostak A, Padgett A, Spears E, Wisniewski S, et al. Tacrolimus- and sirolimus-induced human beta cell dysfunction is reversible and preventable. *JCI Insight*. (2020) 5(1):e130770. doi: 10.1172/jci.insight.130770

40. Transplant NBa. *Interim Report on Pancreas and Islet Transplantation*. England: NHS England (2016 - 2021).

41. Ng NHJ, Tan WX, Koh YX, Teo AKK. Human islet isolation and distribution efforts for clinical and basic research. *OBM Transplant*. (2019) 32(2):068. doi: 10.21926/obm.transplant.1902068

42. McCall M, Shapiro AM. Update on islet transplantation. *Cold Spring Harb Perspect Med*. (2012) 2:a007823. doi: 10.1101/cshperspect.a007823

43. Ricordi C, Goldstein JS, Balamurugan AN, Szot GL, Kin T, Liu C, et al. National institutes of health–sponsored clinical islet transplantation consortium phase 3 trial: manufacture of a complex cellular product at eight processing facilities. *Diabetes*. (2016) 65:3418–28. doi: 10.2337/db16-0234

44. Yamamoto T, Horiguchi A, Ito M, Nagata H, Ichii H, Ricordi C, et al. Quality control for clinical islet transplantation: organ procurement and preservation, the islet processing facility, isolation, and potency tests. *J Hepato-Biliary-Pancreatic Surg*. (2009) 16:131–6. doi: 10.1007/s00534-009-0064-z

45. Yang Y, Wang K, Wu H, Jin Q, Ruan D, Ouyang Z, et al. Genetically humanized pigs exclusively expressing human insulin are generated through custom endonuclease-mediated seamless engineering. *J Mol Cell Biol*. (2016) 8:174–7. doi: 10.1093/jmcb/mjw008

46. Shin JS, Kim JM, Min BH, Yoon IH, Kim HJ, Kim JS, et al. Pre-clinical results in pig-to-non-human primate islet xenotransplantation using anti-CD40 antibody (2C10R4)-based immunosuppression. *Xenotransplantation*. (2018) 25(1):10. doi: 10.1111/xen.12356

47. Anwar IJ, Berman DM, DeLaura I, Gao Q, Willman MA, Miller A, et al. The anti-CD40L monoclonal antibody AT-1501 promotes islet and kidney allograft survival and function in nonhuman primates. *Sci Transl Med*. (2023) 15:eadf6376. doi: 10.1126/scitranslmed.adf6376

48. Coe TM, Markmann JF, Rickert CG. Current status of porcine islet xenotransplantation. *Curr Opin Organ Transpl*. (2020) 25:449–56. doi: 10.1097/MOT.0000000000000794

49. Mohiuddin MM, Singh AK, Corcoran PC, Thomas Iii ML, Clark T, Lewis BG, et al. Chimeric 2C10R4 anti-CD40 antibody therapy is critical for long-term survival of GTKO.hCD46.hTBM pig-to-primate cardiac xenograft. *Nat Commun*. (2016) 7:11138. doi: 10.1038/ncomms11138

50. Ajima K, Tsuda N, Takaki T, Furusako S, Matsumoto S, Shinohara K, et al. A porcine islet-encapsulation device that enables long-term discordant xenotransplantation in immunocompetent diabetic mice. *Cell Rep Methods*. (2023) 3:100370. doi: 10.1016/j.crmeth.2022.100370

51. Matsumoto S, Abalovich A, Wechsler C, Wynyard S, Elliott RB. Clinical benefit of islet xenotransplantation for the treatment of type 1 diabetes. *EBioMedicine* (2016) 12:255–62. doi: 10.1016/j.ebiom.2016.08.034

52. Yang KC, Qi Z, Wu CC, Shirouza Y, Lin FH, Yanai G, et al. The cytoprotection of chitosan based hydrogels in xenogeneic islet transplantation: An in *vivo* study in streptozotocin-induced diabetic mouse. *Biochem Biophys Res Commun*. (2010) 393:818–23. doi: 10.1016/j.bbrc.2010.02.089

53. Basta G, Montanucci P, Luca G, Boselli C, Noya G, Barbaro B, et al. Long-term metabolic and immunological follow-up of nonimmunosuppressed patients with type 1 diabetes treated with microencapsulated islet allografts: four cases. *Diabetes Care*. (2011) 34:2406–9. doi: 10.2337/dc11-0731

54. Matsumoto S, Tan P, Baker J, Durbin K, Tomiya M, Azuma K, et al. Clinical porcine islet xenotransplantation under comprehensive regulation. *Transplant Proc*. (2014) 46:1992–5. doi: 10.1016/j.transproceed.2014.06.008

55. Bochenek MA, Veiseh O, Vegas AJ, McGarrigle JJ, Qi M, Marchese E, et al. Alginate encapsulation as long-term immune protection of allogeneic pancreatic islet cells transplanted into the omental bursa of macaques. *Nat BioMed Eng*. (2018) 2:810–21. doi: 10.1038/s41551-018-0275-1

56. Alagpulinsa DA, Cao JJL, Driscoll RK, Sirbulescu RF, Penson MFE, Sremac M, et al. Alginate-microencapsulation of human stem cell-derived beta cells with CXCL12 prolongs their survival and function in immunocompetent mice without systemic immunosuppression. *Am J Transpl*. (2019) 19:1930–40. doi: 10.1111/ajt.15308

57. Valdes-Gonzalez RA, Dorantes LM, Garibay GN, Bracho-Blanchet E, Mendez AJ, Davila-Perez R, et al. Xenotransplantation of porcine neonatal islets of Langerhans and Sertoli cells: a 4-year study. *Eur J Endocrinol*. (2005) 153:419–27. doi: 10.1530/eje.1.01982

58. Kaneko M, Moriguchi H, Futatsubashi R, Ayano S, Kobayashi G, Ito A. Transplantable cell-encapsulation device using a semipermeable ethylene-vinyl alcohol copolymer membrane in a mouse diabetic model. *J Biosci Bioeng*. (2023) 136:415–22. doi: 10.1016/j.jbiosc.2023.09.001

59. Kumagai-Braesch M, Jacobson S, Mori H, Jia X, Takahashi T, Wernerson A, et al. The TheraCyte device protects against islet allograft rejection in immunized hosts. *Cell Transpl*. (2013) 22:1137–46. doi: 10.3727/096368912X657486

60. Pepper AR, Pawlick R, Gala-Lopez B, MacGillivary A, Mazzuca DM, White DJ, et al. Diabetes is reversed in a murine model by marginal mass syngeneic islet transplantation using a subcutaneous cell pouch device. *Transplantation*. (2015) 99:2294–300. doi: 10.1097/TP.0000000000000864

61. Boettler T, Schneider D, Cheng Y, Kadoya K, Brandon EP, Martinson L, et al. Pancreatic tissue transplanted in TheraCyte encapsulation devices is protected and prevents hyperglycemia in a mouse model of immune-mediated diabetes. *Cell Transpl*. (2016) 25:609–14. doi: 10.3727/096368915X688939

62. Henry Rr, Pettus J, Wilensky J, Shapiro AMJ, Senior PA, Roep B, et al. Initial clinical evaluation of VC-01TM combination product—A stem cell–derived islet replacement for type 1 diabetes (T1D). *Diabetes*. (2018) 67. doi: 10.2337/db18-138-OR

63. Carlsson PO, Espes D, Sedigh A, Rotem A, Zimerman B, Grinberg H, et al. Transplantation of macroencapsulated human islets within the bioartificial pancreas betaAir to patients with type 1 diabetes mellitus. *Am J Transpl*. (2018) 18:1735–44. doi: 10.1111/ajt.14642

64. Shapiro AMJ, Thompson D, Donner TW, Bellin MD, Hsueh W, Pettus J, et al. Insulin expression and C-peptide in type 1 diabetes subjects implanted with stem cell-derived pancreatic endoderm cells in an encapsulation device. *Cell Rep Med*. (2021) 2:100466. doi: 10.1016/j.xcrm.2021.100466

65. *Vertex announces FDA clearance of investigational new drug application for VX-264, a novel encapsulated cell therapy for the treatment of Type 1 diabetes*. Boston: businesswire.com (2023).

66. (2023). Sernova announces positive updated interim phase 1/2 clinical data for the cell pouch system™, in: *American Diabetes Association 83rd Scientific Sessions*, London, Canada, Ontario.

67. Cao R, Avgoustiniatos E, Papas K, de Vos P, Lakey JRT. Mathematical predictions of oxygen availability in micro- and macro-encapsulated human and porcine pancreatic islets. *J BioMed Mater Res B Appl Biomater*. (2020) 108:343–52. doi: 10.1002/jbm.b.34393

68. Kobayashi T, Aomatsu Y, Iwata H, Kin T, Kanehiro H, Hisanaga M, et al. Indefinite islet protection from autoimmune destruction in nonobese diabetic mice by agarose microencapsulation without immunosuppression. *Transplantation*. (2003) 75:619–25. doi: 10.1097/01.TP.0000053749.36365.7E

69. Soetedjo AAP, Lee JM, Lau HH, Goh GL, An J, Koh Y, et al. Tissue engineering and 3D printing of bioartificial pancreas for regenerative medicine in diabetes. *Trends Endocrinol Metab*. (2021) 32(8):609–22. doi: 10.1016/j.tem.2021.05.007

70. Wu S, Wang L, Fang Y, Huang H, You X, Wu J. Advances in encapsulation and delivery strategies for islet transplantation. *Adv Healthc Mater*. (2021) 10:e2100965. doi: 10.1002/adhm.202100965

71. Bruin JE, Rezania A, Xu J, Narayan K, Fox JK, O'Neil JJ, et al. Maturation and function of human embryonic stem cell-derived pancreatic progenitors in macroencapsulation devices following transplant into mice. *Diabetologia*. (2013) 56:1987–98. doi: 10.1007/s00125-013-2955-4

72. Elliott RB, Escobar L, Calafiore R, Basta G, Garkavenko O, Vasconcellos A, et al. Transplantation of micro- and macroencapsulated piglet islets into mice and monkeys. *Transplant Proc*. (2005) 37:466–9. doi: 10.1016/j.transproceed.2004.12.198

73. Ludwig B, Ludwig S, Steffen A, Knauf Y, Zimerman B, Heinke S, et al. Favorable outcome of experimental islet xenotransplantation without immunosuppression in a nonhuman primate model of diabetes. *Proc Natl Acad Sci U S A*. (2017) 114:11745–50. doi: 10.1073/pnas.1708420114

74. Barkai U, Weir GC, Colton CK, Ludwig B, Bornstein SR, Brendel MD, et al. Enhanced oxygen supply improves islet viability in a new bioartificial pancreas. *Cell Transpl*. (2013) 22:1463–76. doi: 10.3727/096368912X657341

75. Ludwig B, Reichel A, Steffen A, Zimerman B, Schally AV, Block NL, et al. Transplantation of human islets without immunosuppression. *Proc Natl Acad Sci U S A*. (2013) 110:19054–8. doi: 10.1073/pnas.1317561110

76. Ramzy A, Thompson DM, Ward-Hartstonge KA, Ivison S, Cook L, Garcia RV, et al. Implanted pluripotent stem-cell-derived pancreatic endoderm cells secrete glucose-responsive C-peptide in patients with type 1 diabetes. *Cell Stem Cell*. (2021) 28:2047–61 e5. doi: 10.1016/j.stem.2021.10.003

77. Keymeulen B, Gillard P, Mathieu C, Movahedi B, Maleux G, Delvaux G, et al. Correlation between beta cell mass and glycemic control in type 1 diabetic recipients of islet cell graft. *Proc Natl Acad Sci U S A*. (2006) 103:17444–9. doi: 10.1073/pnas.0608141103

78. Keymeulen B, De Groot K, Jacobs-Tulleneers-Thevissen D, Thompson DM, Bellin MD, Kroon EJ, et al. Encapsulated stem cell-derived beta cells exert glucose control in patients with type 1 diabetes. *Nat Biotechnol*. (2023). doi: 10.1038/s41587-023-02055-5

79. Reichman TW, Ricordi C, Naji A, Markmann JF, Perkins BA, Wijkstrom M, et al. 836-P: glucose-dependent insulin production and insulin-independence in type 1 diabetes from stem cell–derived, fully differentiated islet cells—Updated data from the VX-880 clinical trial. *Diabetes*. (2023) 72. doi: 10.2337/db23-836-P

80. Hogrebe NJ, Ishahak M, Millman JR. Developments in stem cell-derived islet replacement therapy for treating type 1 diabetes. *Cell Stem Cell*. (2023) 30:530–48. doi: 10.1016/j.stem.2023.04.002

81. Ramzy A, Belmonte PJ, Braam MJS, Ida S, Wilts EM, Levings MK, et al. A century-long journey from the discovery of insulin to the implantation of stem cell-derived islets. *Endocr Rev*. (2023) 44:222–53. doi: 10.1210/endrev/bnac021

82. Melton D. The promise of stem cell-derived islet replacement therapy. *Diabetologia*. (2021) 64:1030–6. doi: 10.1007/s00125-020-05367-2

83. (2023). in: *Vertex presents positive, updated VX-880 results from ongoing phase 1/2 study in Type 1 diabetes at the european association for the study of diabetes 59th annual meeting*. Boston, United States of America: Business Wire.

84. Hu X, White K, Olroyd AG, DeJesus R, Dominguez AA, Dowdle WE, et al. Hypoimmune induced pluripotent stem cells survive long term in fully immunocompetent, allogeneic rhesus macaques. *Nat Biotechnol*. (2023). doi: 10.1038/s41587-023-01784-x

85. Hu X, Gattis C, Olroyd AG, Friera AM, White K, Young C, et al. Human hypoimmune primary pancreatic islets avoid rejection and autoimmunity and alleviate diabetes in allogeneic humanized mice. *Sci Transl Med*. (2023) 15:eadg5794. doi: 10.1126/scitranslmed.adg5794

86. Gerace D, Zhou Q, Kenty JH, Veres A, Sintov E, Wang X, et al. Engineering human stem cell-derived islets to evade immune rejection and promote localized immune tolerance. *Cell Rep Med*. (2023) 4:100879. doi: 10.1016/j.xcrm.2022.100879

87. Parent AV, Faleo G, Chavez J, Saxton M, Berrios DI, Kerper NR, et al. Selective deletion of human leukocyte antigens protects stem cell-derived islets from immune rejection. *Cell Rep*. (2021) 36:109538. doi: 10.1016/j.celrep.2021.109538

88. Yoshihara E, O'Connor C, Gasser E, Wei Z, Oh TG, Tseng TW, et al. Immune-evasive human islet-like organoids ameliorate diabetes. *Nature*. (2020) 586:606–11. doi: 10.1038/s41586-020-2631-z

89. Hu X, White K, Young C, Olroyd AG, Kievit P, Connolly AJ, et al. Hypoimmune islets achieve insulin independence after allogeneic transplantation in a fully immunocompetent non-human primate. *Cell Stem Cell*. (2024), 334–40. doi: 10.1016/j.stem.2024.02.001

90. Moreau A, Varey E, Anegon I, Cuturi MC. Effector mechanisms of rejection. *Cold Spring Harb Perspect Med*. (2013) 3(11):a015461. doi: 10.1101/cshperspect.a015461

91. Deuse T, Hu X, Gravina A, Wang D, Tediashvili G, De C, et al. Hypoimmunogenic derivatives of induced pluripotent stem cells evade immune rejection in fully immunocompetent allogeneic recipients. *Nat Biotechnol*. (2019) 37:252–8. doi: 10.1038/s41587-019-0016-3

92. Gornalusse GG, Hirata RK, Funk SE, Riolobos L, Lopes VS, Manske G, et al. HLA-E-expressing pluripotent stem cells escape allogeneic responses and lysis by NK cells. *Nat Biotechnol*. (2017) 35:765–72. doi: 10.1038/nbt.3860

93. Han X, Wang M, Duan S, Franco PJ, Kenty JH, Hedrick P, et al. Generation of hypoimmunogenic human pluripotent stem cells. *Proc Natl Acad Sci U S A*. (2019) 116:10441–6. doi: 10.1073/pnas.1902566116

94. Xu H, Wang B, Ono M, Kagita A, Fujii K, Sasakawa N, et al. Targeted Disruption of HLA Genes via CRISPR-Cas9 Generates iPSCs with Enhanced Immune Compatibility. *Cell Stem Cell*. (2019) 24:566–78 e7. doi: 10.1016/j.stem.2019.02.005

95. Braud VM, Allan DS, O'Callaghan CA, Soderstrom K, D'Andrea A, Ogg GS, et al. HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. *Nature*. (1998) 391:795–9. doi: 10.1038/35869

96. Sockolosky JT, Dougan M, Ingram JR, Ho CC, Kauke MJ, Almo SC, et al. Durable antitumor responses to CD47 blockade require adaptive immune stimulation. *Proc Natl Acad Sci U S A*. (2016) 113:E2646–54. doi: 10.1073/pnas.1604268113

97. Yi M, Niu M, Xu L, Luo S, Wu K. Regulation of PD-L1 expression in the tumor microenvironment. *J Hematol Oncol*. (2021) 14:10. doi: 10.1186/s13045-020-01027-5

98. *Vertex and CRISPR Therapeutics Announce Licensing Agreement to Accelerate Development of Vertex's Hypoimmune Cell Therapies for the Treatment of Type 1 Diabetes*. Boston and Zug, Switzerland: businesswire.com (2023).

99. Fu Y, Foden JA, Khayter C, Maeder ML, Reyon D, Joung JK, et al. High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. *Nat Biotechnol*. (2013) 31:822–6. doi: 10.1038/nbt.2623

100. Pacesa M, Lin CH, Clery A, Saha A, Arantes PR, Bargsten K, et al. Structural basis for Cas9 off-target activity. *Cell*. (2022) 185:4067–81 e21. doi: 10.1016/j.cell.2022.09.026

101. Hsu PD, Scott DA, Weinstein JA, Ran FA, Konermann S, Agarwala V, et al. DNA targeting specificity of RNA-guided Cas9 nucleases. *Nat Biotechnol*. (2013) 31:827–32. doi: 10.1038/nbt.2647

102. Wienert B, Cromer MK. CRISPR nuclease off-target activity and mitigation strategies. *Front Genome Ed*. (2022) 4:1050507. doi: 10.3389/fgeed.2022.1050507

103. Ibraheim R, Tai PWL, Mir A, Javeed N, Wang J, Rodriguez TC, et al. Self-inactivating, all-in-one AAV vectors for precision Cas9 genome editing via homology-directed repair in vivo. *Nat Commun*. (2021) 12:6267. doi: 10.1038/s41467-021-26518-y

104. Li A, Lee CM, Hurley AE, Jarrett KE, De Giorgi M, Lu W, et al. A self-deleting AAV-CRISPR system for *in vivo* genome editing. *Mol Ther Methods Clin Dev*. (2019) 12:111–22. doi: 10.1016/j.omtm.2018.11.009

105. Li W, Xiang AP. Safeguarding clinical translation of pluripotent stem cells with suicide genes. *Organogenesis*. (2013) 9:34–9. doi: 10.4161/org.24317

106. Harding J, Vintersten-Nagy K, Yang H, Tang JK, Shutova M, Jong ED, et al. Immune-privileged tissues formed from immunologically cloaked mouse embryonic stem cells survive long term in allogeneic hosts. *Nat BioMed Eng*. (2023). doi: 10.1038/s41551-023-01133-y

107. Harding J, Vintersten-Nagy K, Nagy A. Universal stem cells: making the unsafe safe. *Cell Stem Cell*. (2020) 27:198–9. doi: 10.1016/j.stem.2020.07.004

108. Liang Q, Monetti C, Shutova MV, Neely EJ, Hacibekiroglu S, Yang H, et al. Linking a cell-division gene and a suicide gene to define and improve cell therapy safety. *Nature*. (2018) 563:701–4. doi: 10.1038/s41586-018-0733-7

109. Ge X, Wang IN, Toma I, Sebastiano V, Liu J, Butte MJ, et al. Human amniotic mesenchymal stem cell-derived induced pluripotent stem cells may generate a universal source of cardiac cells. *Stem Cells Dev*. (2012) 21:2798–808. doi: 10.1089/scd.2011.0435

110. Fang YH, Wang SPH, Gao ZH, Wu SN, Chang HA-O, Yang PJ, et al. *Efficient cardiac differentiation of human amniotic fluid-derived stem cells into induced pluripotent stem cells and their potential immune privilege*. Switzerland (2020). pp. 1422–0067. doi: 10.3390/ijms21072359.

111. Liu Y-W, Fang IH, Wang SP. Abstract 10121: hypoimmunogenic human induced pluripotent stem cells derived cardiomyocytes for cardiac regeneration. *Circulation*. (2022) 146:A10121–A. doi: 10.1161/circ.146.suppl_1.10121

112. Cai YJ, Huang L, Leung TY, Burd A. A study of the immune properties of human umbilical cord lining epithelial cells. *Cytotherapy*. (2014) 16:631–9. doi: 10.1016/j.jcyt.2013.10.008

113. Wang M, Yang Y, Yang D, Luo F, Liang W, Guo S, et al. The immunomodulatory activity of human umbilical cord blood-derived mesenchymal stem cells in vitro. *Immunology*. (2009) 126:220–32. doi: 10.1111/j.1365-2567.2008.02891.x

114. Cho PS, Messina DJ, Hirsh EL, Chi N, Goldman SN, Lo DP, et al. Immunogenicity of umbilical cord tissue derived cells. *Blood*. (2008) 111:430–8. doi: 10.1182/blood-2007-03-078774

115. Su X, Parikh BH, Chen QF, Liu Z, Barathi V, Lim KL. Hypo-immunogenic human umbilical cord lining derived retinal pigment epithelial (CLiPS-RPE) monolayers for treatment of atrophic maculopathies. *Invest Ophthalmol Visual Sci*. (2023) 64:4615–.

116. Karnieli O, Izhar-Prato Y, Bulvik S, Efrat S. Generation of insulin-producing cells from human bone marrow mesenchymal stem cells by genetic manipulation. *Stem Cells*. (2007) 25:2837–44. doi: 10.1634/stemcells.2007-0164

117. Wang HS, Shyu JF, Shen WS, Hsu HC, Chi TC, Chen CP, et al. Transplantation of insulin-producing cells derived from umbilical cord stromal mesenchymal stem cells to treat NOD mice. *Cell Transpl*. (2011) 20:455–66. doi: 10.3727/096368910X522270

118. Huang F, Lai J, Qian L, Hong W, Li L-c. Differentiation of Uc-MSCs into insulin secreting islet-like clusters by trypsin through TGF-beta signaling pathway. *Differentiation*. (2023) 100744. doi: 10.1016/j.diff.2023.100744

119. Sarang S, Viswanathan C. Umbilical cord derived mesenchymal stem cells useful in insulin production - another opportunity in cell therapy. *Int J Stem Cells*. (2016) 9:60–9. doi: 10.15283/ijsc.2016.9.1.60

120. Park YM, Yang CM, Cho HY. Therapeutic effects of insulin-producing human umbilical cord-derived mesenchymal stem cells in a type 1 diabetes mouse model. *Int J Mol Sci*. (2022) 23(13):6877. doi: 10.3390/ijms23136877

121. Kim SJ, Choi Ys Fau - Ko E-S, Ko Es Fau - Lim S-M, Lim Sm Fau - Lee C-W, Lee Cw Fau - Kim D-I, Kim DI. Glucose-stimulated insulin secretion of various mesenchymal stem cells after insulin-producing cell differentiation. *J Biosci Bioeng*. (2012) 113(6):1347–4421. doi: 10.1016/j.jbiosc.2012.02.007

122. Zhang Y, Shen W Fau - Hua J, Hua J Fau - Lei A, Lei A Fau - Lv C, Lv C Fau - Wang H, Wang H Fau - Yang C, et al. *Pancreatic islet-like clusters from bone marrow mesenchymal stem cells of human first-trimester abortus can cure streptozocin-induced mouse diabetes*. United States of America (2010). pp. 1557–8577.

123. Gao F, Wu D-Q, Hu Y-H, Jin G-X, Li G-D, Sun T-W, et al. *In vitro* cultivation of islet-like cell clusters from human umbilical cord blood-derived mesenchymal stem cells. *Trans Res*. (2008) 151:293–302. doi: 10.1016/j.trsl.2008.03.003

124. Phuc PV, Nhung TH, Loan DTT, Chung DC, Ngoc PK. Differentiating of banked human umbilical cord blood-derived mesenchymal stem cells into insulin-secreting cells. *In Vitro Cell Dev Biol Animal*. (2011) 47:54–63. doi: 10.1007/s11626-010-9356-5

125. Moshtagh PR, Emami SH, Sharifi AM. Differentiation of human adipose-derived mesenchymal stem cell into insulin-producing cells: an in *vitro* study. *J Physiol Biochem*. (2013) 69:451–8. doi: 10.1007/s13105-012-0228-1

126. Wu LF, Wang NN, Liu YS, Wei X. Differentiation of Wharton's jelly primitive stromal cells into insulin-producing cells in comparison with bone marrow mesenchymal stem cells. *Tissue Eng Part A*. (2009) 15:2865–73. doi: 10.1089/ten.tea.2008.0579

127. Ander SE, Diamond MS, Coyne CB. Immune responses at the maternal-fetal interface. *Sci Immunol*. (2019) 4(31):eaat6114. doi: 10.1126/sciimmunol.aat6114

128. Hudson LE, Allen RL. Leukocyte Ig-like receptors - A model for MHC class I disease associations. *Front Immunol*. (2016) 7:281. doi: 10.3389/fimmu.2016.00281

129. Allard M, Oger R, Benlalam H, Florenceau L, Echasserieau K, Bernardeau K, et al. Soluble HLA-I/peptide monomers mediate antigen-specific CD8 T cell activation through passive peptide exchange with cell-bound HLA-I molecules. *J Immunol*. (2014) 192:5090–7. doi: 10.4049/jimmunol.1303226

130. Pietra G, Romagnani C, Moretta L, Mingari MC. HLA-E and HLA-E-bound peptides: recognition by subsets of NK and T cells. *Curr Pharm Des*. (2009) 15:3336–44. doi: 10.2174/138161209789105207

131. Leddon SA, Sant AJ. Generation of MHC class II-peptide ligands for CD4 T-cell allorecognition of MHC class II molecules. *Curr Opin Organ Transpl*. (2010) 15:505–11. doi: 10.1097/MOT.0b013e32833bfc5c

132. Ballou C, Barton F, Payne EH, Berney T, Villard J, Meier RPH, et al. Matching for HLA-DR excluding diabetogenic HLA-DR3 and HLA-DR4 predicts insulin independence after pancreatic islet transplantation. *Front Immunol*. (2023) 14:1110544. doi: 10.3389/fimmu.2023.1110544

133. Lemos JRN, Baidal DA, Poggioli R, Fuenmayor V, Chavez C, Alvarez A, et al. HLA-B matching prolongs allograft survival in islet cell transplantation. *Cell Transpl*. (2023) 32:9636897231166529. doi: 10.1177/09636897231166529

134. Rudolph EN, Dunn TA-O, Mauer D, Noreen H, Sutherland DE, Kandaswamy R, et al. *HLA-A, -B, -C, -DR, and -DQ matching in pancreas transplantation: effect on graft rejection and survival*. United States of America (2016). pp. 1600–6143 (Electronic).

135. Morizane A, Kikuchi T, Hayashi T, Mizuma H, Takara S, Doi H, et al. MHC matching improves engraftment of iPSC-derived neurons in non-human primates. *Nat Commun*. (2017) 8:385. doi: 10.1038/s41467-017-00926-5

136. Ansari D, Bucin D, Nilsson J. Human leukocyte antigen matching in heart transplantation: systematic review and meta-analysis. *Transpl Int*. (2014) 27:793–804. doi: 10.1111/tri.12335

137. Kawamura T, Miyagawa S, Fukushima S, Maeda A, Kashiyama N, Kawamura A, et al. Cardiomyocytes derived from MHC-homozygous induced pluripotent stem cells exhibit reduced allogeneic immunogenicity in MHC-matched non-human primates. *Stem Cell Rep*. (2016) 6:312–20. doi: 10.1016/j.stemcr.2016.01.012

138. Opelz G, Dohler B. Ceppellini Lecture 2012: collateral damage from HLA mismatching in kidney transplantation. *Tissue Antigens*. (2013) 82:235–42. doi: 10.1111/tan.12147

139. Taylor CJ, Peacock S, Chaudhry AN, Bradley JA, Bolton EM. Generating an iPSC bank for HLA-matched tissue transplantation based on known donor and recipient HLA types. *Cell Stem Cell*. (2012) 11:147–52. doi: 10.1016/j.stem.2012.07.014

140. Kurtzberg J, Prasad VK, Carter SL, Wagner JE, Baxter-Lowe LA, Wall D, et al. Results of the Cord Blood Transplantation Study (COBLT): clinical outcomes of unrelated donor umbilical cord blood transplantation in pediatric patients with hematologic Malignancies. *Blood*. (2008) 112:4318–27. doi: 10.1182/blood-2007-06-098020

141. Opelz G, Dohler B. Effect of human leukocyte antigen compatibility on kidney graft survival: comparative analysis of two decades. *Transplantation*. (2007) 84:137–43. doi: 10.1097/01.tp.0000269725.74189.b9

142. Lim WH, Chapman JR, Coates PT, Lewis JR, Russ GR, Watson N, et al. HLA-DQ mismatches and rejection in kidney transplant recipients. *Clin J Am Soc Nephrol*. (2016) 11:875–83. doi: 10.2215/CJN.11641115

143. Hanatani T, Takasu N. CiRA iPSC seed stocks (CiRA's iPSC Stock Project). *Stem Cell Res*. (2020) 50:102033. doi: 10.1016/j.scr.2020.102033

144. Umekage M, Sato Y, Takasu N. Overview: an iPS cell stock at CiRA. *Inflammation Regen*. (2019) 39:17. doi: 10.1186/s41232-019-0106-0

145. Yoshida S, Kato TM, Sato Y, Umekage M, Ichisaka T, Tsukahara M, et al. A clinical-grade HLA haplobank of human induced pluripotent stem cells matching approximately 40% of the Japanese population. *Med*. (2023) 4:51–66 e10. doi: 10.1016/j.medj.2022.10.003

146. Kim JH, Jo HY, Ha HY, Kim YO. Korea national stem cell bank. *Stem Cell Res*. (2021) 53:102270. doi: 10.1016/j.scr.2021.102270

147. Lee S, Huh JY, Turner DM, Lee S, Robinson J, Stein JE, et al. Repurposing the cord blood bank for haplobanking of HLA-homozygous iPSCs and their usefulness to multiple populations. *Stem Cells*. (2018) 36:1552–66. doi: 10.1002/stem.2865

148. Kuebler B, Alvarez-Palomo B, Aran B, Castano J, Rodriguez L, Raya A, et al. Generation of a bank of clinical-grade, HLA-homozygous iPSC lines with high coverage of the Spanish population. *Stem Cell Res Ther*. (2023) 14:366. doi: 10.1186/s13287-023-03576-1

149. Stacey GN, Hao J. Biobanking of human pluripotent stem cells in China. *Cell Prolif*. (2022) 55:e13180. doi: 10.1111/cpr.13180

150. Martins de Oliveira ML, Tura BR, Meira Leite M, Melo Dos Santos EJ, Porto LC, Pereira LV, et al. Creating an HLA-homozygous iPS cell bank for the Brazilian population: Challenges and opportunities. *Stem Cell Rep*. (2023) 18:1905–12. doi: 10.1016/j.stemcr.2023.09.001

151. Clancy J, Hyvarinen K, Ritari J, Wahlfors T, Partanen J, Koskela S. Blood donor biobank and HLA imputation as a resource for HLA homozygous cells for therapeutic and research use. *Stem Cell Res Ther*. (2022) 13:502. doi: 10.1186/s13287-022-03182-7

152. Abberton KM, McDonald TL, Diviney M, Holdsworth R, Leslie S, Delatycki MB, et al. Identification and re-consent of existing cord blood donors for creation of induced pluripotent stem cell lines for potential clinical applications. *Stem Cells Transl Med*. (2022) 11:1052–60. doi: 10.1093/stcltm/szac060

153. Gourraud PA, Gilson L, Girard M, Peschanski M. The role of human leukocyte antigen matching in the development of multiethnic "haplobank" of induced pluripotent stem cell lines. *Stem Cells*. (2012) 30:180–6. doi: 10.1002/stem.772

154. Aron Badin R, Bugi A, Williams S, Vadori M, Michael M, Jan C, et al. MHC matching fails to prevent long-term rejection of iPSC-derived neurons in non-human primates. *Nat Commun*. (2019) 10:4357. doi: 10.1038/s41467-019-12324-0

155. Sackett SD, Brown ME, Tremmel DM, Ellis T, Burlingham WJ, Odorico JS. Modulation of human allogeneic and syngeneic pluripotent stem cells and immunological implications for transplantation. *Transplant Rev (Orlando)*. (2016) 30:61–70. doi: 10.1016/j.trre.2016.02.001

156. Tohyama S, Fujita J, Fujita C, Yamaguchi M, Kanaami S, Ohno R, et al. Efficient large-scale 2D culture system for human induced pluripotent stem cells and differentiated cardiomyocytes. *Stem Cell Rep*. (2017) 9:1406–14. doi: 10.1016/j.stemcr.2017.08.025

157. Gerecht-Nir S, Cohen S, Itskovitz-Eldor J. Bioreactor cultivation enhances the efficiency of human embryoid body (hEB) formation and differentiation. *Biotechnol Bioeng*. (2004) 86:493–502. doi: 10.1002/bit.20045

158. Kwok CK, Sébastien I, Hariharan K, Meiser I, Wihan J, Altmaier S, et al. Scalable expansion of iPSC and their derivatives across multiple lineages. *Reprod Toxicol*. (2022) 112:23–35. doi: 10.1016/j.reprotox.2022.05.007

159. Becerra D, Wu T, Jeffs S, Ott HC. High-throughput culture method of induced pluripotent stem cell-derived alveolar epithelial cells. *Tissue Eng Part C Methods*. (2021) 27:639–48. doi: 10.1089/ten.tec.2021.0174

160. Guha P, Morgan JW, Mostoslavsky G, Rodrigues NP, Boyd AS. Lack of immune response to differentiated cells derived from syngeneic induced pluripotent stem cells. *Cell Stem Cell*. (2013) 12:407–12. doi: 10.1016/j.stem.2013.01.006

161. Morizane A, Doi D, Kikuchi T, Okita K, Hotta A, Kawasaki T, et al. Direct comparison of autologous and allogeneic transplantation of iPSC-derived neural cells in the brain of a non-human primate. *Stem Cell Rep*. (2013) 1:283–92. doi: 10.1016/j.stemcr.2013.08.007

162. Scheiner ZS, Talib S, Feigal EG. The potential for immunogenicity of autologous induced pluripotent stem cell-derived therapies. *J Biol Chem*. (2014) 289:4571–7. doi: 10.1074/jbc.R113.509588

163. Zhao T, Zhang ZN, Westenskow PD, Todorova D, Hu Z, Lin T, et al. Humanized mice reveal differential immunogenicity of cells derived from autologous induced pluripotent stem cells. *Cell Stem Cell*. (2015) 17:353–9. doi: 10.1016/j.stem.2015.07.021

164. Tan LS, Chen JT, Lim LY, Teo AKK. Manufacturing clinical-grade human induced pluripotent stem cell-derived beta cells for diabetes treatment. *Cell Prolif*. (2022) 55(8):e13232. doi: 10.1111/cpr.13232

165. Song X, Wang X, Zhang J, Shen S, Yin W, Ye G, et al. A tunable self-healing ionic hydrogel with microscopic homogeneous conductivity as a cardiac patch for myocardial infarction repair. *Biomaterials*. (2021) 273:120811. doi: 10.1016/j.biomaterials.2021.120811

166. Kim IG, Hwang MP, Park JS, Kim SH, Kim JH, Kang HJ, et al. Stretchable ECM patch enhances stem cell delivery for post-MI cardiovascular repair. *Adv Healthc Mater*. (2019) 8:e1900593. doi: 10.1002/adhm.201900593

167. Bejleri D, Streeter BW, Nachlas ALY, Brown ME, Gaetani R, Christman KL, et al. A bioprinted cardiac patch composed of cardiac-specific extracellular matrix and progenitor cells for heart repair. *Adv Healthc Mater*. (2018) 7:e1800672. doi: 10.1002/adhm.201800672

168. Coon ME, Stephan SB, Gupta V, Kealey CP, Stephan MT. Nitinol thin films functionalized with CAR-T cells for the treatment of solid tumours. *Nat BioMed Eng*. (2020) 4:195–206. doi: 10.1038/s41551-019-0486-0

169. Weiden J, Voerman D, Dolen Y, Das RK, van Duffelen A, Hammink R, et al. Injectable biomimetic hydrogels as tools for efficient T cell expansion and delivery. *Front Immunol*. (2018) 9:2798. doi: 10.3389/fimmu.2018.02798

170. Deuse T, Tediashvili G, Hu X, Gravina A, Tamenang A, Wang D, et al. Hypoimmune induced pluripotent stem cell-derived cell therapeutics treat cardiovascular and pulmonary diseases in immunocompetent allogeneic mice. *Proc Natl Acad Sci U.S.A*. (2021) 118(28):e2022091118. doi: 10.1073/pnas.2022091118

171. Martin RM, Fowler JL, Cromer MK, Lesch BJ, Ponce E, Uchida N, et al. Improving the safety of human pluripotent stem cell therapies using genome-edited orthogonal safeguards. *Nat Commun*. (2020) 11:2713. doi: 10.1038/s41467-020-16455-7

172. Witkowski P, Philipson LH, Buse JB, Robertson RP, Alejandro R, Bellin MD, et al. Islets Transplantation at a Crossroads - Need for Urgent Regulatory Update in the United States: Perspective Presented During the Scientific Sessions 2021 at the American Diabetes Association Congress. *Front Endocrinol (Lausanne)*. (2021) 12:789526. doi: 10.3389/fendo.2021.789526

173. Pittenger MF, Discher DE, Peault BM, Phinney DG, Hare JM, Caplan AI. Mesenchymal stem cell perspective: cell biology to clinical progress. *NPJ Regener Med*. (2019) 4:22. doi: 10.1038/s41536-019-0083-6

174. Staels W, Verdonck Y, Heremans Y, Leuckx G, De Groef S, Heirman C, et al. Vegf-A mRNA transfection as a novel approach to improve mouse and human islet graft revascularisation. *Diabetologia*. (2018) 61:1804–10. doi: 10.1007/s00125-018-4646-7

175. Wrublewsky S, Weinzierl A, Hornung I, Prates-Roma L, Menger MD, Laschke MW, et al. Co-transplantation of pancreatic islets and microvascular fragments effectively restores normoglycemia in diabetic mice. *NPJ Regener Med*. (2022) 7:67. doi: 10.1038/s41536-022-00262-3

176. Hussey AJ, Winardi M, Han XL, Thomas GP, Penington AJ, Morrison WA, et al. Seeding of pancreatic islets into prevascularized tissue engineering chambers. *Tissue Eng Part A*. (2009) 15:3823–33. doi: 10.1089/ten.tea.2008.0682

177. Lim LY, Ding SSL, Muthukumaran P, Teoh SH, Koh Y, Teo AKK. Tissue engineering of decellularized pancreas scaffolds for regenerative medicine in diabetes. *Acta Biomater*. (2023) 157:49–66. doi: 10.1016/j.actbio.2022.11.032

178. Bochenek MA, Walters B, Zhang J, Fenton OS, Facklam A, Kronekova Z, et al. Enhancing the functionality of immunoisolated human SC-betaeta cell clusters through prior resizing. *Small*. (2024) 11:e2307464. doi: 10.1002/smll.202307464

179. Bertuzzi F, Colussi G, Lauterio A, De Carlis L. Intramuscular islet allotransplantation in type 1 diabetes mellitus. *Eur Rev Med Pharmacol Sci*. (2018) 22:1731–6. doi: 10.26355/eurrev_201803_14588

180. Yin Z, Li J, Zheng Y, Wang S, Wang X. Gastric submucosal alleviated pro-inflammation cytokines mediated initial dysfunction of islets allografts. *Transpl Immunol*. (2021) 65:101292. doi: 10.1016/j.trim.2020.101292

181. Tun SBB, Chua M, Hasan R, Kohler M, Zheng X, Ali Y, et al. Islet transplantation to the anterior chamber of the eye-A future treatment option for insulin-deficient type-2 diabetics? A case report from a nonhuman type-2 diabetic primate. *Cell Transpl*. (2020) 29:963689720913256. doi: 10.1177/0963689720913256

182. Leishman DJ, Oppler SH, Stone LLH, O'Brien TD, Ramachandran S, Willenberg BJ, et al. Targeted mapping and utilization of the perihepatic surface for therapeutic beta cell replacement and retrieval in diabetic non-human primates. *Front Transplant*. (2024) 3. doi: 10.3389/frtra.2024.1352777

183. Wang X, Wang K, Yu M, Velluto D, Hong X, Wang B, et al. Engineered immunomodulatory accessory cells improve experimental allogeneic islet transplantation without immunosuppression. *Sci Adv*. (2022) 8:eabn0071. doi: 10.1126/sciadv.abn0071

184. Kenyon NS, Willman MA, Han D, Leeman RS, Rabassa A, Diaz WL, et al. Extended survival versus accelerated rejection of nonhuman primate islet allografts: Effect of mesenchymal stem cell source and timing. *Am J Transpl*. (2021) 21:3524–37. doi: 10.1111/ajt.16693

185. Chou HS, Hsieh CC, Charles R, Wang L, Wagner T, Fung JJ, et al. Myeloid-derived suppressor cells protect islet transplants by B7-H1 mediated enhancement of T regulatory cells. *Transplantation*. (2012) 93(3):272–82. doi: 10.1097/TP.0b013e31823ffd39

186. Lei J, Coronel MM, Yolcu ES, Deng H, Grimany-Nuno O, Hunckler MD, et al. FasL microgels induce immune acceptance of islet allografts in nonhuman primates. *Sci Adv*. (2022) 8:eabm9881. doi: 10.1126/sciadv.abm9881

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The nature of chronic rejection after lung transplantation: a murine orthotopic lung transplant study

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Introduction: Chronic rejection is a major complication post-transplantation. Within lung transplantation, chronic rejection was considered as airway centred. Chronic Lung Allograft Dysfunction (CLAD), defined to cover all late chronic complications, makes it more difficult to understand chronic rejection from an immunological perspective. This study investigated the true nature, timing and location of chronic rejection as a whole, within mouse lung transplantation.

Methods: 40 mice underwent an orthotopic left lung transplantation, were sacrificed at day 70 and evaluated by histology and in vivo µCT. For timing and location of rejection, extra grafts were sacrificed at day 7, 35, 56 and investigated by ex vivo µCT or single cell RNA (scRNA) profiling.

Results: Chronic rejection originated as innate inflammation around small arteries evolving toward adaptive organization with subsequent end-arterial fibrosis and obliterans. Subsequently, venous and pleural infiltration appeared,

followed by airway related bronchiolar folding and rarely bronchiolitis obliterans was observed. Ex vivo μCT and scRNA profiling validated the time, location and sequence of events with endothelial destruction and activation as primary onset.

Conclusion: Against the current belief, chronic rejection in lung transplantation may start as an arterial response, followed by responses in venules, pleura, and, only in the late stage, bronchioles, as may be seen in some but not all patients with CLAD.

KEYWORDS

lung transplantation, chronic rejection, imaging, single-cell profiling, mouse model

Introduction

Lung transplantation is a life-saving treatment for end-stage lung diseases. However, the lung is prone to rejection due to the strong allo-immune response of the specialized mucosal immune barrier of the lung epithelium. Rejection represents the Achilles' heel of lung transplantation, with a survival rate below that of other solid organ transplantations (5-year survival of 59%) (1). Transplant immunologists have classified rejection into three stages depending on the timing post-transplant: hyperacute, acute, and chronic rejection (2), occurring within the first hours, weeks, or more than 6 months after transplantation. Chronic rejection involves cellular and humoral immune activation, is poorly responsive to treatment, and consequently is the main culprit for long-term survival (2). The clinical presentation of chronic rejection is a gradual late allograft dysfunction in which other causes such as infection and malignancy are excluded (3). Pathologically, chronic rejection in organ transplantation is characterized by vascular intimal thickening and fibrosis, resulting in graft necrosis, atrophy, and loss of functionality. In lung transplantation, the destruction of only small airways, pathologically termed obliterative bronchiolitis (OB), was considered the manifestation of chronic rejection (4). Chronic rejection is presumed to be the immunological counterpart of the clinical concept of chronic lung allograft dysfunction (CLAD), uniting all late persisting lung function deteriorations without identifiable cause (3, 5). Understanding how the immunological concept of "specific" rejection fits into the clinical concept of "non-specific" CLAD [bronchiolitis obliterans syndrome (BOS) and restrictive allograft syndrome (RAS)] is essential in determining the true nature of clinical rejection, resulting in better patient management and outcome.

The mouse orthotopic left lung transplant model based on the cuff technique (6, 7) is a unique way to study rejection. This model involves all essential elements to properly study lung transplant rejection "in a controlled way", as it includes the lung as a functioning organ, an immune response responding against an MHC (H2) mismatch, the role of immunosuppression on the lung "architecture", and immune system, and "secondary immunodeficiency". Reports on chronic rejection in orthotopic lung transplantation mostly involved a minor mismatch setting without immunosuppression (8–10); however, we developed a unique model of chronic rejection combining a major genetic mismatch with daily immunosuppression (11, 12).

Our aim was to document the true nature of the immune system "rejecting" the foreign donor lung within a controlled mouse lung transplant setting. This study addresses the timing, the location, and the different elements of the immune system (innate and adaptive) and lung (airways, vessels, parenchyma, and pleura) changes during chronic rejection by using histology, *in vivo* and *ex vivo* μCT imaging, and single-cell RNA profiling.

Abbreviations: A, Allograft; BOS, Bronchiolitis obliterans syndrome; CXCR3, Chemokine CXC receptor 3; CXCL14, Chemokine CXC ligand 14; CLAD, Chronic lung allograft dysfunction; ECM, Extracellular matrix; FBS, Fetal bovine serum; FcR, Immunoglobulin receptor; HU, Hounsfield units; H&E, Hematoxylin–eosin; I, Isograft; Ig, Immunoglobulin; ILD, Interstitial lung disease; μCT, Microcomputed tomography; MHC, Major histocompatibility complex; MT, Masson trichrome; OB, Obliterative bronchiolitis; PGD, Primary graft dysfunction; RAS, Restrictive allograft dysfunction; Treg, Regulatory T lymphocyte.

Methods

Mouse orthotopic left lung transplantation

All mice received human care in compliance with the European Convention on Animal Care and the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH publication 86-23, 1996). The study was approved by the Ethics Committee for Animal Research at KU Leuven (P008/2017). Male C57BL6/N and BALB/C mice, 10–12 weeks old, were purchased from Janvier Labs (France). Orthotopic left lung transplantation was performed as described by Jungraithmayr et al. (7). In summary, following thoracotomy, the artery, vein,

and bronchus were separated from each other, and 10-0 ligatures were placed around the structures. The pulmonary artery and pulmonary vein were closed using 9.0 sutures. First, the vein was anastomosed, followed by the artery, and finally the bronchus. The sutures were released and the lung was inflated. Hereafter, the chest was closed, and the animals were placed on a heating pad after waking up.

Post-transplant study design

A total of 80 mice, consisting of 52 C57BL6/NRj and 28 BALB/cJRj mice, were used. In total, 28 left lung allografts from BALB/c donor mice were transplanted into C57BL/6N recipients. Twelve isograft transplantations were performed with C57BL/6N donor lungs in C57BL/6N recipients as controls. All mice received daily maintenance immunosuppression subcutaneously, consisting of cyclosporine (10 mg/kg/d CsA; Novartis, Belgium) and steroids (1.0 mg/kg/d or 1.6 mg/kg/d methylprednisolone; Pfizer, Belgium). The low dose of steroids is equivalent to the human situation, and the high dose corresponds to other mouse models considering the higher metabolism of mice (11, 12).

In the first set of transplantations, 16 allografts and 8 isografts were monitored daily until sacrifice (day 70). The 8 isografts (I1–I8) and the first 8 allografts received an immunosuppression regimen of CsA with low steroids (A1–A8) ($n = 8$), while the second 8 allografts received CsA with high steroids (A17–A24). The follow-up included the following: daily body weight monitoring, cyclosporine measurement in the blood (retro-orbitally bleeding) at day 56, blood sampling to measure immunoglobulins and complement, and *in vivo* μCT imaging at days 7, 35, and 70 (Figure 1). At sacrifice, a video of the ventilating lung was recorded to document the lung functionality. The macroscopic status of the lungs was classified as failure, extreme deformation, severe deformation, and mild changes (Figure 2). A failed lung had shrunken and was non-ventilating, with or without attachment to the thoracic wall. Within extremely deformed lungs, the lung structures (vessels, airways, and parenchyma) could not be discriminated against anymore, and only a hard, solid fibrotic mass was observed on histology. Within severe deformation, lung structures could still be identified, but no ventilation of the lung was seen. Macroscopically, mildly rejecting lungs were still ventilating and had a normal volume and normal surface appearance (Figure 3, Supplementary Figure S3). In a second group of eight allografts receiving CsA and high steroids, mice were sacrificed earlier to investigate the macroscopic changes and microscopic presentation of the early μCT. Allografts were sacrificed at day 7 (A11; $n = 1$), day 21 (A12 and A16; $n = 2$), day 35 (A9 and A10; $n = 2$), and day 56 (A13–A15; $n = 3$).

A last set of transplantations of three isografts (I10–I12) and three allografts (A25–A27) were sacrificed at days 7, 35, and 70, and the transplanted lungs were used for single-cell RNA sequencing. One healthy/untreated BALB/cJRj and one healthy/untreated C57BL/6NRj left lung were used as baseline controls. Finally, for *ex vivo* imaging, one isograft (I9) and one allograft (A28) were sacrificed at day 70. An overview of the mice and methodology is presented in Figure 1.

Longitudinal *in vivo* μCT imaging

To evaluate the left transplanted lung during follow-up, *in vivo* μCT imaging (days 7, 35, and 70) was performed with a small-animal μCT scanner (SkyScan 1278, Bruker, Belgium; resolution =

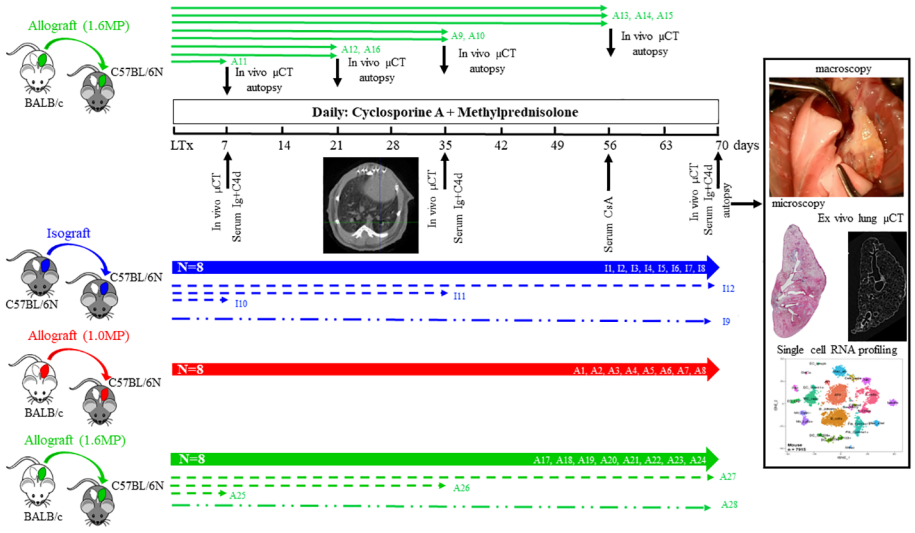

FIGURE 1
The study design of the allograft and isograft orthotopic single left lung transplantation in mice receiving daily immunosuppression of cyclosporine and steroids. Isograft (blue), allograft (low dose of steroids; red), and high dose of steroids (green) were sacrificed at 10 weeks ($n = 8$/group; thick lines). Additional allografts (high dose; $n = 8$) are sacrificed at weeks 1, 3, 5, and 8 (green, thin lines). Evaluation parameters post-transplantation are *in vivo* lung imaging, serum sampling and histology, *ex vivo* lung imaging, and single-cell analysis. Additional mice for single-cell RNA profiling and the *ex vivo* μCT are presented as dotted lines and dot-dashed lines. All animals are coded and reported later on.

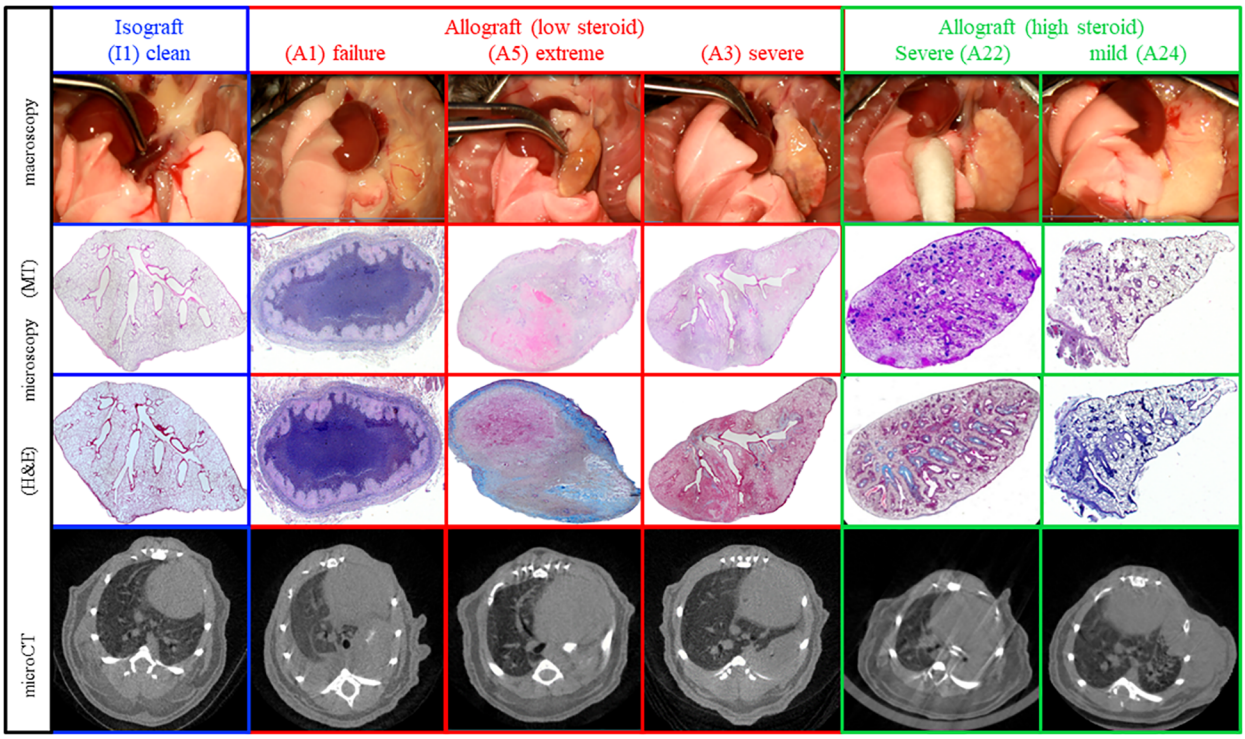

FIGURE 2
Representative macroscopy, microscopy, and *in vivo* µCT of the different pathological presentations at day 70. The different patterns include fully normal lungs, completely destroyed failures, and lungs demonstrating chronic rejection with a spectrum of extreme, severe, and mild rejection.

55 µm³). Mice were anesthetized, and respiratory-gated µCT images of free-breathing animals were acquired. The respiratory cycle was divided into four phases, from the initiation of inspiration to end expiration, and scan parameters were described previously (13) (14) to quantify lung volume and mean lung density for a manually delineated volume of interest (VOI) on the transversal µCT images at end expiration (15). The left transplanted and right native control lungs were analyzed separately to investigate their changes properly.

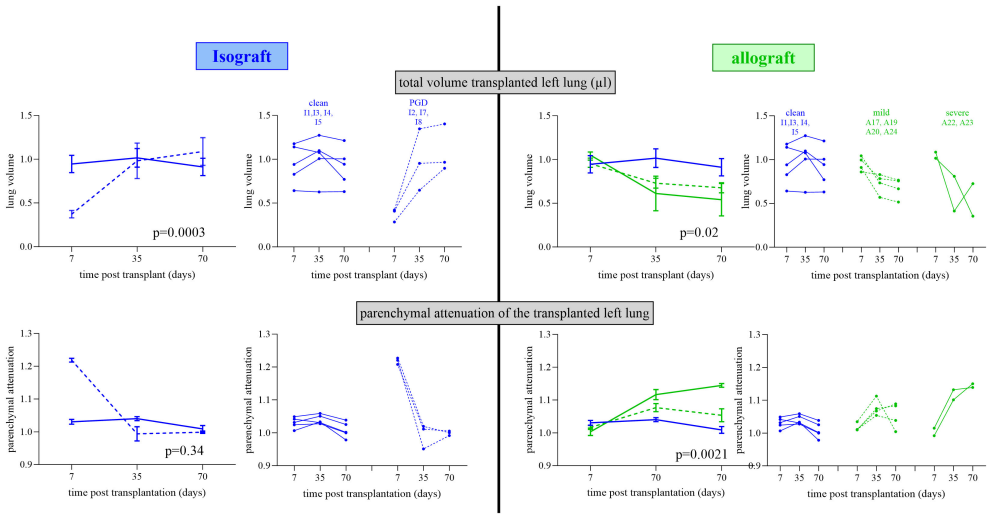

FIGURE 3
Repeated *in vivo* µCT lung evolution of the isograft and allograft groups. *In vivo* µCT lung evolution for lung volume and parenchymal attenuation. µCT parameters are normalized to the reference lungs. The left side shows the isografts (blue lines) stratified according to the occurrence of PGD (dotted line) or not (full line) with group variation and individual evolution. The right side shows isografts (no PGD; blue) and allografts (green) stratified according to mild (dotted line) and severe (full line) rejection with group variation and individual evolution. For group variation, the median with SEM is presented at each time point.

To make the data comparable, untreated C57BL/6N (n = 4) and BALB/c (n = 4) littermates were scanned to create baseline values. Owing to the anatomical differences in lung structure and volume, transplanted lungs of isografts were normalized using C57BL/6N baseline data, and transplanted lungs of allografts were normalized using BALB/c baseline data.

Lung histopathology

Lungs were fixed (10% formalin, 24 h) and paraffin sections (7 µm) were stained with hematoxylin–eosin (H&E) and Masson trichrome (MT), and sections were evaluated by a pathologist to identify the pathological elements of chronic rejection. To observe how lung structural changes parallel the organization of the immune system, mice were sacrificed at different time points to find a sequence of events with respect to lung architecture, as performed previously (11, 12).

Ex vivo µCT

One isograft (I9) and one mildly rejecting allograft (A28) lung collected at day 70 were used for *ex vivo* µCT to reconstruct the early changes in an allograft. The grafted lung was fixated (10% formalin; 24 h), followed by ethanol dehydration (70%/80%/90%/100%) and complete chemical drying in hexamethyldisilazane. Dried lungs were scanned using an *ex vivo* SkyScan 1272 µCT scanner (resolution = 2.5 µm; Bruker) to segment the airway, veins, and arterial lumen by ITK-SNAP (16).

Blood analysis

Cyclosporine blood levels were analyzed with an immunoassay (Dimension® RXL, Diamond Diagnostics, USA). Serum immunoglobulins (IgGA/IgE/IgM/IgG1/IgG2b/IgG2c/IgG3) were measured with a ProcartaPlex Mouse Isotyping Panel (Thermo Fisher, Belgium). Serum complement factor 4d was measured by the conventional ELISA kit C4d (MyBioSource, USA).

Single-cell RNA sequencing

Grafted lungs from three isografts (I10–I12) and three allografts (A25–A27) at days 7, 35, and 70 were excised and immediately processed into single-cell suspension according to the Miltenyi protocol (Miltenyi Lung Dissociation Kit mouse). One BalBc lung was included as a comparison. Single-cell suspensions of the left lung of seven mice (control BALB/c, n = 1; isografts at days 7 and 70, n = 1 per time point; allografts at days 7, 35, and 70, n = 1 per time point) were successfully obtained. Briefly, the lungs were flushed, excised, and cleaned of excess tissue. The MACS enzyme solution was instilled into the lung, and the lobes were transferred into gentle MACS tubes containing the enzyme mix to dissociate cells. Single-cell suspensions were cryopreserved in liquid nitrogen until sequencing. Single-cell RNA sequencing was performed using the 10x Genomics 3-prime-v3 dual index assay using the manufacturer's protocol. Sequencing was performed using an Illumina HiSeq4000. Read alignment was made as previously published (17) using the mouse genome (GRCm39). The gene-cell matrix was inputted into Seurat (v4.0.3) for analysis. The matrix was filtered to remove cells with <1,000 reads or >5% mitochondrial genes, normalized, and scaled with a regression of mitochondrial gene percentage. Clusters were grouped using Louvain clustering, and cell-type clusters were determined using canonical marker genes and FindAllMarkers to identify uniquely expressed genes based on their expression of these marker genes. Cells were then classified into epithelial, endothelial, stromal, and immune groups based on their type. UMAP reduction was used for visualization. Differentially expressed genes were identified using the FindMarkers set to compare allograft time points (e.g., A1W) with all other groups. GO enrichment analysis was performed with *clusterProfiler* (v3.18.0) (18). Up- and downregulated genes of allografts compared to isografts and controls were identified using the *FindMarkers* function in *Seurat* (expression in at least 10% of cells, adjusted p-value < 0.05, and average log2 fold change < −0.25 or >0.25). GO enrichment analysis was performed using enrichGO with default parameters and the org.Mm.eg.db (v3.12.0). Revigo and simRel were used to summarize GO ontology terms (19). Adjusted log10 p-values were visualized using *ggpubr* (v0.4.0). Connectome analysis was performed using the Connectome (v1.0.0) package on github (https://msraredon.github.io/Connectome/). Default parameters were used with the exception of setting the minimum Z-score to 2.6 for visualization. Cellular archetypes were identified with pseudotime analysis using the phateR (v1.0.7) and slingshot (v1.8.0) packages in R to determine cells that show correlated or unique features with disease progression. The visualization of heatmaps was done using ComplexHeatmap (v2.6.2).

Statistics

Data analysis was performed with Prism10 (GraphPad, USA) and expressed as the mean (± SEM). The D'Agostino and Pearson normality test was performed. To compare the different groups, a one-way analysis of variance (ANOVA) was used, and to compare different groups over time, a mixed-effects model with Tukey's multiple comparisons post-hoc test was used. A Mann–Whitney U test was performed to compare the different transplant groups and time effects. A value of 0.05 was considered significant.

Results

Macroscopic and microscopic evaluation of the lung grafts

Macroscopic evaluation of the transplanted lungs revealed differences across and within different groups (Figure 2, Supplementary Figure S1). Evaluation of the 24 transplanted lungs at day 70 revealed five failures, including one isograft (I6), two allografts with low steroid (A2 and A8), and two allografts with

high steroid (A18 and A21). Failures histologically presented necrosis destroying the lung, including parenchyma, airways, and vessels. In three of the graft failures (I6, A1, and A8), an end-stage fibrotic mass with few cells was the only remaining fragment of the lung. To properly study chronic rejection, failures were excluded from further analysis. Macroscopically, all isografts had a normal lung color and morphology and were ventilated well. Within the low-steroid allografts, three allografts showed extreme deformation (A1, A4, and A6), and three allografts showed severe deformation (A3, A5, and A7). Within the high-steroid group, two allografts (A22 and A23) were severely deformed, while four allografts (A17, A19, A20, and A24) were mildly affected. Within the allografts, only mild rejecting allografts were ventilating at day 70 (Supplementary Figure S1). There were no differences in body weight ($p = 0.92$) and cyclosporine levels ($p = 0.35$) between the three groups (Supplementary Figure S2). The cyclosporine level of all mice was 587 ± 35 µg/L. Microscopic evaluation supported the macroscopic observation (Figure 4). Macroscopically, extreme deformation within the low-steroid allografts (A2, A4, and A6) was presented as intense end-stage fibrosis in all compartments. Although the vessels and airways could be located, these were never functional.

In vivo repeated µCT evaluation

Evaluation of lung volume changes showed differences within isografts and allografts over time ($p < 0.0001$) (Figure 5). Lung volume at day 7 was comparable between allografts and isografts. Over time, isografts showed an increasing lung volume, while allografts decreased. Changes in lung volume were observed between mildly and severely affected allografts versus clean isografts ($p = 0.025$), as mildly and severely rejected allografts had volume reductions at day 35 ($p = 0.12$ and $p = 0.39$) and day 70 ($p = 0.18$ and $p = 0.40$) versus isografts (Figure 5). The lung volume difference between inspiration and expiration on µCT scans (a type of tidal volume) was compromised in allografts and was greatly compromised in severely rejecting allografts. Analysis of the right native lung confirmed that the decrease in tidal volume was caused by the graft (Supplementary Figure S3). Parenchymal attenuation of the transplanted lung was different ($p < 0.0001$) between isografts and allografts. Allografts were initially open (day 7) and lung attenuation appeared afterwards (Figure 6). Attenuation was increased in mild and severe rejecting allografts at day 35 ($p = 0.087$ and $p = 0.036$) and day 70 ($p = 0.15$ and $p = 0.0002$) versus isografts. Low-steroid allografts had more attenuation than high-steroid allografts at days 35 and 70 ($p = 0.0011$ and 0.009). The native lung had no increased attenuation, confirming the absence of collateral damage or possible infection (Supplementary Figure S4). Isografts demonstrated a normal lung appearance without attenuation at day 70, but at day 7, some isografts demonstrated attenuation and resembled potential primary graft dysfunction (PGD) (Figure 3). PGD is a type of severe lung injury that occurs within the first 72 h of lung transplantation and is the most common cause of early mortality. PGD decreased towards days 35 and 70. PGD is a graft defect. Repeated µCT revealed lung volume differences between isografts with and without PGD ($p = 0.0003$). While the lung volume in PGD was lower at day 7, it returned to the level of the isografts without PGD at days 35 and 70 (Figure 3).

Pathological pattern of chronic rejection in time and space

Pathological examination of allografts under high immunosuppression revealed an evolutionary pattern of chronic rejection organized by time, location, and immune response (Figure 7). Stage 1, shown in an allograft (A11, day 7), presented neutrophil extravasation into the vessel wall of end-arterioles, reducing the arteriolar lumen without increasing wall thickness. Stage 2 showed inflammation around end-arterioles and end-venules. The innate activation around end-arterioles increased in

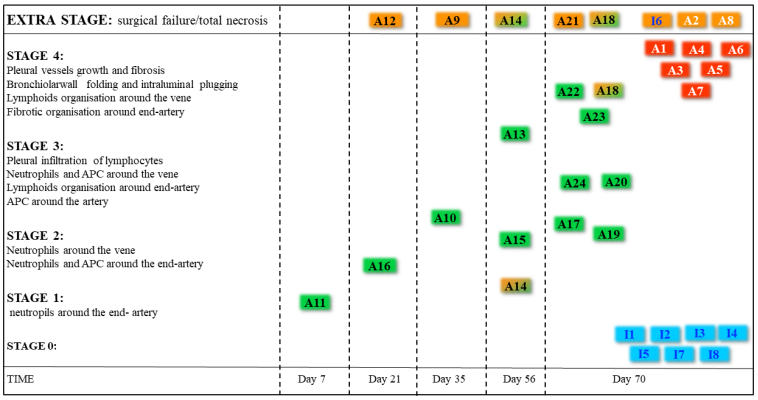

FIGURE 4
The pathological staging of rejection. All allografts were used to identify the stage and were subdivided into stages of rejection. Failures are documented in orange boxes. Two animals demonstrated a part of the lung to be destroyed, and another part presented rejection (half green half orange boxes). The color of the boxes in the included lungs represents the origin of the graft being an isograft (blue, n = 7), an allograft under high-dose steroids (green boxes, n = 13), and an allograft under low-dose steroids (red boxes, n = 6).

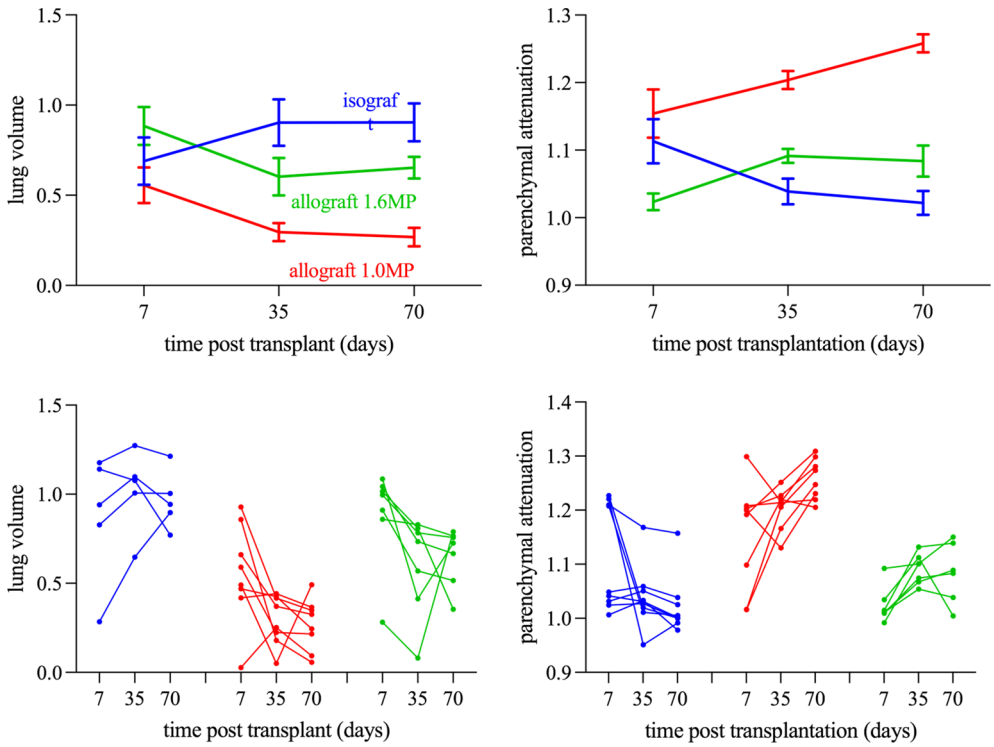

FIGURE 5
Longitudinal morphometric μCT analysis stratified according to the severity of rejection (Tx left lung). **(A)** Total lung volume of the transplanted left lung. **(B)** Parenchymal attenuation of the transplanted left lung. The Left shows the isograft group (blue lines) stratified according to the occurrence of PGD (dotted lines). The right shows the Allograft 1.6MP group (green lines) stratified according to the severity of rejection (mild rejection is denoted as dotted lines and severe rejection is denoted as full lines). All data have been normalized as described in the Methods section.

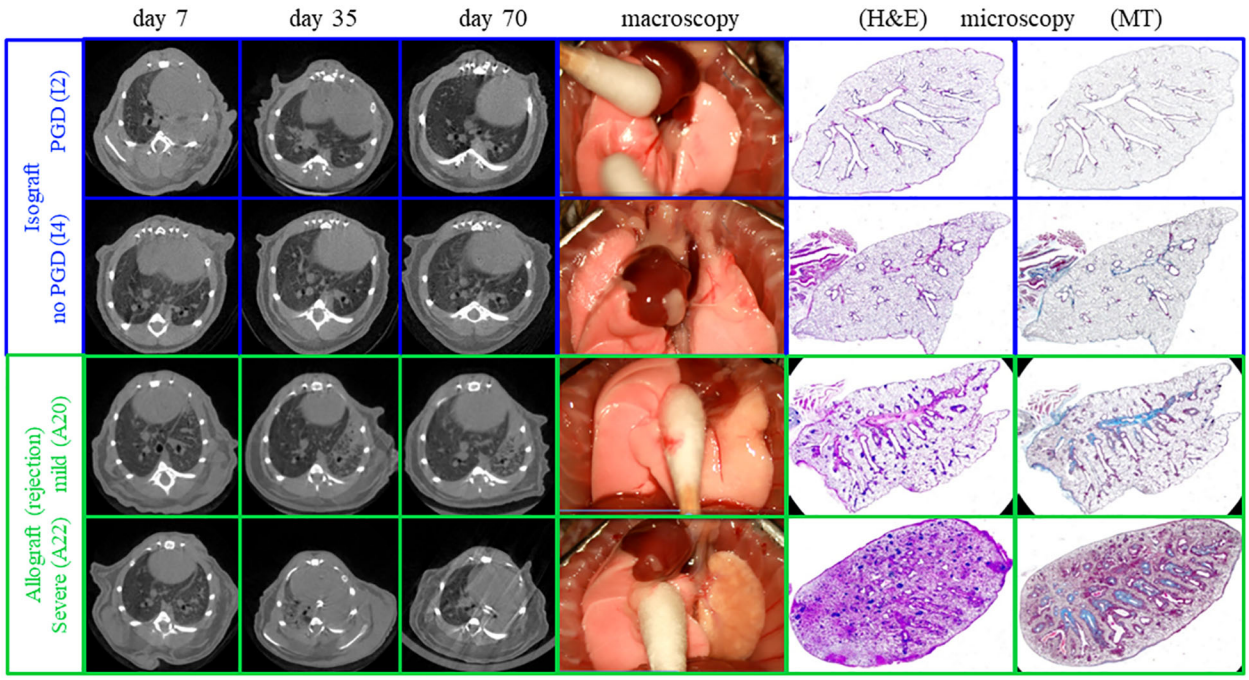

FIGURE 6
Representative macroscopy, microscopy, and *in vivo* μCT imaging of mild and severe chronic rejection. One allograft with mild rejection and one with severe rejection are presented (green). In comparison, a control isograft is present, and an additional isograft demonstrating on μCT at week 1 primary graft dysfunction (PGD).

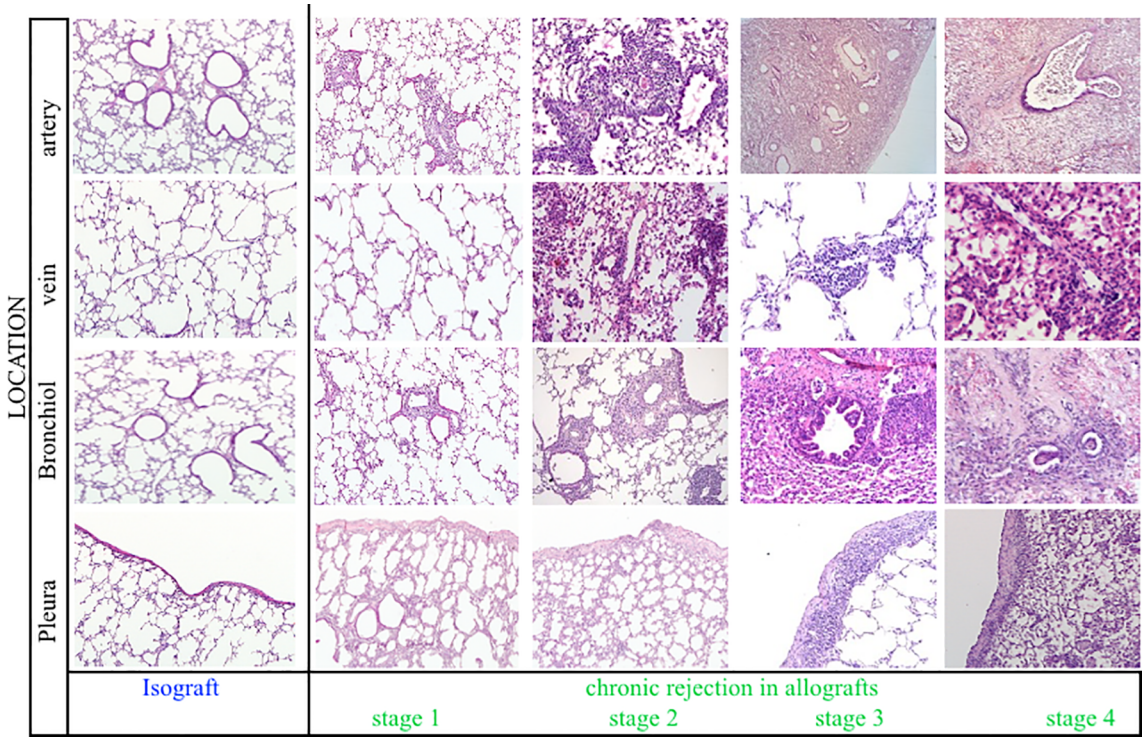

FIGURE 7
Representative histological illustrations of the four stages of chronic rejection. For each stage, the different anatomical lung compartments involved were presented, including arteries, veins, bronchioles, and pleura. Stage 1, Stage 2, Stage 3, and Stage 4 are represented by A11 at day 7, A16 at day 21, A24 at day 70, and A22 at day 70, respectively.

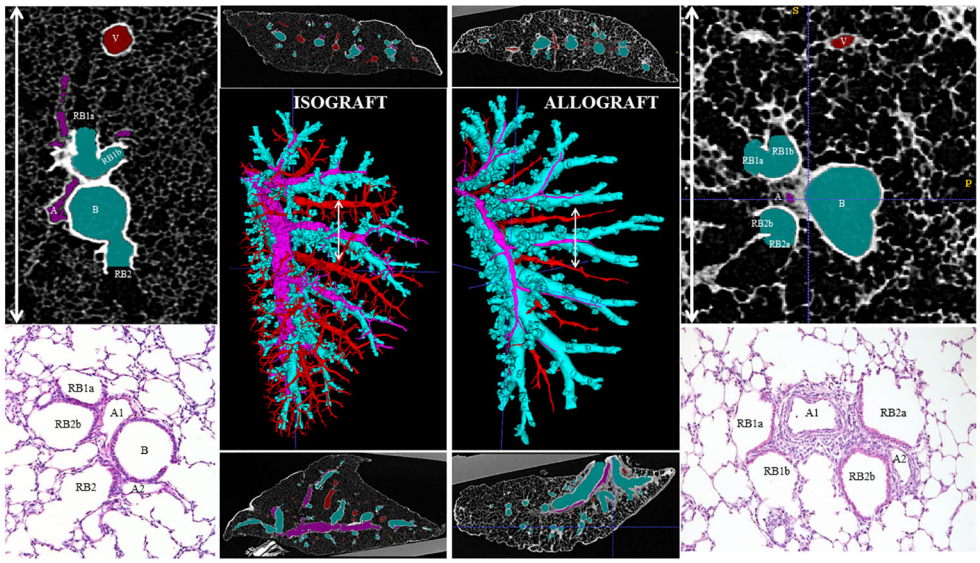

FIGURE 8
An *ex vivo* high-resolution µCT imaging and reconstruction of the organization of chronic rejection. The airways (light blue), arterial vessels (pink), and venous vessels (red) of the transplanted left lung were segmented and reconstructed in 3D (middle large picture) in an isograft (left) and an allograft (right). A transverse and sagittal image of the scans is presented above and below the reconstruction. On the right and left sides of the figure, µCT (top) and histological (bottom) details of the broncho-vascular bundle, specifically of the location of the white arrow line, identify the arterial origin of rejection at the generation where airways go over in respiratory bronchioles.

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size with the influx of antigen-presenting cells. Simultaneously, neutrophils infiltrated the end-venules, but the bronchioles were still not involved. *Ex vivo* µCT confirmed the vessel lumens narrowing for both arteries and veins, while the airway lumen remained unaffected (Figure 8, Supplementary Figure S5). Evolution toward Stage 3 consisted of immune organization around arteries evolving toward adaptive activation. Venules remained innate, but monocytes appeared besides neutrophils. The lumen of the venules decreased, and the pleural compartment started to be infiltrated by lymphocytes. The final stage of rejection (Stage 4) of arterioles evolved toward fibrosis with end-arterial obliterans. The venous compartment remained

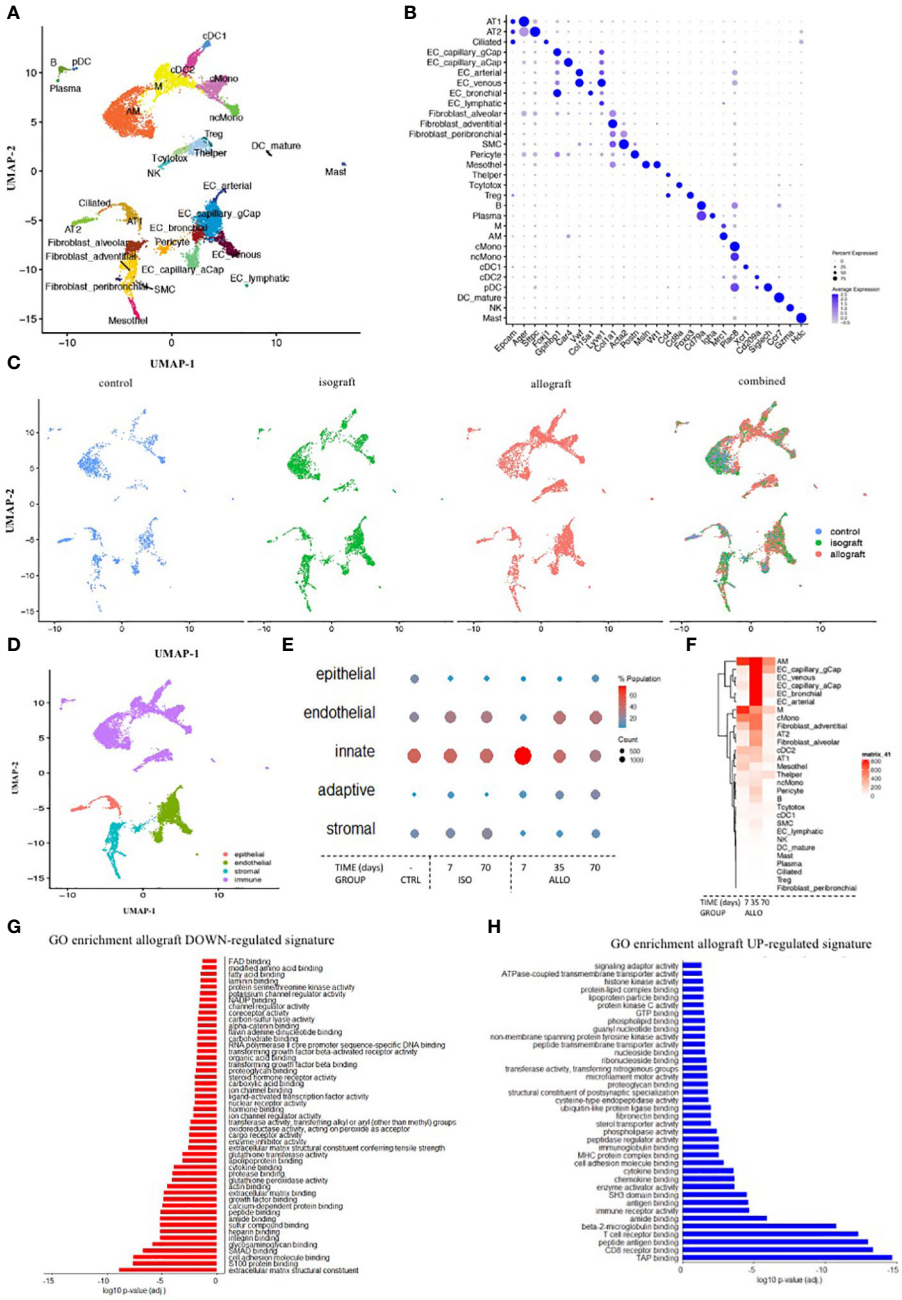

FIGURE 9
Single-cell RNA profiling to validate the sequence of chronic rejection across the different cells involved and subcellular mechanisms. **(A)** UMAP plot of the cells from the left lung of three isografts (7, 35, and 70 days), three allografts (7, 35, and 70 days), and one control BalBc lung color-coded by major cellular lineage. **(B)** Dot plot heatmap of the expression of representative marker genes of cellular lineages. The size and color intensity of each dot represent, respectively, the percentage or average expression of the marker gene in this cell type. Color scale: blue, high expression; white, low expression. **(C)** UMAP plot of lung cells, color-coded for the indicated conditions of the left lung. **(D)** UMAP plot of lung cells, color-coded for the indicated major cell subcluster. **(E)** Dot plot heatmap of the major cell subcluster. The size and color intensity of each dot represent, respectively, the total number and percentage of cells within each cell type. Color scale: red, high expression; blue, low expression. **(F)** Gene expression heatmap of all individual genes in every identified cell type. Color scale: red, high expression; blue, low expression. **(G, H)** A barplot of the GO enrichment analysis of down- and upregulated gene signatures in allograft lungs.

innately immune-organized, and bronchioles affected by the neighboring arteriolar inflammation demonstrated typical bronchiolar folding, but only one allograft presented fibrotic intraluminal plugging (bronchiolitis obliterans).

Single-cell RNA profiling to validate the sequence of chronic rejection

Single-cell profiling sequenced 12,821 cells from seven mice. Each specific cell within control, isograft, and allograft lungs was presented in a UMAP plot and gene marker validation (Figures 9A-C). The different cell types were clustered into structural epithelial/endothelial, innate, adaptive, and stromal cells (Figures 9D, E). The structural cells decreased at day 7 in allografts compared to isografts and controls and recovered gradually at days 35 and 70 (Supplementary Figure S7C). Innate cells increased at day 7 and returned to the isograft and control levels at days 35 and 70 (Figure 9E, Supplementary Figures S7A-C). Cell profiling of eosinophils and neutrophils was unsuccessful and could not be evaluated. Adaptive cells, including T helper and cytotoxic cells, B cells, plasma B cells, and Treg cells, gradually increased toward day 70. Finally, stromal cells started to increase and eventually generated the fibrotic environment (Figure 9E) for chronic rejection. Although adventitial fibroblasts appear to be the leading producers of extracellular matrix (ECM), multiple stromal cells were upregulated.

Overall gene expression in allografts was increased versus isografts and control lungs. Endothelial cells and monocytes/macrophages showed a particular increase in gene expression, suggestive of a key role in the onset and progression of rejection (Figure 9F). Upregulation of signaling pathways linked to the immune response, with innate and adaptive elements such as MHCI/II elements, receptor binding, proteasome formation, and downstream signal transduction (Figure 9H), was found. In the early onset of rejection, macrophages presented an increased expression of MHCII together with CXCR3, CXCL14, and FcRE. T cell receptor-related costimulatory elements are upregulated at an early stage, even prior to the increase and proliferation of the T cells (Supplementary Figure S8D). To examine the temporal evolution of the expression, pseudotime analysis identified genes involved in early and late processes (Supplementary Figures S8E, F). Within the Tc cells, inducer and effector cytokines are increasingly expressed (Supplementary Figure S8C). ECM production was very active in the early period after transplantation and subdued at day 70 (Supplementary Figure S9D). Contractile properties are present in smooth muscle cells, whose expression levels decreased early after transplantation of isografts but recovered later (Supplementary Figure S9D). Connectome analysis confirms these complex changes of lung homeostasis and immune activation with innate cells linked to endothelial cell involvement early on and to adaptive cell involvement later on (Supplementary Figures S9E, F). GO enrichment analysis confirmed that structural cells and stromal cells have very low general gene expression. Downregulation of signaling pathways related to cell homeostasis, integrity, and organization was observed to be involved in the onset of rejection (Figure 9G). Mechanistic clues demonstrated that isografts receiving immunosuppression had lower levels of MHC molecules in the structural cells. However, during rejection, MHC expression increased in allografts above isograft levels (Supplementary Figure S7D). These higher levels of MHCI are confirmed by the increase in proteasome elements, expression of chemokines, and interferon elements (Supplementary Figure S7E). In addition, MHCII expression was also increased, especially during the early phase of rejection.

Systemic humoral involvement

Measurements of humoral components showed large inter-individual variation, making it difficult to reach significance. IgA and IgG1 were below detection, while immunoglobulins IgG1, IgG2, and IgG3 tended to increase in severe rejection. IgG2c and IgG3 were linked to adaptive organization in late and severe rejection. IgM slightly increased early on, while IgE and C4d tended to increase in late severe rejection. Humoral activation was absent in isografts (Supplementary Figure S10).

Discussion

This orthotopic lung transplant model, including a major MHC mismatch with immunosuppression, is the first to examine the nature, timing, and place of chronic rejection after lung transplantation. The methodological approach combining imaging, histology, and transcriptomic profiling allows the observation of chronic rejection from pathology to immunology.

We revealed the true nature of chronic rejection after lung transplantation, originating around vessels and, more precisely, around the arterioles. After innate activation, adaptive activation and fibrosis around arteries resulted in end-arteritis obliterans. Only later did innate venous inflammation, pleural infiltration/fibrosis, and "obliterative bronchiolitis" appear. µCT imaging confirmed that the gradual rejection model within 10 weeks was reproducible. The gradual onset of rejection questions the segregation of rejection into hyperacute, acute, and chronic rejection. Both cellular and humoral immunity may be part of the same immune response to rejection, where only the timing and magnitude differ.

The most important finding is the endothelial origin of chronic rejection, which alludes to abandoning the old enigma of airway-centered rejection, "obliterative bronchiolitis". The concept that recipients' immune cells only identify foreign cells in the small airway as "non-self" and induce the rather limited immune organization of the OB is counterintuitive. Although previous mouse lung transplant studies identified intraluminal airway fibrosis and constriction, including pleuro-parenchymal infiltration and fibrosis (10, 12, 20, 21), this study identified the first site of chronic rejection as being at the arteriole site. This new observation is in line with all solid organ transplantations (22) and is more plausible as recipients' immune cells enter the "foreign" donor via arteries, representing the first contact location.

µCT imaging opened unique insights into the progression of rejection. Early innate onset and adaptive immune activation around the arteries and venous compartment are presented as mild lung attenuation. Severe attenuation is present when fibrotic organization around arteries, venous innate inflammation, and pleural and airway inflammation are histologically found.

Histological imaging of chronic rejection in both time and place was confirmed by single-cell RNA profiling. The earliest event of (chronic) rejection—endothelial activation—was observed by the upregulation of MHC1/2, adhesion molecules, and integrins, initiating extravasation of innate and adaptive immune cells. Within T helper cells, early master, inducer, and effector cytokines were not only increasingly expressed, demonstrating lymphoid activation, but also blocked regulation as Treg cells. Rejection is not only about immune cells controlling homeostasis but also about structural and stromal cells. Low expression levels in structural cells and stromal cells indicate that the lung structure is under pressure and its homeostasis is lost. Although adventitial fibroblasts appear to be the leading producers of ECM in rejection, multiple stromal cells were identified as ECM drivers, supporting the idea that rejection is more than restricted to OB lesions. In addition to the B cell involvement found by histology and cell profiling, humoral elements such as IgG2c and IgG3 confirmed the adaptive response in late rejection, in line with delayed-type hypersensitivity of rejection. Obviously, cell profiling should be more mechanistically validated.

This murine model, with its diagnostic tool, opens new horizons. This study maps (chronic) rejection and confirms its standard immune response nature, as we have only one immune system. Since all cells, cytokines, and so on resemble the classical immune responses, it is difficult to consider the specificity of rejection. Immune responses against microbial and malignant cells or immune responses due to secondary immune deficiency may have seriously biased our understanding of rejection. All can be studied in this controlled setting by paralleling infections, environmental factors, medication non-adherence, and autoimmune-induced immune responses. In addition, this model opens perspectives for immunotherapy research.

Limitations are the low *n*-values, the presence of failures, and the heterogeneity of rejection. To prevent failures, it is important to identify infections, twisted cuffs, and air leaks. The heterogeneity in the progression and severity of rejection may be related to surgical processes such as suturing difficulties, flushing issues, twisted cuffs, and the uptake of immunosuppression.

Our goal to validate the histological and imaging findings of rejection was achieved very elegantly and provided new avenues for research. Where chronic rejection fits into the clinical hallmarks of BOS and RAS is not clear-cut anymore, and how these mouse findings of rejection parallel the spectrum of CLAD remains to be answered. The observed lesions in the mouse are most consistent with RAS. Patients with BOS may experience early chronic rejection, but the pronounced airway pathology caused by immunosuppression or excessive exposure through inhalation of microorganisms and pollution may be confused with rejection. BOS and RAS are different but may have more overlap than identified.

This study described the true nature, timing, and location of chronic rejection after lung transplantation in murine orthotopic lung transplantation using cutting-edge diagnostic tools and opened new horizons for research. It invites researchers to re-explore chronic rejection in the clinical setting of CLAD.

Data availability statement

The single cell RNA dataset presented in the study are deposited in the NCBI SRA bioproject, accession number PRJNA1076139: The nature of chronic rejection after lung transplantation: a murine orthotopic lung transplant study.

Ethics statement

The animal study was approved by the Ethics Committee for Animal Research at KU Leuven. The study was conducted in accordance with local legislation and institutional requirements.

Author contributions

TH: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. JK: Formal analysis, Investigation, Methodology, Writing – review & editing. CA: Formal analysis, Investigation, Methodology, Writing – review & editing. JSe: Investigation, Methodology, Writing – review & editing. YY: Formal analysis, Methodology, Writing – review & editing. VG: Methodology, Writing – review & editing, Investigation, Visualization. AH: Data curation, Formal analysis, Writing – review & editing, Investigation, Methodology. ArV: Investigation, Methodology, Writing – review & editing, Formal analysis. AS: Methodology, Writing – review & editing, Formal analysis, Investigation. SO: Methodology, Writing – review & editing, Formal analysis. AF: Investigation, Methodology, Formal analysis, Writing – review & editing. BS-G: Writing – review & editing, Data curation, Formal analysis, Visualization. JSl: Investigation, Methodology, Writing – review & editing. HB: Investigation, Methodology, Writing – review & editing, Data curation. NA: Methodology, Writing – review & editing, Visualization. MO: Methodology, Writing – review & editing, Investigation. AnV: Investigation, Methodology, Writing – review & editing. SC: Formal analysis, Methodology, Writing – review & editing, Software. DS: Methodology, Writing – review & editing, Formal analysis, Supervision, Validation. GV: Methodology, Writing – review & editing, Formal analysis, Supervision. JSc: Formal analysis, Methodology, Writing – review & editing, Data

curation, Investigation. NK: Methodology, Supervision, Writing – review & editing, Funding acquisition, Investigation, Project administration. MB: Data curation, Formal analysis, Methodology, Writing – review & editing. HK: Funding acquisition, Supervision, Writing – review & editing, Resources. SM: Methodology, Writing – review & editing, Resources, Visualization. LD: Resources, Supervision, Writing – review & editing, Project administration. JV: Methodology, Writing – review & editing, Conceptualization, Supervision. LG: Supervision, Writing – review & editing, Data curation, Methodology. DR: Methodology, Conceptualization, Data curation, Investigation, Supervision, Writing – original draft. WJ: Supervision, Writing – review & editing, Funding acquisition, Project administration, Resources. GG-R: Funding acquisition, Project administration, Supervision, Writing – review & editing, Investigation, Methodology. LC: Investigation, Methodology, Supervision, Writing – review & editing. JM: Data curation, Methodology, Software, Writing – review & editing. EV: Methodology, Conceptualization, Data curation, Investigation, Supervision, Writing – original draft. RV: Conceptualization, Funding acquisition, Supervision, Writing – review & editing. BV: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2024.1369536/full#supplementary-material

SUPPLEMENTARY VIDEO 1
Representative macroscopy, including lung ventilation, microscopy, and µCT of the different pathological presentations. The different patterns include fully normal lungs, completely destroyed failures, and lungs demonstrating chronic rejection with a spectrum of extreme, severe, and mild rejection.

SUPPLEMENTARY FIGURE 2
Postoperative body weight and cyclosporine trough levels. The cyclosporine trough level of all mice was 587 ± 35µg/L, within the aimed range related to a better outcome for mice.

SUPPLEMENTARY FIGURE 4
Morphometric longitudinal *in vivo* µCT lung evolution of the native right lung for lung volume and parenchymal attenuation. At each time point, either individual values or the median with SEM are presented.

SUPPLEMENTARY VIDEO 2
Representative longitudinal *in vivo* µCT lung reconstruction of the representative grafts. I2, I4, A20, and A22 are represented by inspiration and expiration scans, and three rotating and ventilating 3D reconstructions of the scans at 7, 35, and 70 days.

SUPPLEMENTARY VIDEO 3
Representative *ex vivo* µCT lung reconstruction of an isograft and mild allografts. The airways (light blue), arterial (pink), and venous (red) systems of I9 and A28 (day 70) are segmented and reconstructed in 3D.

SUPPLEMENTARY FIGURE 6
Tidal volume of the different presentations of rejection. From the representative graft (I2, I4; A20 A22) the lung volume difference between in and expiration was calculated for each time point of the whole lung (upper panel), the right native lung (left side), and the left transplanted graft lung (right side). In the lower panel, a subdivision is made for PGD in the isograft group and for mild versus severe in the allograft group.

SUPPLEMENTARY FIGURE 7
Single-cell RNA profiling of the different cells of the structural cell subcluster. **(A)** UMAP plot of all cells of the left lung color-coded by major cellular lineage. **(B)** UMAP plot of the structural cells of the left lung, color-coded by the cellular subcluster of the structural cells. **(C)** Dot plot heatmap of the structural cell subcluster. The size and color intensity of each dot represent, respectively, the percentage of cells within each cell type. Color scale: red, high expression; blue, low expression. **(D)** A heatmap of the expression of mouse MHC1/2 complex H-2 genes within the endothelial cell lineages, where each line represents a specific endothelial cell and the color intensity represents the expression of the specific H2 gene. Color scale: yellow, high expression; purple, low expression. **(E)** Violin plots of proteasome elements, chemokines, and interferon pathway members were divided into the control mouse and isograft and allograft at days 7, 35, and 70. **(F)** UMAP plot of lung cells, color-coded for different adhesion molecules. Color scale: purple, high expression; grey, low expression. G/UMAP plot of lung cells for controls, isografts, and allografts, color-coded for the adhesion molecule CD34. Color scale: purple, high expression; grey, low expression.

SUPPLEMENTARY FIGURE 8
Single-cell RNA profiling of the different cells of the immune cell subcluster. **(A)** UMAP plot of all cells of the left lung, color-coded by major cellular subcluster. **(B)** UMAP plot of the immune cells of the left lung color-coded by the cellular subcluster of the immune cells. **(C)** Dot plot heatmap of the

immune cell subcluster. The size and color intensity of each dot represent, respectively, the percentage of cells within each cell type. Color scale: red, high expression; blue, low expression. **(D)** Violin plots of T cell activation elements specific to the T cell subcluster divided into the control mouse, isograft, and allograft at days 7, 35, and 70. **(E)** and **(G)** Heatmaps of gene expression of genes correlated with pseudotime disease progression (Pseudotime: red to blue) within innate macrophages or adaptive T cells. Gene expression was scaled and plotted from low (purple) to high (yellow) expression.

SUPPLEMENTARY FIGURE 9

Single-cell RNA profiling of the different cells of the stromal cell subcluster. **(A)** UMAP plot of all cells of the left lung, color-coded by major cellular cluster. **(B)** UMAP plot of the structural cells of the left lung, color-coded by the cellular cluster of the stromal cells. **(C)** Dot plot heatmap of the stromal cell subcluster. The size and color intensity of each dot represent, respectively, the percentage of cells within each cell type. Color scale: red, high expression; blue, low expression. **(D)** Dot plot heatmap of expression of representative marker genes of contractility and extracellular matrix proteins for the different groups, including control, isograft, and allograft (with time points). The size and color intensity of each dot represent respectively the percentage or average expression of the marker gene in this cell type. Color scale: blew, high expression; white, low expression. **(E)** Connectome showing ligand-receptor pairs identified within control, isograft, or allograft mice. Colors denote the different cell types. **(F)** Connectome showing ligand-receptor pairs identified in the different allograft timepoints (A1W, A5W, or A10W). It should be noted that collagen ligands were highly connected in the early stages of rejection.

SUPPLEMENTARY FIGURE 10

Serial evaluation of systemic immunoglobulins and complement factors. Immunoglobulins included are IgG1, IgG2b, IgG2c, IgG3, IgM, IgA, and IgE accompanied by IgG against double strained DNA, BL6 DNA, and BalBc DNA. For complement, C4d was used.

References

1. Sayegh MH, Carpenter CB. Transplantation 50 years later - progress, challenges, and promises. *New Engl J Med*. (2004) 351:2761–6. doi: 10.1056/NEJMon043418

2. Li XC, Jevnikar AM. *Transplant immunology*. Houston, USA: Wiley-Blackwell (2015). doi: 10.1002/9781119072997

3. Verleden GM, Glanville AR, Lease ED, Fisher AJ, Calabrese F, Corris PA, et al. Chronic lung allograft dysfunction: Definition, diagnostic criteria, and approaches to treatment-A consensus report from the Pulmonary Council of the ISHLT. *J Heart Lung Transplant*. (2019) 38:493–503. doi: 10.1016/j.healun.2019.03.009

4. Barker AF, Bergeron A, Rom WN, Hertz MI. Obliterative bronchiolitis. *N Engl J Med*. (2014) 370:1820–8. doi: 10.1056/NEJMra1204664

5. Sato M, Waddell TK, Wagnetz U, Roberts HC, Hwang DM, Haroon A, et al. Restrictive allograft syndrome (RAS): a novel form of chronic lung allograft dysfunction. *J Heart Lung Transplant*. (2011) 30:735–42. doi: 10.1016/j.healun.2011.01.712

6. Okazaki M, Krupnick AS, Kornfeld CG, Lai JM, Ritter JH, Richardson SB, et al. A mouse model of orthotopic vascularized aerated lung transplantation. *Am J Transplant*. (2007) 7:1672–9. doi: 10.1111/j.1600-6143.2007.01819.x

7. Jungraithmayr WM, Korom S, Hillinger S, Weder W. A mouse model of orthotopic, single-lung transplantation. *J Thorac Cardiovasc Surg*. (2009) 137:486–91. doi: 10.1016/j.jtcvs.2008.10.007

8. Suzuki H, Fan L, Wilkes DS. Development of obliterative bronchiolitis in a murine model of orthotopic lung transplantation. *J Vis Exp*. (2012) 65. doi: 10.3791/3947

9. Oishi H, Martinu T, Sato M, Matsuda Y, Hirayama S, Juvet SC, et al. Halofuginone treatment reduces interleukin-17A and ameliorates features of chronic lung allograft dysfunction in a mouse orthotopic lung transplant model. *J Heart Lung Transplant*. (2016) 35:518–27. doi: 10.1016/j.healun.2015.12.003

10. Martinu T, Oishi H, Juvet SC, Cypel M, Liu M, Berry GJ, et al. Spectrum of chronic lung allograft pathology in a mouse minor-mismatched orthotopic lung transplant model. *Am J Transplant*. (2019) 19:247–58. doi: 10.1111/ajt.15167

11. De Vleeschauwer S, Jungraithmayr W, Wauters S, Willems S, Rinaldi M, Vaneylen A, et al. Chronic rejection pathology after orthotopic lung transplantation in mice: the development of a murine BOS model and its drawbacks. *PloS One*. (2012) 7:e29802. doi: 10.1371/journal.pone.0029802

12. Yamada Y, Vandermeulen E, Heigl T, Somers J, Vaneylen A, Verleden S, et al. The role of recipient derived interleukin-17A in a murine orthotopic lung transplant model of restrictive chronic lung allograft dysfunction. *Transplant Immunol*. (2016) 39:10–7. doi: 10.1016/j.trim.2016.10.001

13. Berghen N, Dekoster K, Marien E, Dabin J, Hillen A, Wouters J, et al. Radiosafe micro-computed tomography for longitudinal evaluation of murine disease models. *Sci Rep*. (2019) 9:17598. doi: 10.1038/s41598-019-53876-x

14. De Langhe E, Vande Velde G, Hostens J, Himmelreich U, Nemery B, Luyten FP, et al. Quantification of lung fibrosis and emphysema in mice using automated micro-computed tomography. *PloS One*. (2012) 7:e43123. doi: 10.1371/journal.pone.0043123

15. Vande Velde G, Poelmans J, De Langhe E, Hillen A, Vanoirbeek J, Himmelreich U, et al. Longitudinal micro-CT provides biomarkers of lung disease that can be used to assess the effect of therapy in preclinical mouse models and reveal compensatory changes in lung volume. *Dis Models Mechanisms*. (2016) 9(1):91–8. doi: 10.1242/dmm.020321

16. Yushkevich PA, Piven J, Hazlett HC, Smith RG, Ho S, Gee JC, et al. User-guided 3D active contour segmentation of anatomical structures: Significantly improved efficiency and reliability. *NeuroImage*. (2006) 31:1116–28. doi: 10.1016/j.neuroimage.2006.01.015

17. Adams TS, Schupp JC, Poli S, Ayaub EA, Neumark N, Ahangari F, et al. Single-cell RNA-seq reveals ectopic and aberrant lung-resident cell populations in idiopathic pulmonary fibrosis. *Sci Adv*. (2020) 6:eaba1983. doi: 10.1126/sciadv.aba1983

18. Yu G, Wang L-G, Han Y, He Q-Y. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS*. (2012) 16:284–7. doi: 10.1089/omi.2011.0118

19. Supek F, Bošnjak M, Škunca N, Šmuc T. REVIGO summarizes and visualizes long lists of gene ontology terms. *PloS One*. (2011) 6:e21800. doi: 10.1371/journal.pone.0021800

20. Fan L, Benson HL, Vittal R, Mickler EA, Presson R, Jo Fisher A, et al. Neutralizing IL-17 prevents obliterative bronchiolitis in murine orthotopic lung transplantation. *Am J Transplant*. (2011) 11:911–22. doi: 10.1111/j.1600-6143.2011.03482.x

21. Misumi K, Wheeler DS, Aoki Y, Combs MP, Braeuer RR, Higashikubo R, et al. Humoral immune responses mediate the development of a restrictive phenotype of chronic lung allograft dysfunction. *JCI Insight*. (2020) 5. doi: 10.1172/jci.insight.136533

22. Libby P, Pober JS. Chronic rejection. *Immunity*. (2001) 14:387–97. doi: 10.1016/S1074-7613(01)00119-4

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Elucidating T cell dynamics and molecular mechanisms in syngeneic and allogeneic islet transplantation through single-cell RNA sequencing

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Islet transplantation is a promising therapy for diabetes treatment. However, the molecular underpinnings governing the immune response, particularly T-cell dynamics in syngeneic and allogeneic transplant settings, remain poorly understood. Understanding these T cell dynamics is crucial for enhancing graft acceptance and managing diabetes treatment more effectively. This study aimed to elucidate the molecular mechanisms, gene expression differences, biological pathway alterations, and intercellular communication patterns among T-cell subpopulations after syngeneic and allogeneic islet transplantation. Using single-cell RNA sequencing, we analyzed cellular heterogeneity and gene expression profiles using the Seurat package for quality control and dimensionality reduction through t-SNE. Differentially expressed genes (DEGs) were analyzed among different T cell subtypes. GSEA was conducted utilizing the HALLMARK gene sets from MSigDB, while CellChat was used to infer and visualize cell-cell communication networks. Our findings revealed genetic variations within T-cell subpopulations between syngeneic and allogeneic islet transplants. We identified significant DEGs across these conditions, highlighting molecular discrepancies that may underpin rejection or other immune responses. GSEA indicated activation of the interferon-alpha response in memory T cells and suppression in CD4+ helper and γδ T cells, whereas TNFα signaling via NFκB was particularly active in regulatory T cells, γδ T cells, proliferating T cells, and activated CD8+ T cells. CellChat analysis revealed complex communication patterns within T-cell subsets, notably between proliferating T cells and activated CD8+ T cells. In conclusion, our study provides a comprehensive molecular landscape of T-cell diversity in islet

transplantation. The insights into specific gene upregulation in xenotransplants suggest potential targets for improving graft tolerance. The differential pathway activation across T-cell subsets underscores their distinct roles in immune responses posttransplantation.

KEYWORDS

diabetes, islet transplantation, allotransplantation, single-cell RNA sequencing, T-cell, immunomodulation, transplant rejection, immune tolerance

1 Introduction

Islet transplantation has become a promising therapy for certain endocrine disorders, particularly type 1 diabetes mellitus (T1DM), which affects approximately 1.6 million Americans, and the incidence of this disease continues to increase globally (1–3). Despite advances in insulin therapy and continuous glucose monitoring, achieving optimal glycemic control remains a challenge for many patients, leading to long-term complications and increased mortality (4, 5). The limitations of current treatments underscore the urgent need for alternative approaches, such as islet transplantation, to restore endogenous insulin production and achieve tighter glycemic control. However, the success of such transplantations is often limited by immune rejection and the scarcity of donor islets. Recent advancements have explored the feasibility of using personalized endoderm stem cell-derived islets, which may provide a renewable source of islet tissues tailored to individual patient needs, potentially overcoming the limitations of donor availability and improving the compatibility and longevity of grafts (6). Moreover, Encapsulation techniques, which protect transplanted islets from the immune system using biomaterials, offer a potential solution to enhance graft survival and function (7). Targeted local drug delivery systems have also been developed to modulate immune responses directly at the transplantation site, thereby improving transplant outcomes by addressing non-specific, alloantigen-specific, and autoimmune rejection pathways (8).

Allogeneic islet transplantation has demonstrated efficacy in restoring insulin independence in T1DM patients; however, donor scarcity and the necessity for chronic immunosuppression limit its widespread application (8–10). Moreover, allogeneic islet transplantation is also accompanied by significant immunological challenges, primarily due to robust T-cell-mediated rejection (11). The critical role of T-cell dynamics in islet transplantation is underscored by their central involvement in immune tolerance and rejection processes. Understanding the molecular mechanisms governing T-cell responses is crucial for improving graft survival and function. A detailed study of these dynamics can provide insights into more effective immunosuppressive therapies and long-term graft survival, addressing both the immediate and prolonged challenges that impact the success of allogeneic transplants.

The critical role of T-cell dynamics in islet transplantation is underscored by their central involvement in immune tolerance and rejection processes. Detailed study of these dynamics can provide insights into more effective immunosuppressive therapies and long-term graft survival.

The heterogeneity of T-cell subpopulations and their distinct roles in transplantation immunobiology has been studied. For instance, regulatory T cells have been shown to promote graft tolerance (12), while effector T cells contribute to graft rejection (11). The phenotypic characterization of T-cell subpopulations in the context of islet transplantation has revealed potential targets for immunomodulatory therapies, indicating the potential of these cell types for improving transplantation outcomes (13).

In this study, we utilized cutting-edge single-cell RNA sequencing (scRNA-seq) technology (14) to dissect the molecular mechanisms, gene expression profiles, biological pathway alterations, and intercellular communication patterns among T-cell subgroups in both allogeneic and syngeneic islet transplantation models. This approach provided a high-resolution view of the cellular heterogeneity and dynamic changes within the T-cell community, essential for pinpointing the critical factors influencing transplantation outcomes. Our comprehensive analysis using scRNA-seq, along with Gene Set Enrichment Analysis (GSEA) and CellChat, enabled us to uncover significant genetic variations and differences in gene expression between transplantation conditions, revealing the activation or suppression of specific biological processes and signaling pathways within different T-cell subpopulations. These findings offer new insights into the complex communication patterns among T-cell subgroups and with other cell types, highlighting differences in signaling activities between allogeneic and syngeneic transplants that could be pivotal for developing targeted therapeutic strategies.

2 Materials and methods

2.1 Single-cell data analysis of islet grafts

The analysis of single-cell RNA sequencing (scRNA-seq) data from syngeneic and allogeneic islet grafts was meticulously conducted to understand the cellular heterogeneity and

underlying molecular mechanisms that differentiate these two transplant types by Seurat (version 4.1.0) (15). We obtained the scRNA-seq datasets from our previous study (GSE198865) (16). These datasets included samples from both syngeneic (genetically identical donor and recipient) and allogeneic (genetically different donor and recipient) islet transplantation. Cells were filtered using the Seurat package according to the following criteria (1): Cells with fewer than 200 detected genes or more than 4,500 genes were removed to exclude empty droplets or multiplets, respectively. (2) Cells with a mitochondrial gene content exceeding 15% were also excluded to avoid cells undergoing apoptosis or those with damaged membranes. (3) Genes not detected in at least 3 cells were removed to focus the analysis on biologically relevant transcripts. Following rigorous computational quality filtering, we successfully obtained the transcriptomes of 19,640 single cells, comprising 11,870 cells derived from allografts and 7,770 cells originating from syngeneic grafts. PCA was performed to reduce the dimensionality of the dataset and to highlight the genetic variances that differentiate cells. We utilized the "RunHarmony" function (17) within Seurat to correct for potential batch effects across different samples, ensuring that subsequent analyses were not confounded by technical variations.

The t-SNE was used to visualize the data (18). Based on known marker genes, cells were annotated to identify specific cell types, such as lymphocytes, endothelial cells, islet cells, mesenchymal cells, and myeloid cells. Following the initial preprocessing and clustering, further analysis was conducted to delineate the cellular subtypes of T cells (including 6471 cells) and understand their functional roles within the grafts.

The analysis revealed distinct T cell populations in the transcriptome data. Among these, the CD4+ Tconv (Conventional CD4+ T Cells) were characterized by the presence of Cd4 and Tnfsf8 markers. The Activated CD8+ T Cells were identified using Cd8a and Klrc1 markers. Regulatory T Cells, also known as Tregs, were distinguished by Il2ra and Foxp3 markers. The Dividing T Cells were recognized by Stmn1 and Top2a markers, indicating their proliferative state. Memory T Cells were characterized by Sell and Ccr7 markers. Finally, Gamma Delta T Cells were identified based on the presence of Blk, Cd163l1, and Rorc markers.

2.2 Analysis of differentially expressed genes

To explore the molecular differences between T-cell subsets derived from syngeneic and allogeneic islet grafts, we used a rigorous approach to identify differentially expressed genes (DEGs). Initially, DEGs were screened using the Wilcoxon rank-sum test. This nonparametric test was chosen for its efficacy in identifying differences between two independent samples, which is essential for our study comparing two distinct graft conditions. After initial screening, the limma package (version 3.59.1) was used to refine our DEG analysis. Limma provides a robust framework for analyzing gene expression data, particularly through its ability to fit linear models for comparisons of interest and its empirical Bayes smoothing of standard errors, which enhances the reliability of DEG identification. Genes were considered differentially expressed based on two key criteria. We set the adjusted p-value threshold of less than 0.05 to ensure that the findings were statistically significant while controlling for multiple testing errors. A cutoff for a |log2-fold change| of more than 0.25 was applied. This threshold helped in identifying genes with meaningful expression differences, avoiding those with minor fluctuations that are less likely to be biologically significant. This extensive DEG analysis helped reveal the molecular variations within and across major T-cell clusters and subcell types, with detailed expression profiles visualized in various figures. We generated heatmaps to visually represent the DEGs between the syngeneic and allogeneic grafts within each T-cell subset. Heatmaps are particularly effective for this purpose because they provide a clear and intuitive visualization of the expression levels across multiple genes and conditions, facilitating quick identification of patterns and outliers in the data. This methodology not only ensures a robust analysis of gene expression differences but also helps in understanding the functional implications of these differences in the context of T-cell behavior and the immune response in islet transplantation.

2.3 Pathway enrichment analysis in T cell subsets

To elucidate the functional implications of differentially expressed genes (DEGs) identified within T-cell subsets from syngeneic and allogeneic islet grafts, we conducted a comprehensive pathway enrichment analysis using the HALLMARK gene set collection from the Molecular Signatures Database (MSigDB). This analysis aimed to identify key biological processes and signaling pathways that are differentially activated or suppressed across these cell subsets.

For each T-cell subset, we performed gene set enrichment analysis (GSEA) using the preranked list of genes based on their log2-fold changes. Specifically, we used the irGSEA software (version 2.1.5) and MSigDB's mh.all.v2023.2.Mm.symbols.gmt as the gene set database. This analysis helps in identifying whether HALLMARK pathways show differences between syngeneic and allogeneic islet grafts. Pathway enrichment analysis provided detailed insights into the molecular mechanisms underlying T-cell responses in syngeneic versus allogeneic islet grafts. This comprehensive pathway enrichment analysis not only delineated the specific pathways activated or repressed in different T-cell subsets but also provided a molecular framework for understanding the potential impacts of these pathways on the fate of islet grafts.

2.4 Analysis of cell communication patterns in T-cell populations using CellChat

We then applied CellChat (version 2.0.0) to analyze intercellular communication by quantifying and visualizing the contributions of different ligands and receptors expressed by these cells (19). This

allowed us to delineate the cellular hierarchies and communication dynamics within the T-cell population. We utilized CellChat to detect and interpret complex communication patterns among the T-cell subgroups. This included the identification of four incoming signaling patterns. These patterns represent the pathways through which T cells receive signals from other cells, helping us understand how external signals influence cell behavior and function. Three outgoing signaling patterns: These patterns illustrate how T cells send signals to other cells, indicating their role in modulating immune responses and cellular environments. This algorithm enabled the distinction between autocrine (self-signaling within the same cell type) and paracrine (signaling between different cell types) communication modes. The analysis provided a structured understanding of which cell types are the predominant senders and receivers of signals, which is critical for identifying key regulatory nodes within the immune system.

To effectively communicate our findings, we used CellChat's built-in visualization functions. Network plots: Network plots showing the overall signaling network and highlighting the most influential cell types and pathways. Sankey diagrams: These diagrams depict the flow of signals between different cell groups, providing a clear representation of communication from senders to receivers. Heatmaps and chord diagrams: These visualizations quantified and compared the strength and frequency of interactions across different signaling pathways, emphasizing the contributions of each cell type to the overall communication network. We integrated the results from CellChat in our study to compare the signaling activities between syngeneic and allogeneic grafts. This integrative approach helped in pinpointing differential signaling pathways that might be responsible for the distinct immune responses observed between the two graft types. By employing this comprehensive methodology, we were able to uncover nuanced insights into the cellular communication landscape, revealing how specific signaling pathways are orchestrated within T-cell populations and their impact on graft outcomes.

2.5 Statistical analysis

All analyses were performed in R (version 4.2.1). We established statistical significance at $P < 0.05$.

3 Results

3.1 Workflow of this study

The workflow of this study is shown in Figure 1. Step 1: Our study's workflow begins with the analysis of single-cell datasets containing both syngeneic and allogeneic islet grafts. Initial quality checks, data standardization, and preliminary dimensionality reduction set the foundation for deeper analysis using T-SNE to identify distinct cellular clusters within the grafts. Further subdivision revealed five major cell types, which were expanded into ten subtypes, enabling detailed cellular profiling and differential gene expression analysis. In-depth scRNA-seq analysis of T-cell populations revealed six transcriptionally distinct clusters. We further expanded this classification into 20 distinct subcell types.

Step 2: We then conducted a comprehensive analysis of differentially expressed genes (DEGs) across these cell types and subtypes to explore molecular differences.

Step 3: We performed pathway enrichment analyses to identify signaling pathways such as HALLMARK INTERFERON ALPHA, GAMMA RESPONSES, and TNFA SIGNALING VIA NFKB. These pathways were particularly notable for their varied expression across memory T cells, Tregs, and other T-cell types, highlighting both autocrine and paracrine signaling.

Step 4: Cell–cell interaction analyses further revealed significant ligand–receptor interactions between various T-cell subsets, emphasizing the communication dynamics within the T-cell populations. Pattern recognition techniques map these interactions, distinguishing cells as signal senders or receivers through specified signaling patterns, thus providing a comprehensive view of the communication and signaling mechanisms at play within T-cell populations. This multifaceted approach not only clarified the internal communication patterns among T cells but also linked these patterns to broader immune responses in islet transplants. Our study included a detailed analysis of the MIF signaling pathway, focusing on its impact across various T-cell subgroups. We explored autocrine signaling within the Treg and activated CD8+ T-cell groups and compared it to the paracrine signaling observed in the CD4+ Tconv, dividing T, memory T, and γδT cell groups. This process helped us assess the number of cells involved and the likelihood of communication within each subgroup.

3.2 Analysis of single-cell datasets

In the initial phase of our research, we obtained single-cell datasets that included syngeneic and allogeneic islet grafts from our previous study (GSE198865) (16). We conducted thorough quality checks, standardized the data, and performed initial steps to reduce dimensionality. Subsequently, we applied the T-SNE technique to achieve further dimension reduction, which allowed us to clearly distinguish cellular clusters specific to syngeneic versus allogeneic islet grafts.

Our analysis identified five main cell types, depicted in Figure 2A, which included lymphocytes, endothelial cells, islet cells, mesenchymal cells, and myeloid cells. We extended our analysis to categorize these cells into 10 detailed subtypes, as shown in Figure 2B, and their markers are displayed in Figure 2C. These subtypes consist of B cells, endothelial cells, islet cells, mesenchymal cells, CD4+ T cells, macrophages, CD8+ T cells, regulatory T cells (Tregs), natural killer (NK) cells, and dendritic cells (DCs), providing a detailed view of the cell variety within the grafts. The distribution of each cell type across the samples is thoroughly documented in Figure 2D. Additionally, we performed a differential gene expression analysis, the results of which are shown in a volcano plot in Figure 2E.

In-depth scRNA-seq analysis of T-cell populations revealed six transcriptionally distinct clusters: CD4+ Tconv cells, Tregs,

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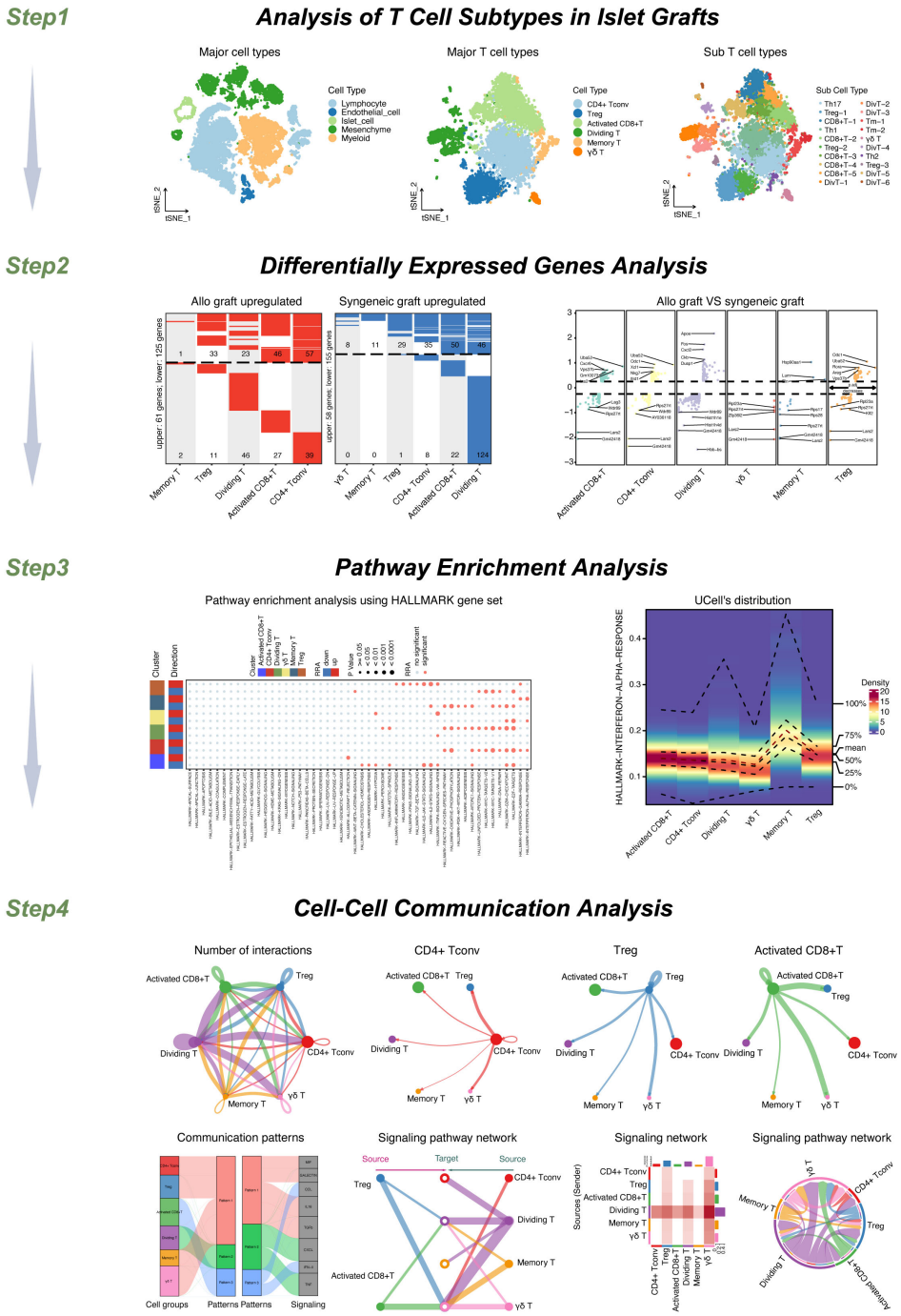

FIGURE 1
Study workflow diagram.

activated CD8+ T cells, dividing T cells, memory T cells, and γδ T cells, as shown in Figure 3A. We expanded this classification into 20 distinct subcell types, as depicted in Figure 3B, with the markers shown in Figures 3C, D. These subtypes, which include various forms of activated CD8+ T cells, dividing T cells, memory T cells, regulatory T cells, and different T helper cell types, provide a detailed perspective on the cellular diversity within grafts. The proportions of these subcell types across the samples are detailed in Figure 3E.

3.3 Comparative analysis of DEGs in T cells between syngeneic and allogeneic islet transplants

To investigate the molecular differences between T cells from syngeneic versus allogeneic islet grafts, we conducted a thorough differential gene expression analysis. This approach enabled us to identify and characterize the DEGs across six major distinct clusters of T cells (Figures 4A–C) and 17 subcell types (Figures 4D–F).

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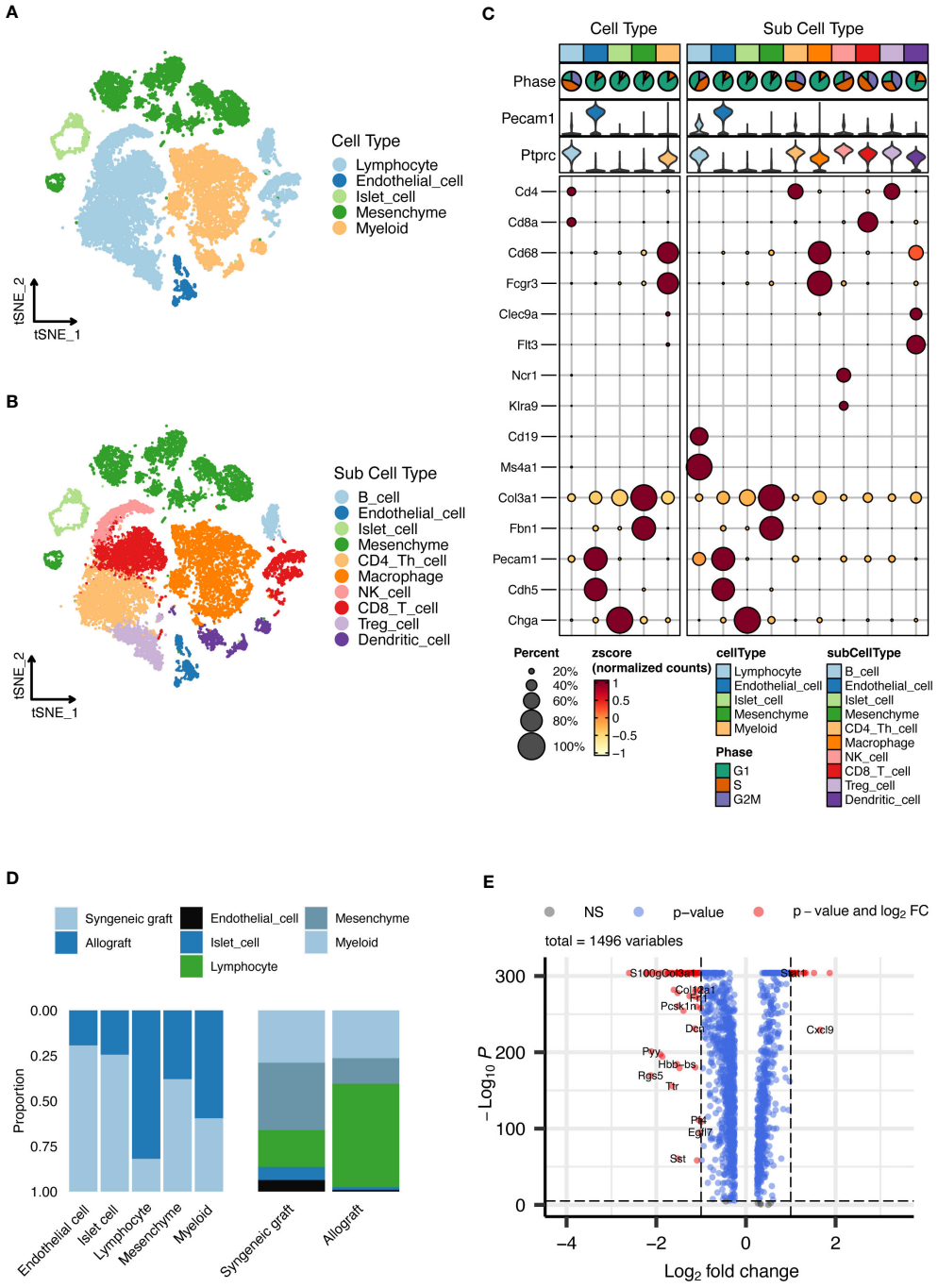

FIGURE 2
Single-cell RNA sequencing analysis of islet grafts. **(A)** Cellular clusters identified in islet grafts display the five main cell types identified within the islet grafts, which are lymphocytes, endothelial cells, islet cells, mesenchymal cells, and myeloid cells. **(B)** Subdivision of cell types showing the ten detailed subtypes of the main cell types for further cellular profiling and analysis. **(C)** Marker expression profiles showing the expression markers for each of the ten cell subtypes, providing insight into the cellular identity and function within the grafts. **(D)** The distribution of cell types documents the distribution of each cell type across the sampled grafts, highlighting variations between syngeneic and allogeneic samples. **(E)** Volcano plot of differential gene expression showing the results of the differential gene expression analysis, identifying significantly upregulated and downregulated genes.

Statistical significance was established using the Wilcoxon rank-sum test and refined through the limma package, with genes considered significantly differentially expressed at an adjusted p-value < 0.05 and a |log2 fold change| > 0.25. By employing bioinformatics tools, we generated heatmaps to visually represent the DEGs between the syngeneic and allogeneic islet grafts within each T-cell subset. The upregulated DEGs within six primary T-cell clusters in allogeneic islet grafts are presented in Figure 4A. The upregulated DEGs within six primary T-cell clusters in syngeneic islet grafts are presented in Figure 4B. The detailed expression of

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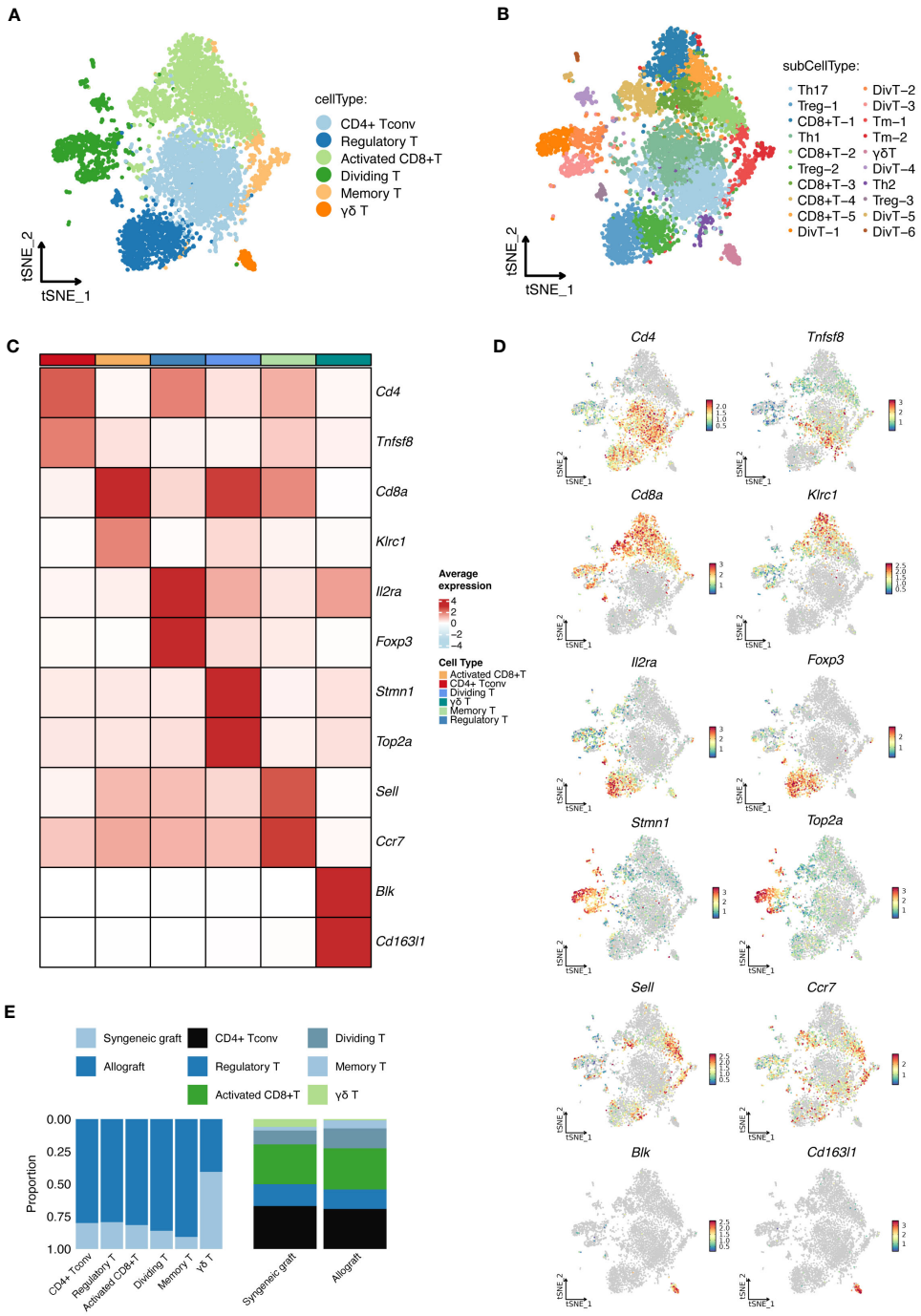

FIGURE 3
Detailed scRNA-seq analysis of T-Cell populations. **(A)** Cellular clusters of T cells identified in islet grafts show the six main cell types identified within the islet grafts. **(B)** Subdivision of T-cell types showing the 20 detailed subtypes of the main T-cell types for further cellular profiling and analysis. **(C)** The heatmap shows the specific marker profiles of different T-cell types. **(D)** The t-SNE analysis shows the specific marker profiles of different T-cell types. **(E)** The proportions of different T-cell types distributed across samples. DivT, Dividing T; Tm, Memory T.

DEGs within six primary T cells from syngeneic versus allogeneic islet grafts is presented in Figure 4C. The upregulated DEGs within 17 subcell types of T cells in allogeneic islet grafts are presented in Figure 4D. The upregulated DEGs within 17 subcell types of T cells in syngeneic islet grafts are presented in Figure 4E. The detailed expression of DEGs within 17 subcell types of T cells from syngeneic versus allogeneic islet grafts is presented in Figure 4F.

3.4 Pathway enrichment in T cell subsets

Furthermore, we conducted pathway enrichment analysis on these cellular subsets (Figure 5A). This analysis highlighted that the HALLMARK INTERFERON ALPHA RESPONSE pathway was predominantly activated in memory T cells, whereas it was suppressed in CD4+ Tconv cells and γδT cells. Similarly, the

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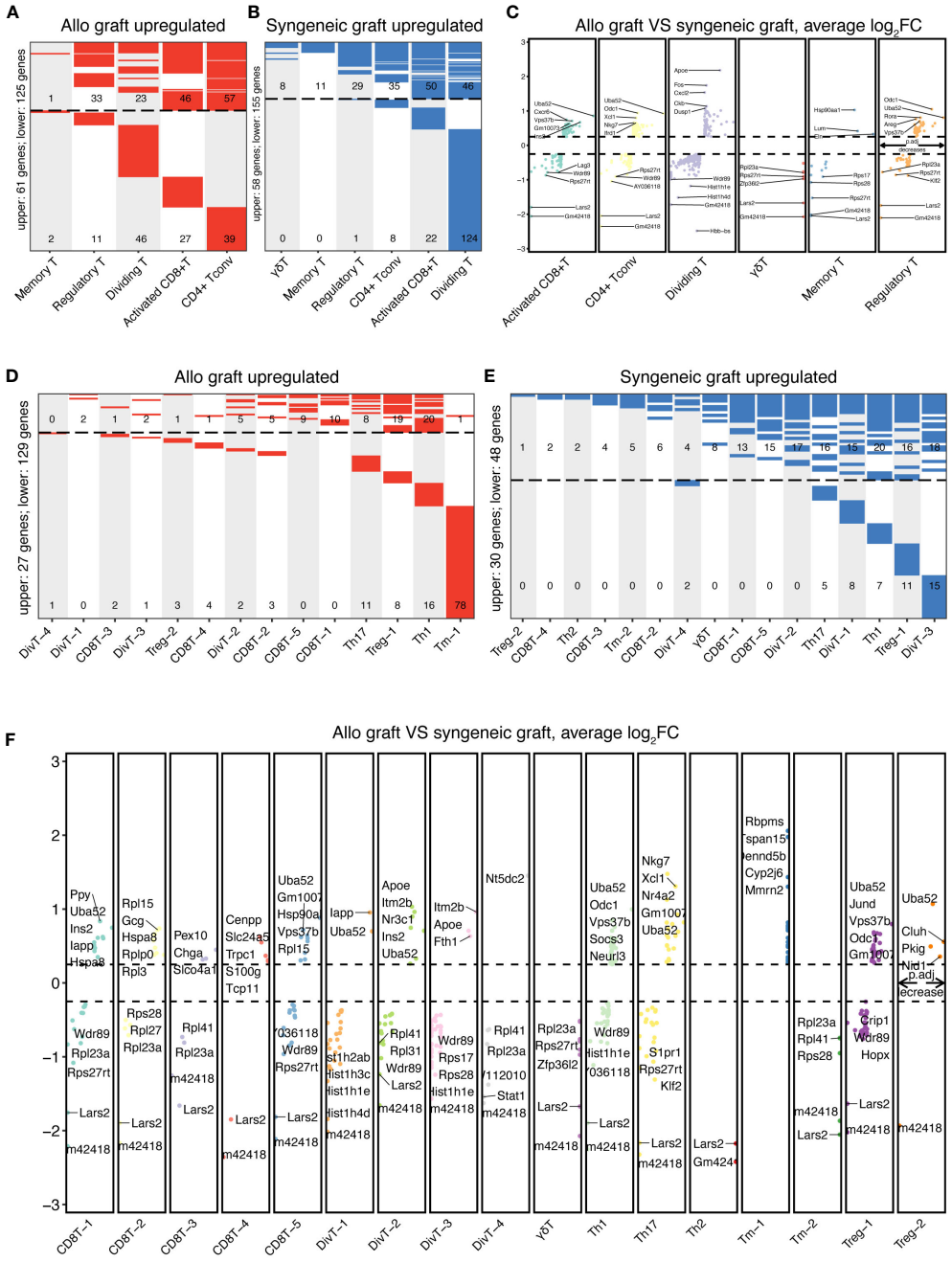

FIGURE 4
Comparative DEG analysis in T cells. The differentially expressed genes in T-cell clusters and subtypes between syngeneic and allogeneic transplants were visualized using heatmaps. **(A)** Upregulated DEGs in allogeneic T-cell clusters are upregulated within five primary T-cell clusters from allogeneic islet grafts, highlighting genes with significant expression changes. No upregulated genes were identified in γδ T cells. **(B)** Upregulated DEGs in syngeneic T-cell clusters illustrate upregulated DEGs within six primary T-cell clusters from syngeneic islet grafts, emphasizing genes with notable increases in expression. **(C)** Comparative DEG expression in T-cell clusters. Detailed comparisons of DEG expression within six primary T-cell clusters from both syngeneic and allogeneic islet grafts are presented, providing a direct visual contrast of molecular differences. **(D)** Upregulated DEGs in allogeneic T-cell subtypes across 17 subcell types of T cells in allogeneic islet grafts, delineating the specific genes that are predominantly expressed. **(E)** Upregulated DEGs in syngeneic T-cell subtypes displayed upregulated DEGs across 17 subcell types of T cells in syngeneic islet grafts, revealing genes with increased expression. **(F)** Comparative DEG expression in T-cell subtypes revealed by a detailed visual comparison of DEG expression across 17 subcell types of T cells from syngeneic versus allogeneic islet grafts, highlighting molecular distinctions.

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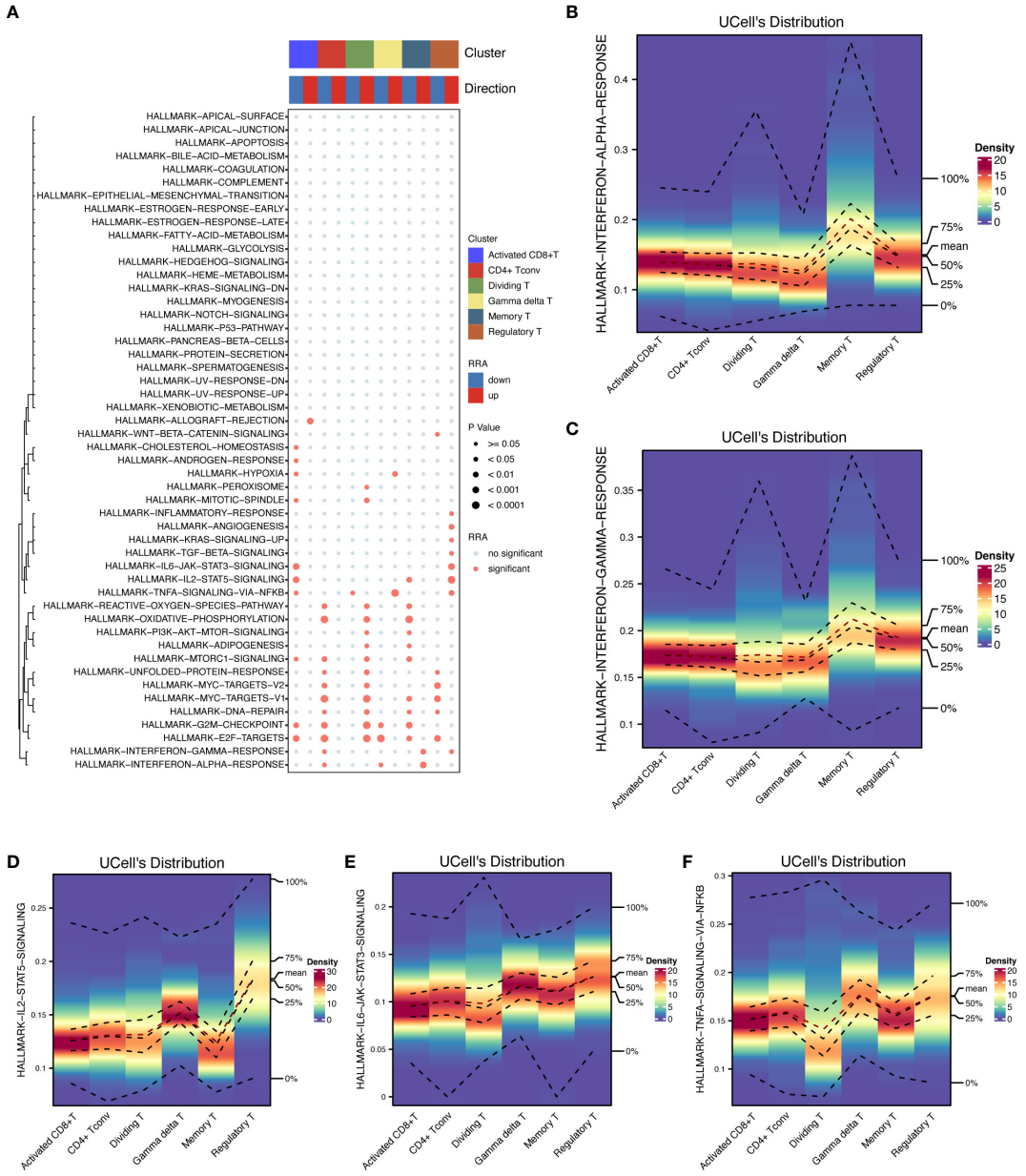

FIGURE 5
Pathway enrichment analysis. The activation of key HALLMARK signaling pathways in T cells was shown to provide insight into their roles in immune responses. **(A)** Pathway enrichment analysis details the results of pathway enrichment analysis across T-cell subsets, highlighting pathways that are differentially activated or suppressed. **(B)** HALLMARK INTERFERON ALPHA response in memory T cells quantifies the INTERFERON ALPHA response activation in memory T cells. **(C)** HALLMARK INTERFERON GAMMA Response in T cells shows an elevated INTERFERON GAMMA response in memory T cells and Tregs and reduced levels in CD4+ Tconv cells. **(D)** HALLMARK IL2-STAT5 signaling in Treg cells emphasizes strong IL2-STAT5 pathway activation in Treg cells. **(E)** HALLMARK IL6-STAT3 signaling in Treg cells is highly active in the IL6-STAT3 signaling pathway in Treg cells. **(F)** HALLMARK TNFA SIGNALING VIA NFKB in Treg cells illustrates the activation of TNFA SIGNALING VIA NFKB in Tregs, γδT cells, dividing T cells, and activated CD8+ T cells.

HALLMARK INTERFERON GAMMA RESPONSE was elevated in memory T cells and Tregs but reduced in CD4+ Tconv cells. Additionally, the HALLMARK TNFA SIGNALING VIA NFKB pathway was prominently active in Tregs, γδT cells, dividing T cells, and activated CD8+ T cells. The HALLMARK IL2 STAT5 SIGNALING pathway exhibited increased activity in Tregs but decreased activity in both memory T cells and activated CD8+ T cells.

In terms of the expression levels and distribution of differentially expressed genes among the cell subsets, the highest intensity of genes in the memory T cells was detected in the HALLMARK INTERFERON ALPHA (Figure 5B) and GAMMA RESPONSES (Figure 5C) subsets, each of which was marked at approximately 0.2 in the respective figures. Treg cells showed the most significant changes in the expression of HALLMARK IL2-STAT5 (Figure 5D), IL6-STAT3 SIGNALING (Figure 5E), and HALLMARK TNFA SIGNALING VIA NFKB (Figure 5F), with intensities of approximately 0.15, 0.1, and 0.15, respectively.

3.5 Analysis of cell–cell interactions in T cells

Pronounced ligand–receptor interactions were observed across various T-cell types, with notable exchanges between dividing T cells and activated CD8+ T cells, Tregs, CD4+ Tconv cells, and γδT cells (Figures 6A, B). The number of interactions among these cells is shown in Figure 6A, and the interaction weights/strengths are shown in Figure 6B. Figures 6C–H shows the details of the ligand–receptor interactions between various T-cell types. Figure 6C shows strong interactions between CD4+ Tconvs and Tregs and between CD4+ Tconvs and γδT cells. Figure 6D shows the strong interactions between Tregs and γδT cells. Figure 6E shows the strong interactions between activated CD8+ T cells and both γδT cells and Tregs. Figure 6F shows strong interactions between dividing T cells and γδT cells, Tregs, CD4+ Tconvs and activated

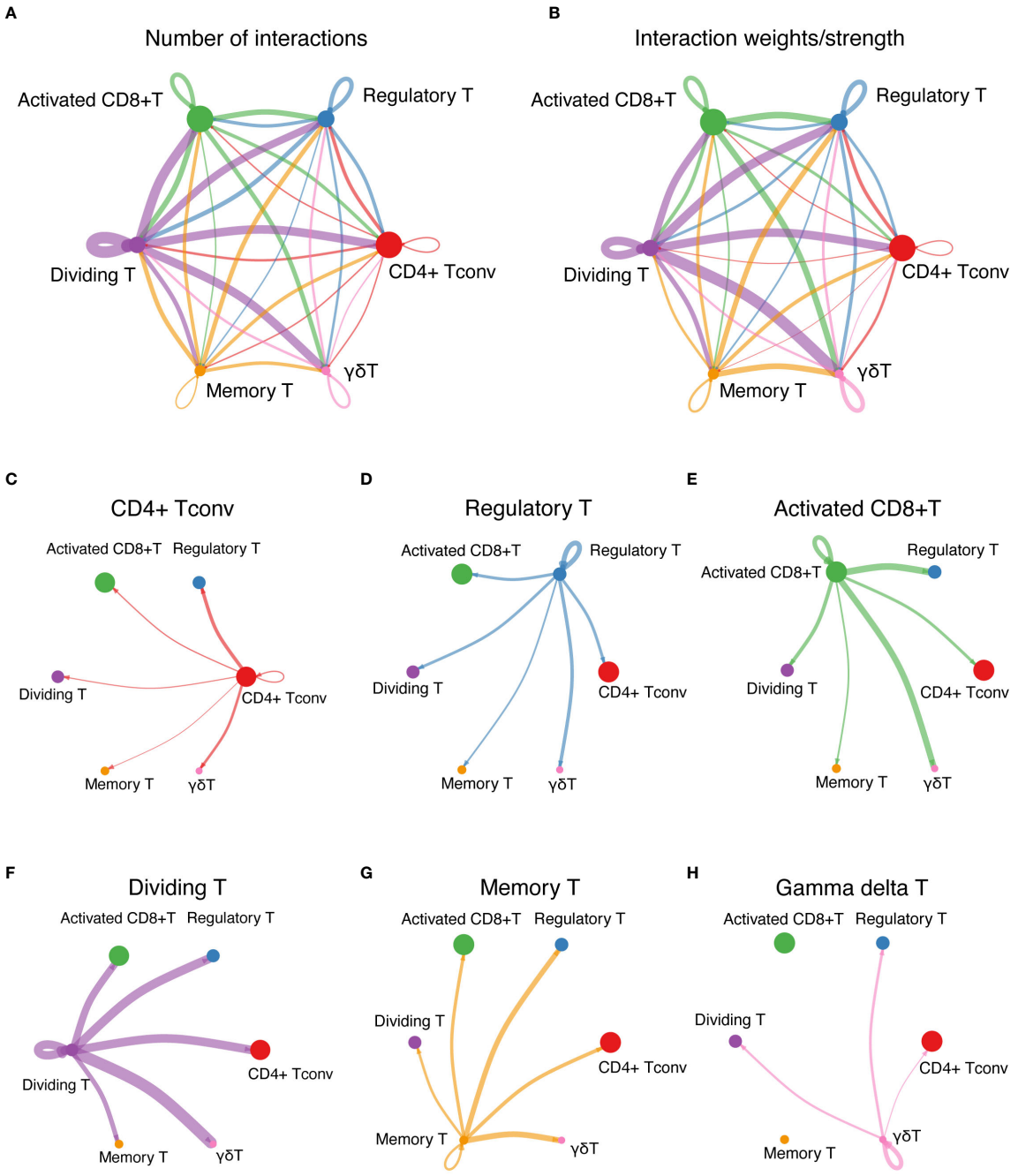

FIGURE 6
Cell-cell interaction network. **(A)** The interactions among T cells represent the number of ligand-receptor interactions among various T-cell types, emphasizing network complexity. **(B)** Interaction strength among T cells shows the interaction weights or strengths among various T-cell types, providing insights into the intensity of cellular communication. **(C-H)** Detailed ligand–receptor interactions. **(C)** through **(H)** show the strong interactions between specific pairs of T-cell types, revealing the key pathways and mediators involved in cellular communication.

CD8+ T cells. Figure 6G shows strong interactions between memory T cells and Tregs and between memory T cells and γδT cells. Figure 6H shows the strong interactions between γδT cells and Tregs and between γδT cells and dividing T cells.

3.6 Analyzing cell communication patterns and signaling pathways in T-cell populations

The patterns of cell communication for groups that primarily acted as signal receivers (cells stimulated by ligands) are shown in Figure 7A. The width of the flow in the diagram indicates the contribution of each element to the pattern. CD4+ Tconv, Treg, memory T, and γδT cells mainly receive stimuli through pattern #1, which includes the MIF, GALECTIN, IL16, and TGFb signaling pathways. Activated CD8+ T cells predominantly receive stimuli through pattern #3, which consists solely of the CCL and IFN-II signaling pathways. Dividing T cells primarily receive stimuli through pattern #2, which includes only the CXCL and TNF signaling pathways.

The pattern of cell communication in groups primarily serving as signal senders (secretion ligands) is shown in Figure 7B. Most of the CD4+ Tconv cells that secrete ligands communicate via pattern #4, which includes the IL16 signaling pathway. All activated CD8+ T cells and memory T cells communicate via pattern #1, which encompasses the GALECTIN and CXCL signaling pathways. All dividing T cells communicate via pattern #3, which includes the CCL, TGFb, and TNF signaling pathways. All γδT cells communicate via pattern #2, which includes the IFN-II signaling pathway.

By comparing Figures 7A, B, we can distinguish between autocrine and paracrine links. Specifically, the MIF and TGFb signaling pathways primarily mediate autocrine signaling, while the CCL and CXCL pathways mainly facilitate paracrine communication between activated CD8+ T cells and dividing T cells.

3.7 Analyzing autocrine and paracrine signaling in T cells via the MIF signaling pathway

The MIF signaling pathway was further analyzed, revealing its impact on various T-cell subgroups (Figure 7C). On the left, the diagram illustrates autocrine signaling within the Treg and activated CD8+ T-cell groups, while the right side shows their paracrine signaling. Figure 7D divides the focus into two types of signaling affecting CD4+ Tconv cells: dividing T cells, memory T cells, and γδT cells. The left side of the diagram represents paracrine signaling in these groups, and the right side illustrates autocrine signaling in the same groups. The size of the circles in the diagram indicates the number of cells in each group, and the width of the lines suggests the likelihood of cell communication occurring.

Detailed visualizations that complement the insights into the MIF signaling pathway are provided in Figures 7E–G. A ring chart illustrates the distribution and prominence of various T-cell groups within the MIF signaling pathway, highlighting their respective contributions and interactions (Figure 7E). The positioning and size of each segment within the ring chart correlate with the role prominence of each cell group, as derived from the heatmap (Figure 7F). A chord diagram was further used to map the intricate network of cell communication within the MIF signaling pathway (Figure 7G). This diagram shows the connections between different T-cell groups, with the thickness of each chord representing the strength and frequency of interactions between the groups. As in Figure 7F, where the heatmap depicts the likelihood of role assumption, the chord diagram in Figure 7G visually emphasizes the central role of γδT cells, as they act as the main mediators managing signal flow during cell communication. Together, these results underscore the dynamics of cellular interactions, and the crucial roles certain T-cell groups play within the signaling pathway.

4 Discussion

Islet transplantation has emerged as a promising therapeutic strategy for patients suffering from type 1 diabetes (20, 21). Allogeneic islet transplantation offers potential cures, yet they are hindered by immune rejection and the scarcity of compatible donors (8). This study aimed to address these challenges by dissecting the molecular mechanisms underlying T-cell responses in both transplantation scenarios, highlighting the necessity for a deeper understanding of immune dynamics to improve transplant outcomes.

Immune rejection in allogeneic islet transplantation is directly linked to the loss of functional pancreatic islets (22, 23). By elucidating the T-cell-mediated immune responses that contribute to islet graft rejection, this research endeavors to unlock new avenues for enhancing graft survival and function (24). The insights gained from our investigation into the differential gene expression and signaling pathways of T-cell subsets in allogeneic and syngeneic transplants could shed light on novel immunomodulatory therapies, potentially revolutionizing the management of type 1 diabetes and improving patient prognoses.

Given the complexity of immune responses in islet transplantation, identifying differentially expressed genes (DEGs) in T-cell clusters is crucial. The upregulation of specific genes within the major T-cell clusters and subtypes in syngeneic transplants suggests a unique molecular signature potentially linked to graft rejection or other immune responses. These DEGs could serve as biomarkers for transplant outcomes or therapeutic targets to enhance graft survival.

Our single-cell transcriptomic analysis has provided significant insights into T-cell heterogeneity and its molecular mechanisms in islet transplantation. Notably, the enrichment of the HALLMARK INTERFERON ALPHA RESPONSE pathway in memory T cells indicates a heightened antiviral defense, which is crucial for graft survival. Conversely, the suppression of this pathway in CD4+ helper and γδ T cells likely represents a regulatory mechanism to prevent tissue damage. Additionally, the widespread activation of

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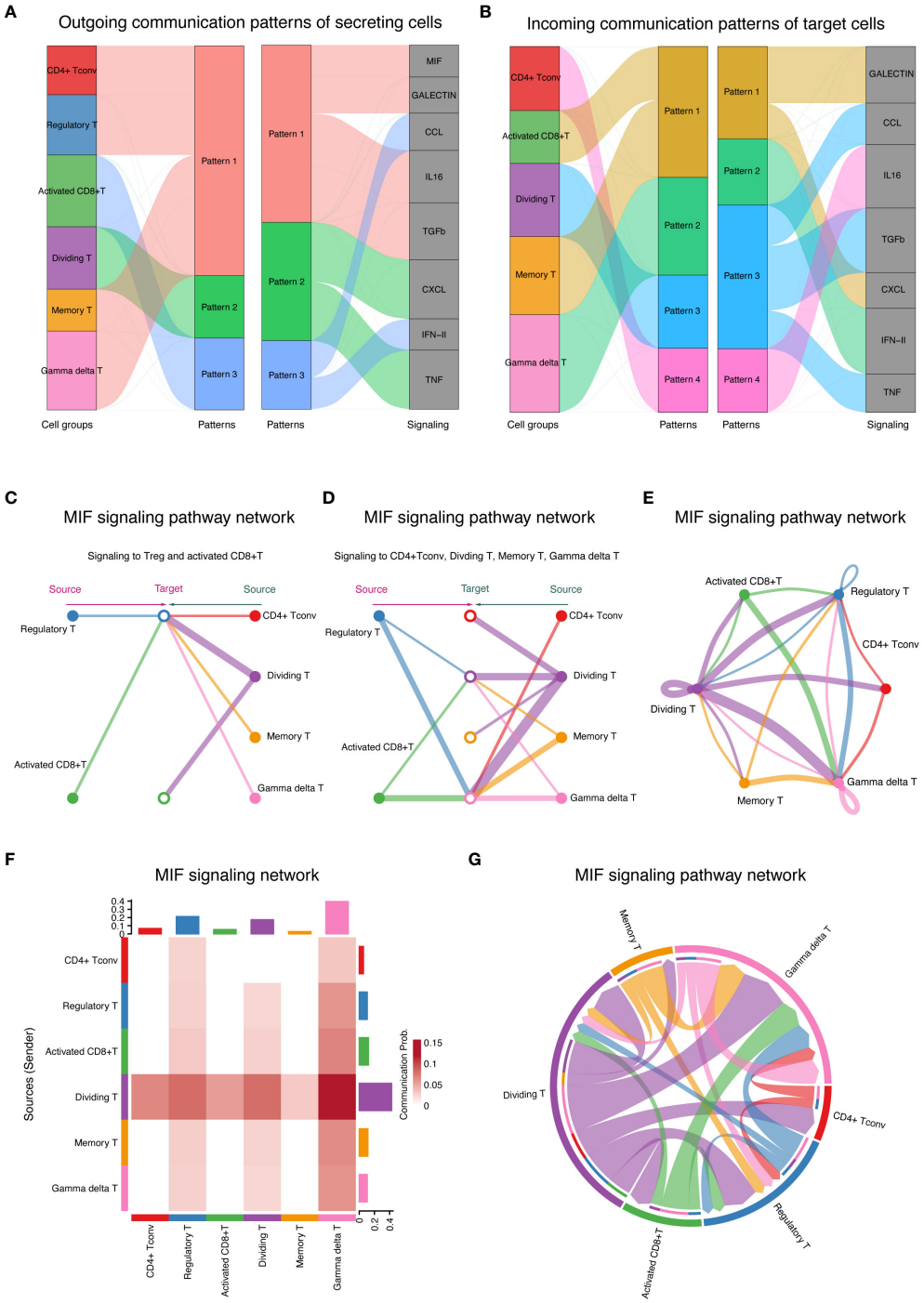

FIGURE 7
Cell communication patterns and signaling pathways **(A)** Signaling patterns for signal receivers showing signaling patterns for T-cell groups acting as signal receivers, detailing the pathways stimulated by ligands. **(B)** Signaling Patterns for Signal Senders Illustrate signaling patterns for T-cell groups acting as signal senders, highlighting the pathways through which these cells secrete ligands. **(C)** MIF signaling pathway autocrine and paracrine signaling in T cells demonstrates the impact of the MIF signaling pathway on Tregs and activated CD8+ T cells, showing autocrine (left) and paracrine (right) signaling. **(D)** Signaling modulation among T-cell subtypes depicts autocrine and paracrine signaling within CD4+ Tconv cells, dividing T cells, memory T cells, and γδT cells. The circle sizes and line widths represent the number of cells and the potential strength of communication, respectively. **(E)** Ring chart visualization of the distribution of the MIF signaling pathway in the T-cell group. A ring chart showing the distribution and role prominence of various T-cell groups within the MIF signaling pathway. **(F)** The heatmap of role prominence in MIF signaling displays a heatmap showing the likelihood of role assumption by different T-cell groups, with color intensity indicating each group's role prominence. **(G)** Chord diagram of T-cell communication in MIF signaling. A chord diagram maps the network of T-cell communications within the MIF signaling pathway, with chord thickness reflecting the strength and frequency of interactions, particularly highlighting the role of γδT cells as central mediators.

the HALLMARK TNFA SIGNALING VIA NFKB pathway across various T-cell subsets, including regulatory T (Treg) cells, γδ T cells, proliferating T cells, and activated CD8+ T cells, highlights the critical role of TNFα in mediating inflammatory responses, which may be targeted to modulate graft rejection and enhance tolerance. The activation of the HALLMARK TNFA SIGNALING VIA NFKB across multiple T-cell subsets suggests a heightened inflammatory state which could predispose to graft rejection or dysfunction. Conversely, the suppression of the INTERFERON ALPHA RESPONSE in CD4+ Tconv and γδT cells may represent a compensatory, regulatory mechanism aimed at tempering the immune response to avoid overactivation and potential graft damage.

The interaction between proliferating T cells and activated CD8 + T cells, as revealed through ligand–receptor analysis using CellChat, underscores the complexity of communication within the T-cell community. These interactions are pivotal for orchestrating the immune response to transplanted tissues, highlighting their potential as targets for enhancing graft acceptance and preventing rejection. Similar challenges are observed in other types of organ transplantation, where post-transplantation diabetes mellitus (PTDM) emerges as a serious complication affecting graft and patient survival (25).

Furthermore, the analysis revealed the critical role of the MIF signaling pathway in modulating interactions among various T-cell subtypes. This pathway is influenced by autocrine signaling in Tregs and activated CD8+ T cells, as well as paracrine signaling in CD4+ conventional T (CD4+ Tconv), proliferating T, memory T, and γδ T cells. This complex signaling pathway highlights the integral role of MIF in immune regulation and suggests that detailed insights into this pathway could inform strategies to modulate immune responses in transplant settings. Moreover, the significant role of MIF in various biological processes and immune responses, particularly its impact on different T-cell types, underscores its potential as a biomarker or therapeutic target in islet transplantation. Compared to findings from the previous study (11), our study further elaborates on the role of γδ T cells in graft environments, providing a deeper understanding of their dual role in immunoregulation and inflammation.

Our findings highlight the potential of targeting specific T-cell signaling pathways, such as TNFA via NFKB, to modulate the immune response in islet transplantation. These pathways hold promise as therapeutic targets to enhance graft tolerance. However, the limited sample size in our study may affect the generalizability of these results. To address this, further studies should investigate the role of these pathways in larger cohorts to explore the mechanistic basis of their modulation using targeted therapies or genetic techniques. Additionally, we recommend conducting future clinical trials designed to assess interventions aimed at the TNFA and interferon pathways, which have been identified as critical in our study. Such trials could provide deeper insights into their potential to improve transplantation outcomes and validate our findings across a broader population, thereby enhancing their applicability and impact in clinical settings.

In summary, our study highlights the intricate interplay of T-cell subsets and their communication networks, which are crucial for understanding immune responses in pancreatic islet transplants. Through detailed analyses using GSEA and CellChat, we identified specific biological processes and signaling pathways that are differentially regulated across T-cell subpopulations. These insights not only deepen our understanding of T-cell behavior in the context of transplantation but also offer potential avenues for developing targeted immunomodulatory therapies aimed at improving transplant tolerance and longevity.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Author contributions

HZ: Writing – original draft, Writing – review & editing. ZP: Conceptualization, Writing – review & editing, Funding acquisition. YL: Formal analysis, Writing – review & editing. PZ: Funding acquisition, Writing – review & editing. HY: Project administration, Writing – review & editing, Funding acquisition. LM: Conceptualization, Funding acquisition, Project administration, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Subramanian S, Khan F, Hirsch IB. New advances in type 1 diabetes. *BMJ*. (2024) 384:e075681. doi: 10.1136/bmj-2023-075681

2. Quattrin T, Mastrandrea LD, Walker LSK. Type 1 diabetes. *Lancet*. (2023) 401:2149–62. doi: 10.1016/S0140-6736(23)00223-4

3. Martens P-J, Mathieu C. Type 1 diabetes mellitus: A brave new world. *Nat Rev Endocrinol*. (2024) 20:71–2. doi: 10.1038/s41574-023-00936-y

4. Kaur J, Seaquist ER. Hypoglycaemia in type 1 diabetes mellitus: Risks and practical prevention strategies. *Nat Rev Endocrinol*. (2023) 19:177–86. doi: 10.1038/s41574-022-00762-8

5. Herold KC, Delong T, Perdigoto AL, Biru N, Brusko TM, Walker LSK. The immunology of type 1 diabetes. *Nat Rev Immunol*. (2024) 24:435–51. doi: 10.1038/s41577-023-00985-4

6. Wu J, Li T, Guo M, Ji J, Meng X, Fu T, et al. Treating a type 2 diabetic patient with impaired pancreatic islet function by personalized endoderm stem cell-derived islet tissue. *Cell Discovery*. (2024) 10:45. doi: 10.1038/s41421-024-00662-3

7. Kioulaphides S, García AJ. Encapsulation and immune protection for type 1 diabetes cell therapy. *Adv Drug Delivery Rev*. (2024) 207:115205. doi: 10.1016/j.addr.2024.115205

8. Lansberry TR, Stabler CL. Immunoprotection of cellular transplants for autoimmune type 1 diabetes through local drug delivery. *Adv Drug Delivery Rev*. (2024) 206:115179. doi: 10.1016/j.addr.2024.115179

9. Mullard A. FDA approves first cell therapy for type 1 diabetes. *Nat Rev Drug Discovery*. (2023) 22:611. doi: 10.1038/d41573-023-00113-w

10. Cayabyab F, Nih LR, Yoshihara E. Advances in pancreatic islet transplantation sites for the treatment of diabetes. *Front Endocrinol (Lausanne)*. (2021) 12:732431. doi: 10.3389/fendo.2021.732431

11. Short S, Lewik G, Issa F. An immune atlas of T cells in transplant rejection: pathways and therapeutic opportunities. *Transplantation*. (2023) 107:2341–52. doi: 10.1097/TP.0000000000004572

12. Wang J, Zhao X, Wan YY. Intricacies of TGF-β signaling in Treg and Th17 cell biology. *Cell Mol Immunol*. (2023) 20:1002–22. doi: 10.1038/s41423-023-01036-7

13. Bernaldo-de-Quirós E, López-Abente J, Camino M, Gil N, Panadero E, López-Esteban R, et al. The presence of a marked imbalance between regulatory T cells and effector T cells reveals that tolerance mechanisms could be compromised in heart transplant children. *Transplant Direct*. (2021) 7:e693. doi: 10.1097/TXD.0000000000001152

14. Wen L, Li G, Huang T, Geng W, Pei H, Yang J, et al. Single-cell technologies: From research to application. *Innovation (Camb)*. (2022) 3:100342. doi: 10.1016/j.xinn.2022.100342

15. Hao Y, Hao S, Andersen-Nissen E, Mauck WM, Zheng S, Butler A, et al. Integrated analysis of multimodal single-cell data. *Cell*. (2021) 184:3573–3587.e29. doi: 10.1016/j.cell.2021.04.048

16. Chen P, Yao F, Lu Y, Peng Y, Zhu S, Deng J, et al. Single-cell landscape of mouse islet allograft and syngeneic graft. *Front Immunol*. (2022) 13:853349. doi: 10.3389/fimmu.2022.853349

17. Korsunsky I, Millard N, Fan J, Slowikowski K, Zhang F, Wei K, et al. Fast, sensitive and accurate integration of single-cell data with Harmony. *Nat Methods*. (2019) 16:1289–96. doi: 10.1038/s41592-019-0619-0

18. Laurens van der M, Hinton G. Visualizing Data using t-SNE. *Mach Learn Res*. (2008) 9:2579–605.

19. Jin S, Guerrero-Juarez CF, Zhang L, Chang I, Ramos R, Kuan C-H, et al. Inference and analysis of cell-cell communication using CellChat. *Nat Commun*. (2021) 12:1088. doi: 10.1038/s41467-021-21246-9

20. Chetboun M, Drumez E, Ballou C, Maanaoui M, Payne E, Barton F, et al. Association between primary graft function and 5-year outcomes of islet allogeneic transplantation in type 1 diabetes: A retrospective, multicentre, observational cohort study in 1210 patients from the Collaborative Islet Transplant Registry. *Lancet Diabetes Endocrinol*. (2023) 11:391–401. doi: 10.1016/S2213-8587(23)00082-7

21. Bornstein SR, Ludwig B, Steenblock C. Progress in islet transplantation is more important than ever. *Nat Rev Endocrinol*. (2022) 18:389–90. doi: 10.1038/s41574-022-00689-0

22. Chen Q-D, Liu L, Zhao X-H, Liang J-B, Li S-W. Challenges and opportunities in the islet transplantation microenvironment: a comprehensive summary of inflammatory cytokine, immune cells, and vascular endothelial cells. *Front Immunol*. (2023) 14:1293762. doi: 10.3389/fimmu.2023.1293762

23. Wang Q, Huang Y-X, Liu L, Zhao X-H, Sun Y, Mao X, et al. Pancreatic islet transplantation: current advances and challenges. *Front Immunol*. (2024) 15:1391504. doi: 10.3389/fimmu.2024.1391504

24. Kaufman DB, Platt JL, Rabe FL, Dunn DL, Bach FH, Sutherland DE. Differential roles of Mac-1+ cells, and CD4+ and CD8+ T lymphocytes in primary nonfunction and classic rejection of islet allografts. *J Exp Med*. (1990) 172:291–302. doi: 10.1084/jem.172.1.291

25. Wu J, Li T, Guo M, Ji J, Meng X, Fu T, et al. Progress of new-onset diabetes after liver and kidney transplantation. *Cell Discovery*. (2023) 10:1091843. doi: 10.3389/fendo.2023.1091843

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Exploring the molecular mechanisms of macrophages in islet transplantation using single-cell analysis

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Background: Islet transplantation is a promising treatment for type 1 diabetes that aims to restore insulin production and improve glucose control, but long-term graft survival remains a challenge due to immune rejection.

Methods: ScRNA-seq data from syngeneic and allogeneic islet transplantation grafts were obtained from GSE198865. Seurat was used for filtering and clustering, and UMAP was used for dimension reduction. Differentially expressed genes were analyzed between syngeneic and allogeneic islet transplantation grafts. Gene set variation analysis (GSVA) was performed on the HALLMARK gene sets from MSigDB. Monocle 2 was used to reconstruct differentiation trajectories, and cytokine signature enrichment analysis was used to compare cytokine responses between syngeneic and allogeneic grafts.

Results: Three distinct macrophage clusters (Mø-C1, Mø-C2, and Mø-C3) were identified, revealing complex interactions and regulatory mechanisms within macrophage populations. The significant activation of macrophages in allogeneic transplants was marked by the upregulation of allograft rejection-related genes and pathways involved in inflammatory and interferon responses. GSVA revealed eight pathways significantly upregulated in the Mø-C2 cluster. Trajectory analysis revealed that Mø-C3 serves as a common progenitor, branching into Mø-C1 and Mø-C2. Cytokine signature enrichment analysis revealed significant differences in cytokine responses, highlighting the distinct immunological environments created by syngeneic and allogeneic grafts.

Conclusion: This study significantly advances the understanding of macrophage roles within the context of islet transplantation by revealing the interactions

between immune pathways and cellular fate processes. The findings highlight potential therapeutic targets for enhancing graft survival and function, emphasizing the importance of understanding the immunological aspects of transplant acceptance and longevity.

KEYWORDS

type 1 diabetes (T1D), islet transplantation, macrophages, immune rejection, single-cell RNA sequencing (scRNA-seq), syngeneic transplantation, allogeneic transplantation, cytokine

1 Introduction

Islet transplantation, a promising treatment for type 1 diabetes (T1D), aims to restore insulin production and achieve better glucose control (1). Although allogeneic islet transplantation has been approved and utilized in several countries for many years, the recent FDA approval of Lantidra in the United States marked a significant milestone in T1D treatment (2–4). According to a 20-year report of islet transplantation, significant progress has been made in improving graft survival and function, with advancements in immunosuppressive protocols and transplantation techniques contributing to better outcomes (5). Despite this progress, long-term graft survival and functionality remain challenging, primarily due to immune rejection (6, 7). Traditional approaches involve immunosuppressants, which have significant side effects, including increased infection and tumor risk (8–10).

The application of single-cell RNA sequencing (scRNA-seq) technology in islet transplantation is particularly novel and urgent due to its unparalleled ability to provide detailed insights into cellular heterogeneity and dynamic gene expression profiles at single-cell resolution. This technology allows us to dissect the complex immune microenvironment within transplanted islets, specifically focusing on macrophages, which play a pivotal role in graft acceptance and rejection. Previous methodologies, such as bulk RNA sequencing, lack the ability to identify distinct cellular subtypes and their specific functions within the graft microenvironment. In contrast, scRNA-seq enables the identification and characterization of diverse macrophage subsets and their roles in modulating immune responses.

The complexity of the immune microenvironment extends beyond the immediate challenges of immune rejection and immunosuppressant usage to include the elaborate interplay between transplanted islet cells and host immune cells, such as T cells (46), B cells, macrophages, dendritic cells, NK cells and neutrophils (11–13). Macrophages are critical in islet transplantation due to their dual role in promoting tissue repair and mediating immune responses (14). These versatile cells are involved in various processes, including phagocytosis, antigen presentation, and cytokine production, which influence graft survival and function. The ability of these cells to polarize into either proinflammatory (M1) or anti-inflammatory (M2) phenotypes significantly influences graft outcomes (15). Previous studies have highlighted the conflicting roles of macrophages in islet transplantation, with some reports indicating their contribution to graft rejection and others suggesting their involvement in immune tolerance. However, these studies were limited by their inability to precisely characterize macrophage subsets and their functional states within the transplant microenvironment.

Recent research underscores the therapeutic potential of macrophages due to their plasticity and diverse functions. Macrophage-based cell therapy can be engineered for tissue repair, immune modulation, and targeting specific diseases (14). Alpha-1 antitrypsin has been shown to suppress proinflammatory macrophage activity, improving islet graft survival (15). Polylysine-bilirubin conjugates support islet viability and promote M2 macrophage polarization, aiding transplant acceptance (16). Islet transplantation can modulate macrophage activity to induce immune tolerance and promote angiogenesis, enhancing transplant success (17). Additionally, immunomodulatory injectable silk hydrogels maintain functional islets and promote M2 macrophage polarization, facilitating graft acceptance (18).

Our previous research identified three distinct macrophage subsets (Mø-C1, Mø-C2, and Mø-C3) through scRNA-seq, revealing their complex involvement in immune rejection and tolerance processes (19). Building on this foundational work, the current study presents a reanalysis of an existing scRNA-seq dataset from mouse transplantation models to characterize macrophage phenotypes associated with syngeneic and allogeneic islet grafts. The aim of this study was to further elucidate the mechanisms by which these macrophages contribute to transplantation outcomes. By comparing key pathways, we sought to uncover the specific roles of macrophage subsets in graft outcomes. This detailed profiling not only enhances our understanding of macrophage biology in islet transplantation but also identifies potential therapeutic targets to improve transplant success and longevity.

2 Materials and methods

2.1 Single-cell data analysis of islet grafts

ScRNA-seq data of syngeneic islet transplantation and allogeneic islet transplantation grafts were obtained from GSE198865 (19). Seurat (version 4.4.0) was used for filtering and subsequent clustering (20). Cells with RNA feature counts less than 200 or greater than 4500 and a mitochondrial content exceeding 15% were excluded as poor-quality cells. Genes not detected in at least 3 cells were removed from subsequent analysis. These thresholds were set to eliminate low-quality cells and potential doublets, ensuring the reliability of downstream analyses. The mitochondrial content threshold is based on the principle that high mitochondrial gene expression may indicate stressed or dying cells, which could bias the results.

Uniform manifold approximation and projection for dimension reduction (UMAP) (21) was performed using the Seurat R package with the first 75 principal components after performing principal component analysis (PCA) on the 2000 most highly expressed genes. Identification of significant clusters was performed using the FindClusters algorithm in the Seurat package with the resolution set to 0.6. Batch effect correction was performed using the "RunHarmony" function (22). Cell subtypes were annotated according to cell markers from the original study (19).

2.2 Differentially expressed genes (DEG) analyzed

For the analysis of DEGs, we used the Wilcoxon rank-sum test for comparisons between two groups. This nonparametric test is suitable for comparing two independent groups and is robust for single-cell RNA sequencing data. The analysis was further refined using the limma package in R (version 4.2.2), where genes were identified as differentially expressed based on two criteria: fold change > 0.25 and an adjusted P value < 0.05. Venn diagrams and heatmaps were generated to visualize the interactions between the DEGs and key pathway gene sets. Heatmaps were generated to visualize the results.

2.3 Gene set variation analysis (GSVA)

Pathway analyses were predominantly performed on the HALLMARK gene sets described in the Molecular Signatures Database (MSigDB) and exported using the MSigDB package (version 7.5.1). We applied GSVA using standard settings, as implemented in the GSVA package (version 1.46.0) (23). Differences in pathway activity per cell according to GSVA among the different macrophage clusters. To correct for multiple comparisons, we employed the Benjamini-Hochberg method to control the false discovery rate (FDR). This correction is crucial for minimizing type I errors when conducting multiple statistical tests simultaneously.

2.4 Analyzing the role of key gene sets in macrophages

To explore the roles of related gene sets in macrophages identified in GSVA, we conduct a specialized analysis of the expression patterns of these gene sets to uncover their potential role in transplant immune responses. Venn diagrams are used to display the intersection genes between each gene set and the DEGs in various macrophage subgroups from both syngeneic and allogeneic transplants.

2.5 Reconstruction of differentiation trajectories using Monocle 2

Using the R package Monocle 2 (version 2.8.0) (24), differentiation hierarchies within different clusters were reconstructed. Cell fate decisions and differentiation trajectories were reconstructed with the Monocle 2 package, which utilized reverse graph embedding based on a user-defined gene list to generate a pseudotime plot that could account for both branched and linear differentiation processes.

2.6 Cytokine signature enrichment analysis

To assess the cytokine signatures of macrophage subsets (Mø-C1, Mø-C2, and Mø-C3) in syngeneic and allogeneic islet transplantation grafts, we utilized the Dictionary of Immune Responses to Cytokines at single-cell resolution. This approach was based on the transcriptional response data to individual cytokine stimulation collected by Cui et al. (25). We compared the cytokine signatures of macrophage subsets after allogeneic islet transplantation to those after syngeneic islet transplantation. Immune response enrichment analysis (IREA) (25) was subsequently conducted to calculate enrichment scores for each cytokine. This analysis identified the 86 cytokines with the enrichment for each macrophage subset.

2.7 Statistical analysis

For the analysis of gene expression in the scRNA-seq data, all single-cell sequencing data statistical analyses were performed in the R Seurat package (version 4.4.0). Heatmaps were generated from the row-scaled expression values using the heatmap package in R (version 4.2.1). We established statistical significance at $P < 0.05$.

3 Results

3.1 The workflow of this study

The workflow of this study is illustrated in Figure 1. We began by acquiring single-cell datasets from GSE198865 covering both syngeneic and allogeneic islet grafts. Following stringent quality control, normalization, and initial dimensionality reduction, we used uniform manifold approximation and projection (UMAP) to

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distinguish cellular clusters from syngeneic and allogeneic islet grafts. ScRNA-seq analysis of macrophages revealed three distinct clusters (Mø-C1, Mø-C2, and Mø-C3) with their marker genes. We then conducted differential gene expression analysis across macrophage clusters (Mø-C1, Mø-C2, and Mø-C3) to identify differentially expressed genes (DEGs) between syngeneic and allogeneic grafts. Gene set variation analysis (GSVA) revealed pathway activity differences, with eight pathways upregulated in Mø-C2 macrophages. Intersection analysis identified key genes involved in pathways across Mø-C1, Mø-C2, and Mø-C3, as visualized through Venn diagrams and heatmaps. Trajectory analysis using Monocle 2 and cytokine signature enrichment analysis further elucidated macrophage dynamics and immune responses in islet transplantation.

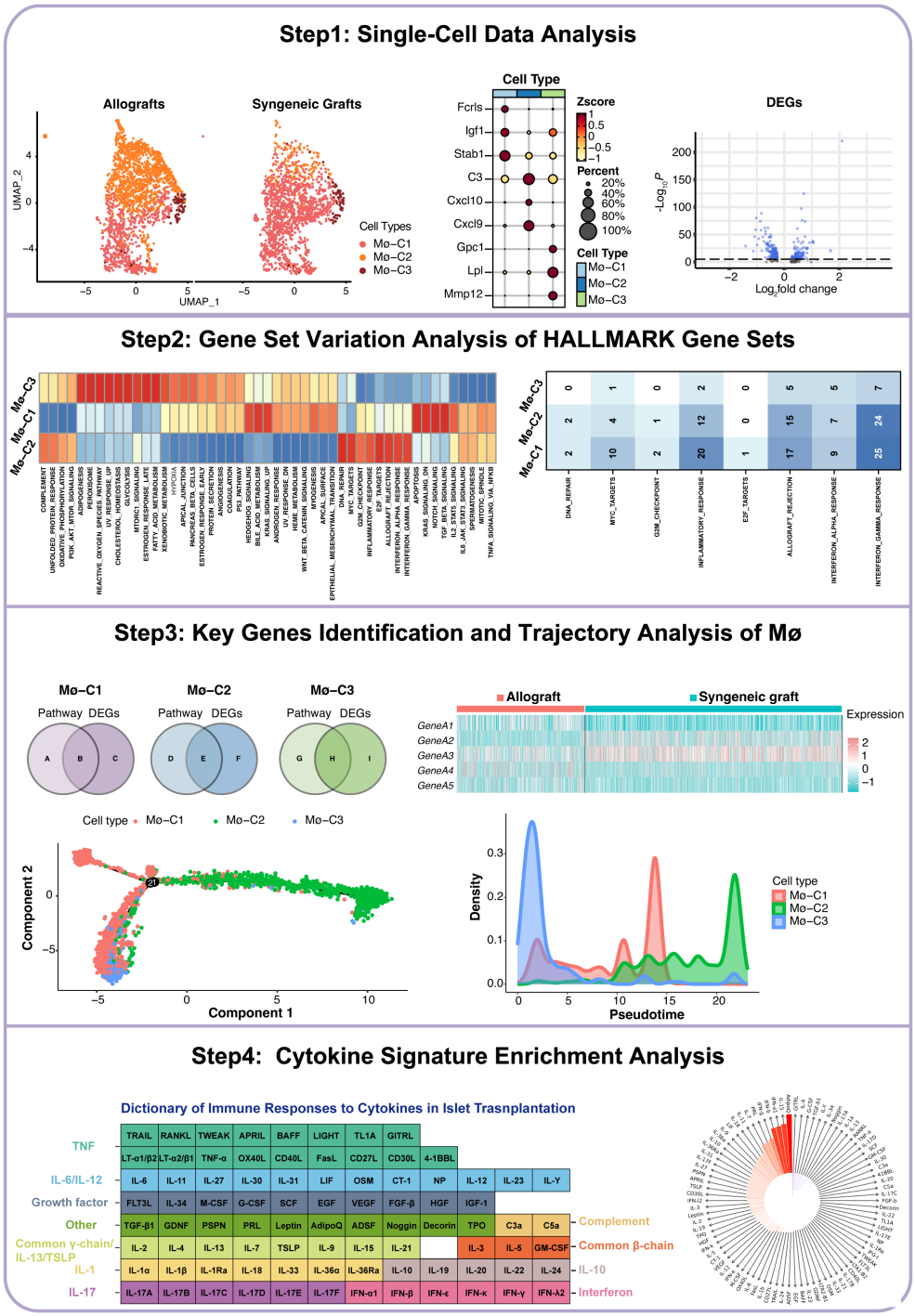

FIGURE 1
Workflow of this study. Mø, macrophage.

3.2 Analysis of single-cell datasets

In the foundational stage of our study, we accessed single-cell datasets encompassing both syngeneic and allogeneic islet grafts sourced from GSE198865 (19). After implementing rigorous quality control measures (Figures 2A, B), normalization processes, and initial dimensionality reduction steps, we utilized UMAP for dimension reduction. Cellular gene markers from the original dataset were used to categorize six primary cell types (Figure 2C): :lymphocytes(markers: *Cd3e, Cd4* and *Cd8*),endothelial cells (markers: *Pecam1, Egfl7* and *Plvap*), islet cells (markers: *Ins1, Chga* and *Scg2*), mesenchymal cells (markers: *Col3a1, Col1a1* and *Col1a2*), myeloid cells (markers: *Cd68, Gzma* and *Cd7*), and acinar cells (markers: *Amy2a, Ptf1a* and *Mist1*). These major cell types were further partitioned into 11 subcell types (Figure 2D): B cells (markers: *Cd19, Cd79a* and *Ms4a1*), endothelial cells, islet cells, mesenchymal cells, CD4+ Th cells (markers: *Cd4, Tnfsf8* and *Lat*), CD8+T cells (markers: *Cd8a, Cd8b1* and *Ms4a4b*), regulatory T cells (Tregs, markers: *Il2ra, Ctla4* and *Cd2*), macrophages (markers: *Cd68, Csf1r* and *Pla2g7*), natural killer cells (NK, markers: *Gzma, Cd7* and *Klrb1c*), acinar cells and dendritic cells (DCs, markers: *Clec9a, Xcr1* and *Cd24a*).

Meticulous scRNA-seq analysis of macrophage populations revealed three transcriptionally unique clusters, namely, Mø-C1, Mø-C2, and Mø-C3, providing deep insights into the heterogeneity and functional specialization of macrophage communities in the context of islet transplantation (Figure 2E). The marker genes of Mø-C1, Mø-C2, and Mø-C3 are shown in Figure 2F. The proportions of Mø-C1, Mø-C2, and Mø-C3 are shown in Figure 2G. The proportion of Mø-C1 cells was significantly greater in syngeneic grafts, whereas the proportion of Mø-C2 cells was considerably greater in allogeneic grafts. The proportion of Mø-C3 cells was similar in both the syngeneic and allogeneic grafts. This granular view of cellular landscapes sets the stage for a nuanced understanding of the immunological intricacies governing graft survival and acceptance.

3.3 Comparative analysis of DEGs in macrophages between syngeneic and allogeneic islet transplants

To investigate the molecular differences between macrophages from syngeneic versus allogeneic islet grafts, we conducted a thorough differential gene expression analysis. This approach enabled us to identify and characterize the DEGs across three distinct clusters of macrophages: Mø-C1 (Figure 2H, Supplementary Table 1), Mø-C2 (Figure 2I, Supplementary Table 2), and Mø-C3 (Figure 2J, Supplementary Table 3). By employing bioinformatics tools, we generated volcano plots to visually represent the DEGs between the syngeneic and allogeneic islet grafts within each macrophage subset. The analysis revealed significant differences in the expression of genes involved in critical pathways associated with graft acceptance, immune response modulation, and islet cell survival.

3.4 Key pathways upregulated in Mø-C2 macrophages during islet-allograft transplantation

Pathway analyses primarily utilized HALLMARK gene sets from the Molecular Signatures Database (MSigDB), which are exported via the MSigDB package. GSVA scores per cell revealed pathway activity differences across macrophage clusters (Mø-C1, Mø-C2, and Mø-C3). Notably, eight pathways were significantly upregulated in Mø-C2 cells, underscoring their critical role in islet-allograft transplantation. These pathways included DNA repair, MYC targets, G2M checkpoint, inflammatory response, E2F targets, allograft rejection, interferon alpha response, and interferon gamma response (Figure 3A). The number of DEGs within these pathways is detailed in Figure 3B.

3.5 Signaling pathway dynamics in Mø-C1

Intersection analysis identified key genes involved in eight distinct pathways within the Mø-C1 macrophage cluster, as illustrated by the Venn diagram (Figure 4A). These pathways include DNA repair (including 2 genes), MYC targets (including 10 genes), G2M checkpoint (including 2 genes), inflammatory response (including 20 genes), E2F targets (including 1 gene), allograft rejection (including 17), interferon alpha response (including 9 genes), and interferon gamma response (including 25 genes). The heatmaps shows the up-regulated DEGs (Figure 4B) and down-regulated DEGs (Figure 4C) in allograft compared with syngeneic graft within these pathways. Detailed information on all DEGs in pathways related to Mø-C1 is provided in Supplementary Tables 4, 5. This analysis highlights the involvement of diverse genes in crucial pathways, shedding light on the multifaceted roles of Mø-C1 macrophages in islet grafts.

3.6 Pathway analysis in Mø-C2

A Venn diagram (Figure 5A) was generated to identify genes significantly enriched in seven pathways within the Mø-C2 cluster. These pathways included DNA repair (including 2 genes), MYC targets (including 4 genes), G2M checkpoint (including 1 gene), inflammatory response (including 12 genes), allograft rejection (including 15 genes), interferon alpha response (including 7 genes), and interferon gamma response (including 24 genes). The heatmaps was generated to visualize the up-regulated DEGs (Figure 5B) and down-regulated DEGs (Figure 5C) in these pathways. Detailed information on all DEGs in pathways related to Mø-C2 is provided in Supplementary Tables 4, 5. This analysis underscores the significant activation of inflammatory and immune response pathways in Mø-C2 macrophages, particularly in the context of allograft rejection.

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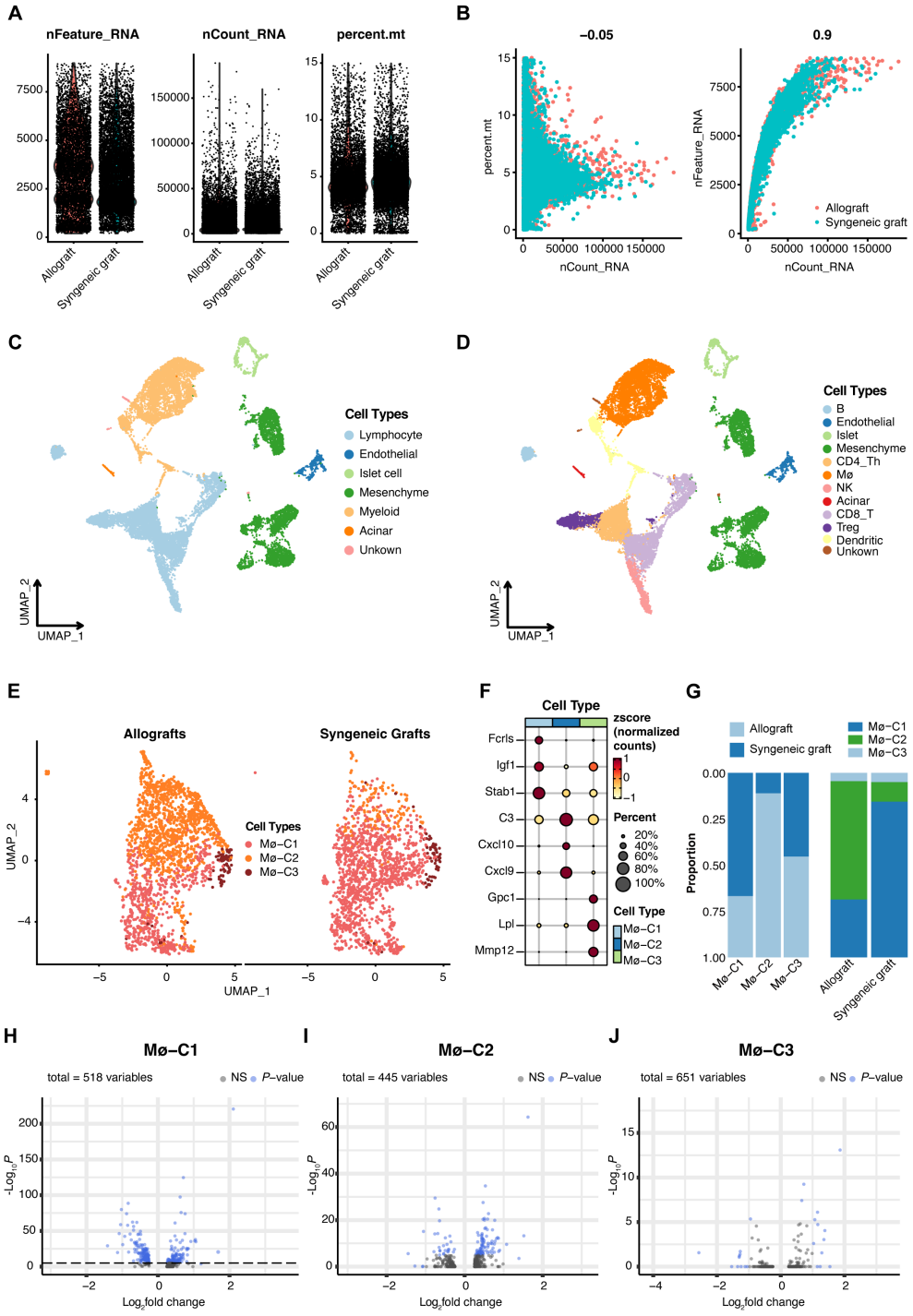

FIGURE 2
Single-cell RNA sequencing (scRNA-seq) insights into islet transplantation. **(A)** Quality control metrics for scRNA-seq of syngeneic and allogeneic islet grafts. **(B)** The number of detected genes showed no correlation with the percentage of mitochondrial content but was significantly correlated with sequencing depth. **(C)** Uniform manifold approximation and projection (UMAP) visualization highlights six predominant cell types within islet grafts, underscoring the diverse cellular landscape. **(D)** Further UMAP analysis revealed 11 subcell types, providing a detailed view of cellular diversity within the grafts. **(E)** Comparative UMAP plots of three macrophage clusters (Mø-C1, Mø-C2, and Mø-C3) in allogeneic (left panel) versus syngeneic (right panel) islet grafts reveal distinct cellular distributions. **(F)** Maker genes of three macrophage clusters. **(G)** The proportions of Mø-C1, Mø-C2, and Mø-C3. The number of Mø-C2 cells in allografts was significantly greater than that in syngeneic grafts. **(H–J)** The variance in gene expression between syngeneic and allogeneic grafts across macrophage clusters (Mø-C1, Mø-C2, and Mø-C3) is depicted, emphasizing the differential expression landscape. Mø, macrophage.

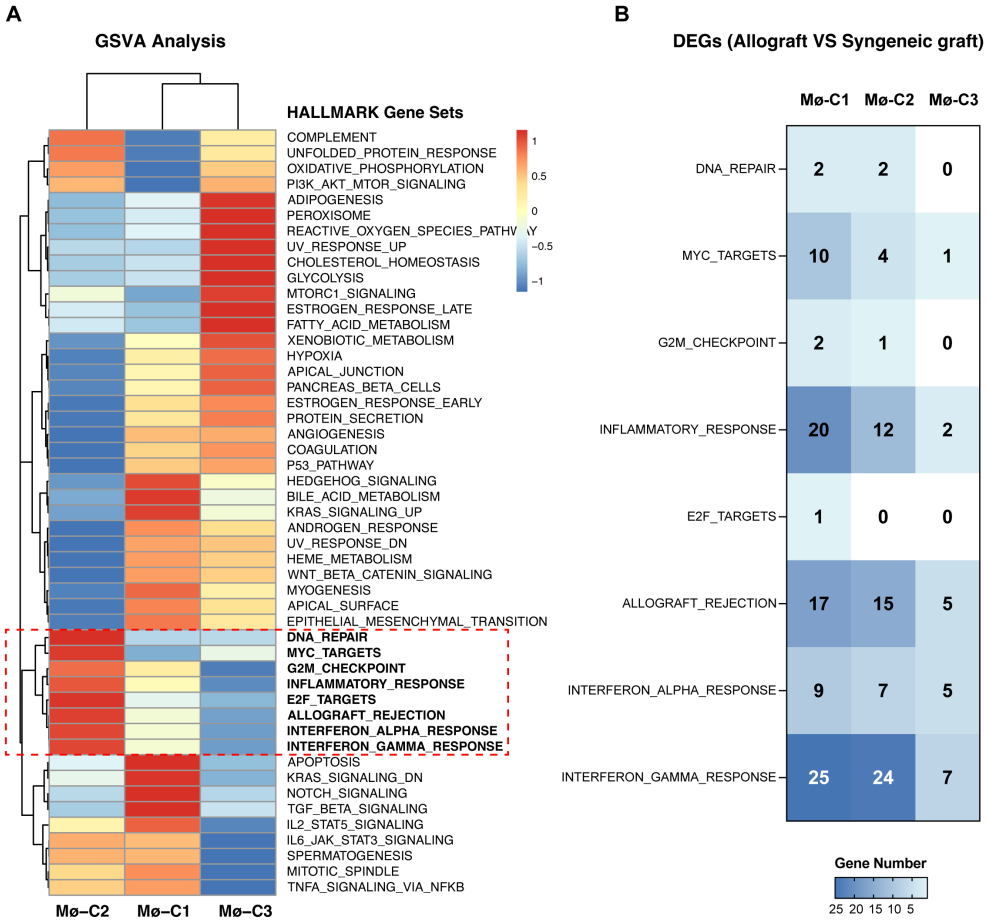

FIGURE 3
Gene set variation analysis (GSVA). **(A)** Pathway analyses primarily utilized HALLMARK gene sets from the Molecular Signatures Database (MSigDB), which were exported via the MSigDB package. GSVA scores per cell revealed pathway activity differences across macrophage clusters (Mø-C1, Mø-C2, and Mø-C3). The red box highlights eight pathways significantly upregulated in Mø-C2 cells, underscoring their critical role in islet-allograft transplantation. These pathways included DNA repair, MYC target, G2M checkpoint, inflammatory response, E2F target, allograft rejection, interferon alpha response, and interferon gamma response pathways. **(B)** The number of differentially expressed genes (DEGs) within pathways significantly upregulated in Mø-C2 macrophages. Mø, macrophage.

3.7 Pathway insights for Mø-C3 macrophages

Key genes associated with five pathways in the Mø-C3 cluster were identified through intersection analysis, as shown in the Venn diagram (Figure 6A). These pathways included MYC targets (including 1 gene), inflammatory response genes (including 2 genes), allograft rejection genes (including 5 genes), interferon alpha response genes (including 5 genes), and interferon gamma response genes (including 7 genes). The heatmaps shows the up-regulated DEGs (Figure 6B) and down-regulated DEGs (Figure 6C) within these pathways. Detailed information on all DEGs in pathways related to Mø-C3 cells is provided in Supplementary Tables 4, 5. This analysis revealed the significant roles of the interferon response and allograft rejection pathways in Mø-C3 macrophages, contributing to the understanding of their function in the immune response to transplantation.

3.8 Macrophage transcriptional state bifurcation and cell fate of three clusters (Mø-C1, Mø-C2, and Mø-C3)

Trajectory manifold analysis of macrophages from islet grafts was conducted using the Monocle 2 algorithm, which identified distinct cellular trajectories or fates based on expression profiles (Figure 7A). The analysis revealed that macrophages primarily originate from the Mø-C3 cluster, which branches into the Mø-C1 and Mø-C2 clusters. Comparative trajectory analysis of macrophages from syngeneic and allogeneic grafts (Figure 7B) further elucidated these dynamics, showing that Mø-C3s serve as common progenitors for both transplant types.

The density plots (Figures 7C, D) illustrate the pseudotime projections of transcriptional changes for the three macrophage clusters (Mø-C1, Mø-C2, and Mø-C3). The proportion of Mø-C3

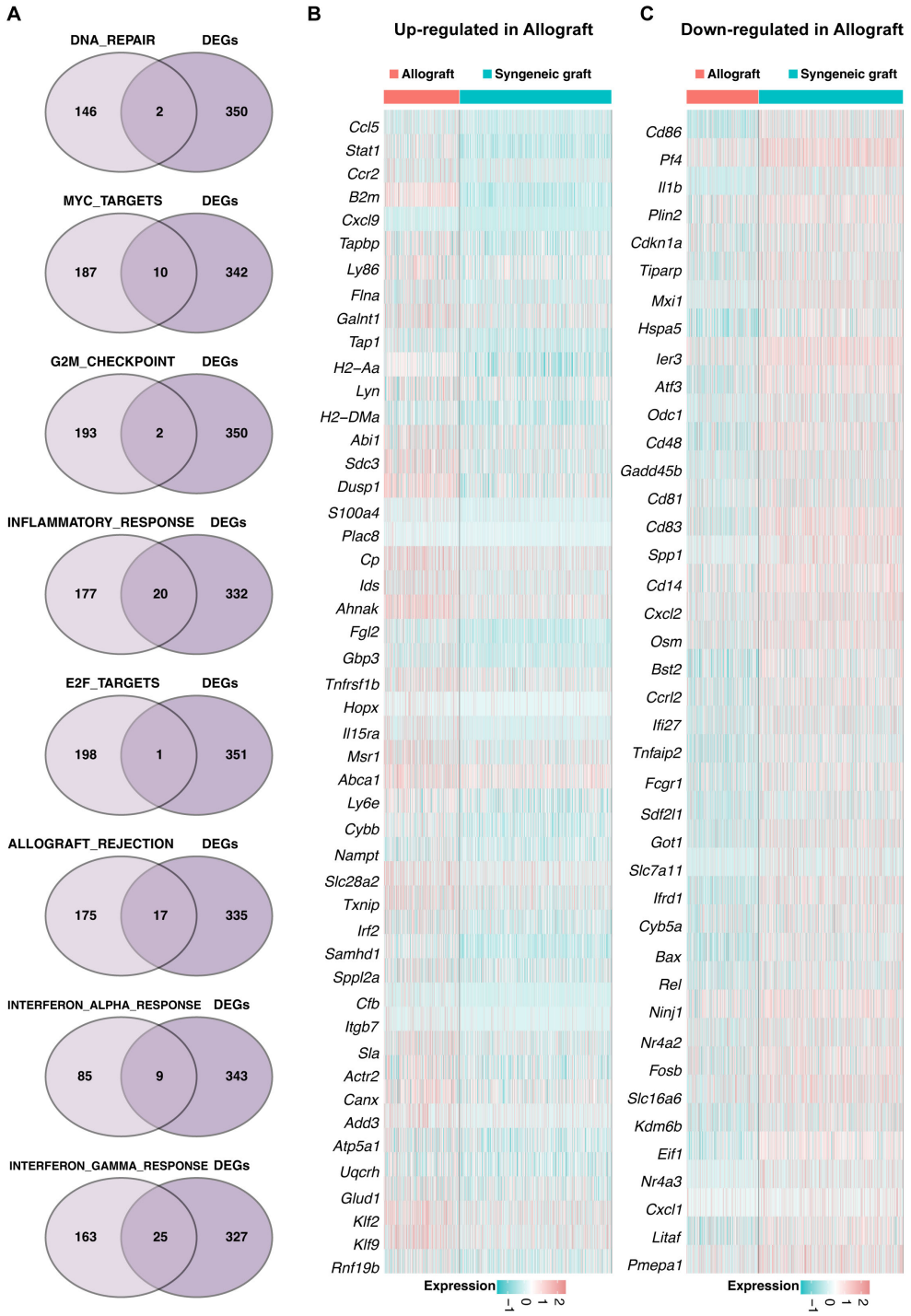

FIGURE 4
Signaling pathway dynamics in Mø-C1. **(A)** Intersection analysis highlights key genes involved in eight distinct pathways within the Mø-C1 macrophage cluster, as shown in the Venn diagram. These pathways included DNA repair, MYC target, G2M checkpoint, inflammatory response, E2F target, allograft rejection, interferon alpha response, and interferon gamma response pathways. **(B)** Heatmap of up-regulated DEGs in allograft involved in these pathways. **(C)** Heatmap of down-regulated DEGs in allograft involved in these pathways. Mø, macrophage.

macrophages was relatively similar in both syngeneic and allogeneic grafts (Figure 2G), indicating that a stable progenitor state was unaffected by the type of graft. However, Mø-C1 macrophages were found in greater proportions in syngeneic grafts (Figure 2G), suggesting that they play a role in promoting graft tolerance. Conversely, Mø-C2 macrophages were more prevalent in allogeneic grafts (Figure 2G), which is indicative of their involvement in inflammatory responses and graft rejection.

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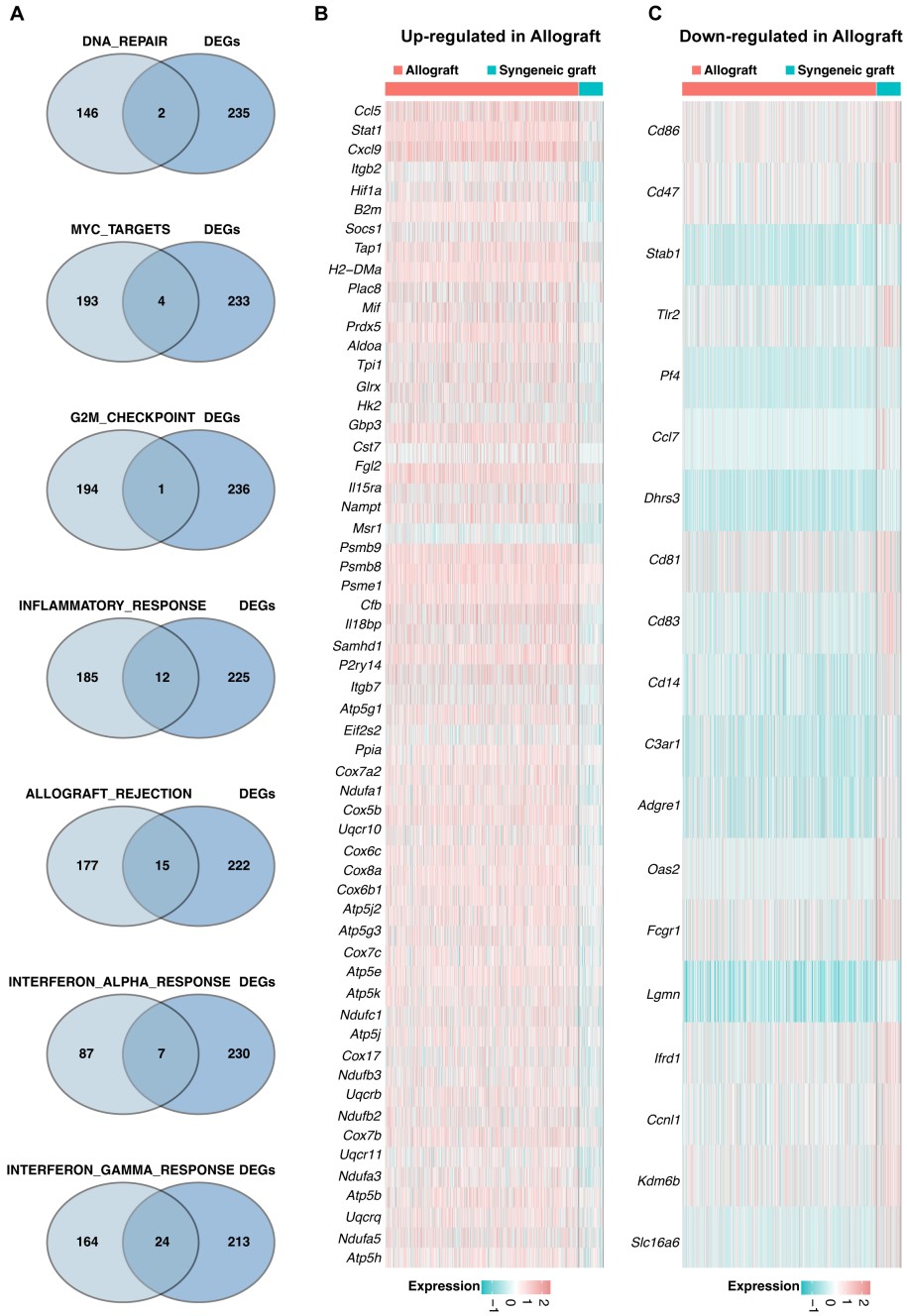

FIGURE 5
Pathway analysis in Mø-C2 macrophages. **(A)** Venn diagram identifying significant genes across seven pathways within the Mø-C2 cluster. These pathways include DNA repair, MYC targets, G2M checkpoint, inflammatory response, allograft rejection, interferon alpha response, and interferon gamma response pathways. **(B)** The heatmap visualizes the up-regulated DEGs in allograft involved in these pathways. **(B, C)** The heatmap visualizes the down-regulated DEGs in allograft involved in these pathways. Mø, macrophage.

3.9 Cytokine signature enrichment in macrophages

To evaluate macrophages in islet grafts, we employed a comprehensive dictionary of immune responses to cytokines. Responses to 86 cytokines were analyzed by comparing syngeneic and allogeneic grafts (Figure 8A). The immune response enrichment analysis (IREA) cytokine enrichment plot (Figure 8B) displays the enrichment score (ES) for each cytokine response across the three macrophage clusters (Mø-C1, Mø-C2, and Mø-C3) in syngeneic versus allogeneic grafts. The bar length represents the ES, while shading indicates the FDR-adjusted *P* value from a two-sided Wilcoxon rank-sum test, with darker shades reflecting greater statistical significance (red for allografts, blue for syngeneic grafts).

This evaluation is based on data collected by Cui et al. (25), where transcriptional responses to individual cytokine stimulation were measured. For Mø-C1s, the top 10 cytokines with the strongest

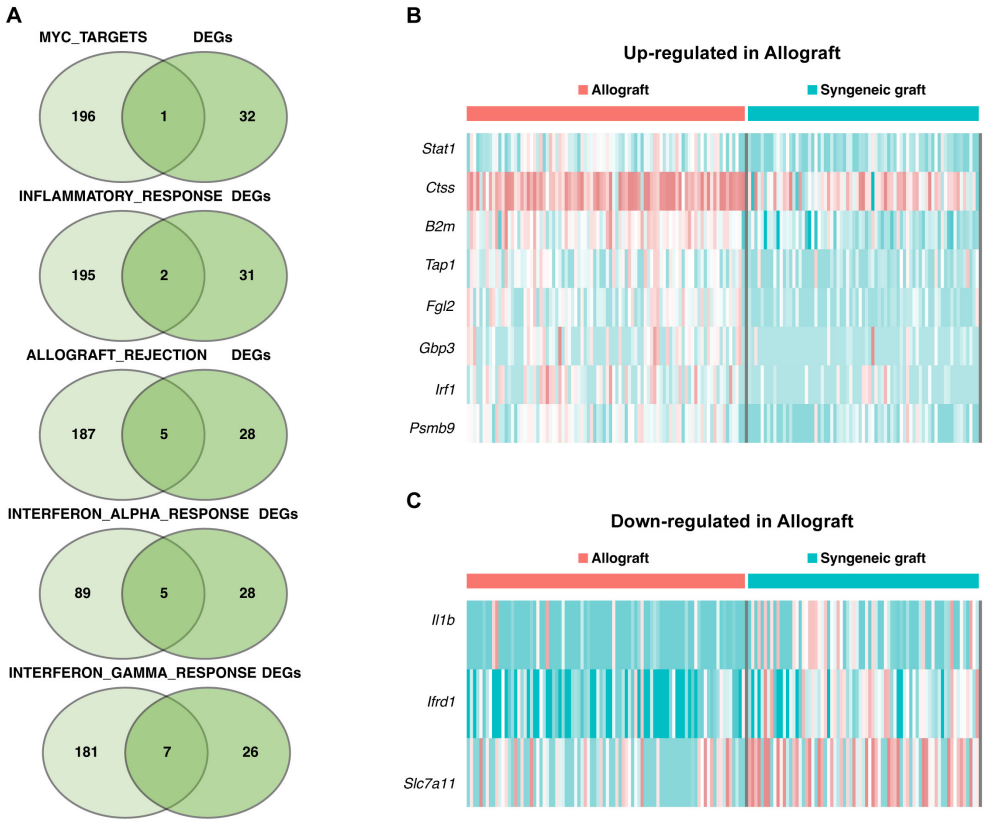

FIGURE 6
Pathway insights for Mø-C3 macrophages. **(A)** Key genes associated with five pathways in the Mø-C3 cluster were identified through intersection analysis. These pathways include MYC targets, inflammatory response, allograft rejection, interferon alpha response, and interferon gamma response. **(B)** Heatmap showing the up-regulated DEGs in allograft within these pathways. **(C)** Heatmap showing the down-regulated DEGs in allograft within these pathways. Mø, macrophage.

enrichment in allografts compared to syngeneic grafts were adiponectin, IL15, IFNα1, IFNβ, IFNγ, prolactin, IL7, IL11, IL18, and LIF (detailed results are provided in Supplementary Table 6). For Mø-C2s, the top 10 cytokines with the strongest enrichment in allografts were IFNγ, IFNα1, IFNβ, IL15, IL18, adiponectin, IL27, IL12, IL11, and IL36α (detailed results are provided in Supplementary Table 7). For Mø-C3s, the top 10 cytokines with the strongest enrichment in allografts were IFNγ, IFNα1, IFNβ, IL15, IL18, adiponectin, IL2, IL12, IL36α, and IFNκ (detailed results are provided in Supplementary Table 8).

These analyses collectively offer a comprehensive view of the molecular underpinnings that define the macrophage-mediated response in syngeneic and allogeneic islet transplantation. By shedding light on the specific pathways and genes differentially expressed in various macrophage populations, this research underscores the complexity of the immune response to transplantation and points toward potential therapeutic targets for enhancing graft survival and function.

4 Discussion

Single-cell RNA sequencing (scRNA-seq) technology has already found extensive applications in immunology (26) and transplantation (27) research due to its ability to provide high-resolution insights into cellular heterogeneity and the distinct functional states of individual cells. In this study, we leveraged single-cell RNA sequencing to thoroughly examine macrophage dynamics and molecular mechanisms in islet transplantation by comparing syngeneic and allogeneic grafts. By analyzing data from GSE198865 (19), we identified three distinct macrophage clusters (Mø-C1, Mø-C2, and Mø-C3) and explored their differential gene expression and pathway activities. Our detailed single-cell analysis revealed complex interactions and regulatory mechanisms within macrophage populations that were not previously captured by bulk RNA sequencing studies. This detailed view of cellular heterogeneity and functional specialization provides deeper insights into the molecular underpinnings of immune responses in transplantation, thereby the existing research.

Recent studies have demonstrated that macrophage heterogeneity and the distinct functional roles of various macrophage subpopulations are critical in shaping immune responses in different tissue contexts (28). For instance, research has shown that tissue-resident macrophages exhibit unique gene expression profiles and functional specializations depending on their tissue of origin and local microenvironment (29). Furthermore, the diversity of macrophage activation states, ranging from proinflammatory to anti-inflammatory and tissue

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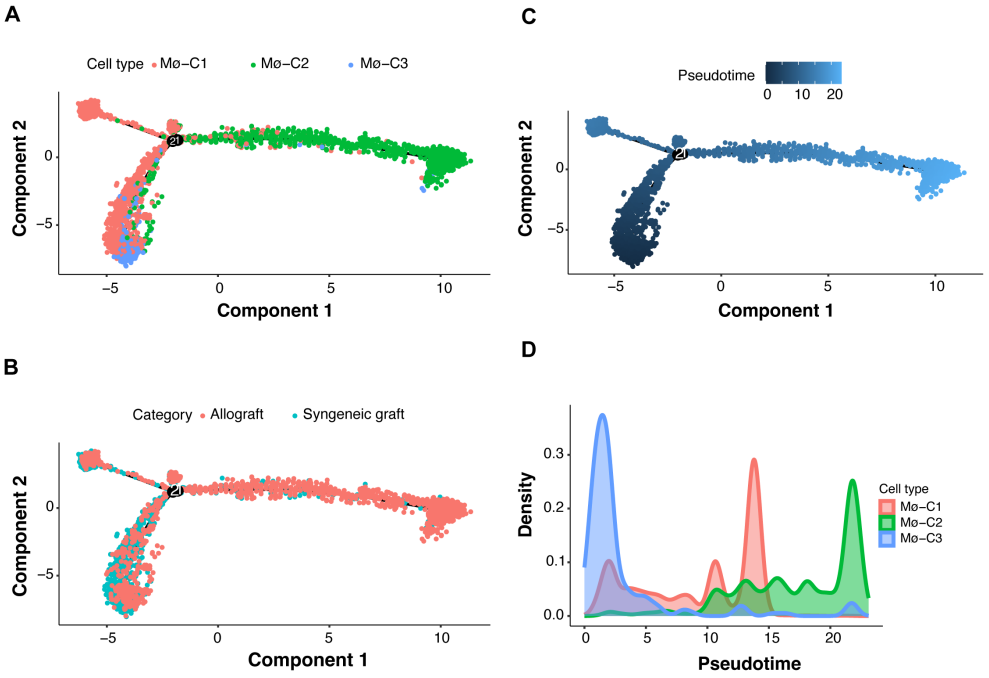

FIGURE 7
Macrophage transcriptional state bifurcation and cell fate of three clusters (Mø-C1, Mø-C2, and Mø-C3). **(A)** Multifold trajectories of macrophages from islet grafts analyzed using the Monocle 2 algorithm. Solid and dotted lines indicate distinct cellular trajectories or fates as determined by their expression profiles. **(B)** Comparative trajectory analysis of macrophages from syngeneic and allogeneic grafts using the Monocle 2 algorithm. **(C, D)** Density plots illustrating pseudotime projections of transcriptional changes for the three macrophage clusters (Mø-C1, Mø-C2, and Mø-C3). Mø, macrophage.

repair phenotypes, underscores the complexity of their roles in immune regulation (30).

By comparing our findings with those in the literature, we observe both the confirmation and expansion of previously reported results. The observed upregulation of allograft rejection, inflammatory response (31, 32), and interferon (33) signaling pathways in allogeneic transplants corroborates previous studies emphasizing the central role of these pathways in mediating inflammatory responses and immune rejection. However, our study extends these findings by providing a more nuanced understanding of the differences in macrophage polarization states between syngeneic and allogeneic transplants, highlighting a skew toward a more inflammatory phenotype in allogeneic settings. Our analysis revealed significant activation of macrophages in allogeneic transplants, marked by the upregulation of allograft rejection-related genes and pathways involved in inflammatory and interferon responses, supporting the hypothesis that immune rejection in allogeneic transplants is driven by the host's immune response to foreign antigens.

Gene set variation analysis (GSVA) is a nonparametric, unsupervised method that assesses pathway activity changes over a sample population in an expression dataset. GSVA transforms gene expression data from a gene-centric to a pathway-centric view, enabling the evaluation of pathway-level changes across samples (23). It has been widely utilized in immunological studies to elucidate the involvement of various signaling pathways in immune responses, disease mechanisms, and therapeutic interventions, providing insights into the functional context of gene expression alterations in immune cells (34). We used GSVA methods and identified eight pathways that were significantly upregulated in the Mø-C2 cluster, namely, DNA repair, MYC target, G2M checkpoint, inflammatory response, E2F target, allograft rejection, interferon alpha response, and interferon gamma response pathways. These pathways are crucial for understanding the immune response dynamics in islet transplantation and highlight potential therapeutic targets to modulate macrophage activity and improve graft outcomes.

Detailed intersection analyses and heatmaps of differentially expressed genes (DEGs) across the macrophage clusters provided insights into the polarization states of macrophages. These findings suggest a shift toward a proinflammatory phenotype in allogeneic transplants, which may contribute to graft rejection. This insight is crucial for developing targeted therapies that could reprogram macrophages to a more tolerogenic state.

Monocle 2, originally described by Qiu et al., utilizes a technique called reverse graph embedding to reconstruct the trajectories of single cells as they progress through different states (24). This method is particularly powerful for revealing the dynamic changes in cell fate decisions over time. Monocle 2 constructs a trajectory of single-cell transcriptomes by ordering cells along a pseudotime axis, which helps in understanding the progression and differentiation of cells in various biological processes.

Recent studies have demonstrated the application of Monocle 2 in various research contexts. For instance, Wang et al. (35) used Monocle 2 to create a single-cell transcriptome atlas of human

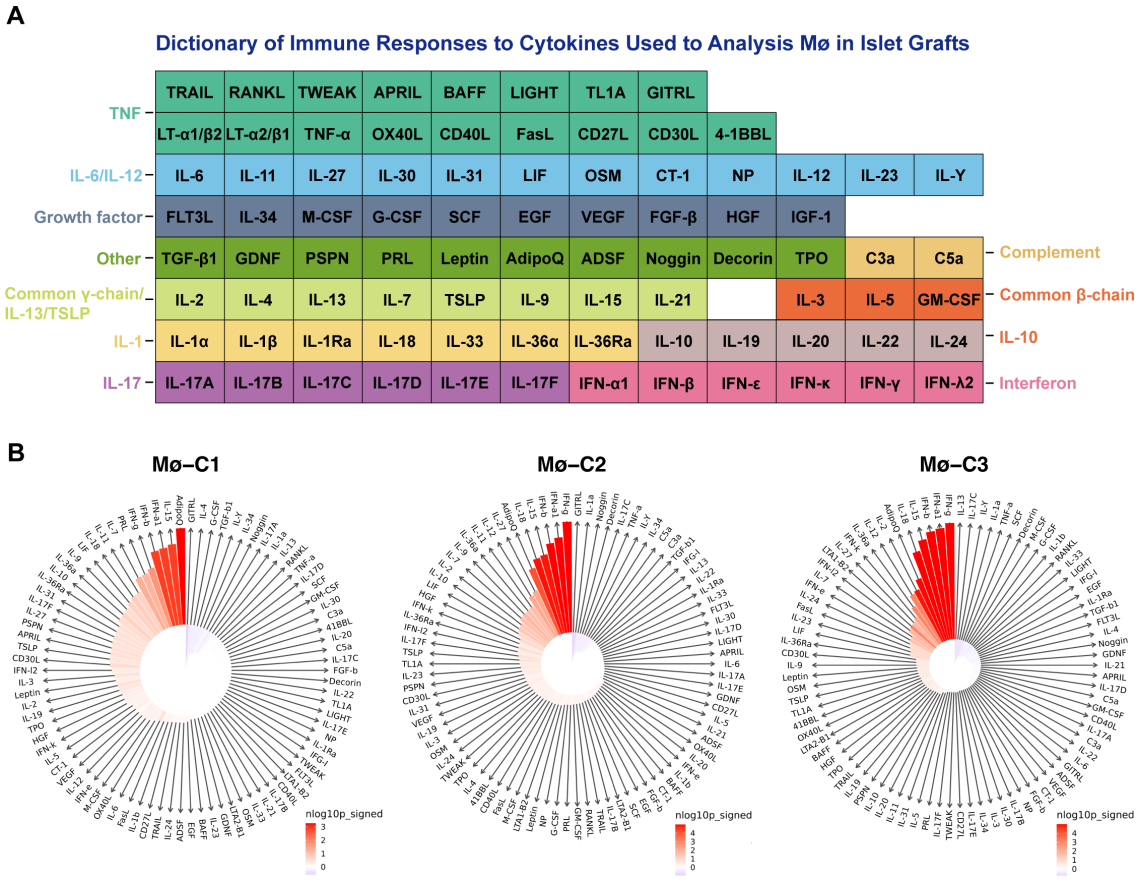

FIGURE 8
Cytokine signature enrichment in macrophages. **(A)** A comprehensive dictionary of immune responses to cytokines was utilized to evaluate macrophages in islet grafts. We analyzed the responses to 86 cytokines by comparing syngeneic and allogeneic grafts. **(B)** The IREA cytokine enrichment plot displays the enrichment score (ES) for each of the 86 cytokine responses across three macrophage clusters (Mø-C1, Mø-C2, and Mø-C3) in allogeneic versus syngeneic grafts. The length of the bars indicates the ES, while the shading reflects the FDR-adjusted P value from a two-sided Wilcoxon rank-sum test, with darker shades denoting greater statistical significance (red for allografts, blue for syngeneic grafts). Mø, macrophage.

euploid and aneuploid blastocysts, providing insights into early human development and chromosomal abnormalities. Huang et al. (36) applied Monocle 2 to explore the molecular landscape of sepsis severity in infants, revealing that enhanced coagulation, innate immunity, and T-cell repression are key factors. Additionally, Su et al. (37) conducted a direct comparison of mass cytometry and single-cell RNA sequencing of human peripheral blood mononuclear cells using Monocle 2 to elucidate cellular heterogeneity and immune responses. Walzer et al. (38) employed Monocle 2 to study the transcriptional control of the Cryptosporidium life cycle, shedding light on the parasite's developmental stages and potential therapeutic targets. Furthermore, Wu et al. (39) integrated single-cell sequencing and bulk RNA-seq to identify and develop a prognostic signature related to colorectal cancer stem cells, utilizing Monocle 2 to trace the differentiation pathways of cancer stem cells. These applications highlight Monocle 2's versatility and effectiveness in tracing cell fate decisions and understanding complex biological processes, making it a valuable tool in both basic and translational research.

Trajectory analysis using Monocle 2 revealed distinct cellular trajectories and fate decisions within macrophage populations, further elucidating the complexity of macrophage responses in islet grafts. The analysis showed that Mø-C3 serves as a common progenitor, branching into Mø-C1 and Mø-C2. Interestingly, the proportion of Mø-C3 cells was similar in both the syngeneic and allogeneic grafts, indicating that the baseline macrophage state was unaffected by the transplant type. In contrast, Mø-C1 cells were predominantly present in syngeneic grafts, while Mø-C2 cells were more abundant in allogeneic grafts. This suggests that Mø-C1 macrophages are more strongly associated with a tolerogenic environment, whereas Mø-C2 macrophages are linked to a more inflammatory response characteristic of graft rejection.

Our cytokine signature enrichment analysis revealed notable differences in cytokine responses between syngeneic and allogeneic grafts. For Mø-C1 macrophages, the top 10 cytokines with the strongest enrichment in allografts compared to syngeneic grafts included adiponectin, IL15, IFNα1, IFNβ, IFNγ, prolactin, IL7, IL11, IL18, and LIF. These cytokines are known to play diverse roles in immune modulation and inflammation. For example, IFNγ (40, 41) and IFNβ (42) are critical for enhancing antigen presentation and promoting a Th1 immune response, which is often associated with graft rejection. Similarly, IL15 and IL18 (43) are potent

activators of NK cells and T cells, further contributing to the inflammatory milieu.

In Mø-C2 macrophages, the top 10 enriched cytokines in allografts were IFNγ, IFNα1, IFNβ, IL15, IL18, adiponectin, IL27, IL12, IL11, and IL36α. The presence of IL27 (44) and IL12 (45) suggests their strong involvement in promoting Th1 and Th17 responses, which are crucial for initiating and sustaining immune responses against transplanted tissues. IL36α, a member of the IL-1 cytokine family, is known for its role in amplifying inflammatory responses and has been implicated in autoimmune diseases, suggesting its potential involvement in graft rejection mechanisms.

For Mø-C3 macrophages, the top 10 enriched cytokines in allografts were IFNγ, IFNα1, IFNβ, IL15, IL18, adiponectin, IL2, IL12, IL36α, and IFNκ. IL2 is essential for T-cell proliferation and survival, indicating a supportive environment for effector T-cell responses in allogeneic grafts (45). The enrichment of IFNκ (42), an interferon involved in antiviral responses, further highlights the complexity and multifaceted nature of the immune response in allogeneic grafts.

The enrichment of these cytokines in allografts underscores their critical roles in mediating immune responses and promoting inflammatory environments that are conducive to graft rejection. In contrast, syngeneic grafts, which are genetically identical to the host, do not provoke such robust inflammatory cytokine responses, allowing for better graft acceptance. Our findings align with previous studies showing that proinflammatory cytokines, such as IFNγ, are upregulated in allogeneic transplants, contributing to graft rejection. Conversely, the role of anti-inflammatory cytokines such as IL-10 in supporting graft acceptance is well documented, highlighting their importance in creating a tolerogenic environment in syngeneic transplants.

The distinct cytokine profiles observed in our study highlight the importance of cytokine signaling pathways in shaping the immune landscape during transplantation. Targeting specific cytokines or their signaling pathways could offer new therapeutic strategies to balance immune activation and tolerance, thereby improving graft survival and function. Future research should focus on developing targeted therapies that modulate these cytokine responses to improve transplant outcomes.

While our study provides substantial insights, it is essential to acknowledge several limitations. Primarily, the reliance on animal models necessitates careful consideration when extrapolating findings to human clinical scenarios. Validation in human transplant samples is crucial to ensure clinical relevance. Additionally, single-cell RNA sequencing captures a snapshot of gene expression, which may not fully represent dynamic cellular processes.

Despite rigorous statistical methods, including the Wilcoxon rank-sum test and the limma package, there remains significant variability in gene expression profiles across different macrophage clusters and transplantation models. Differences in cell capture efficiency, sequencing depth, and batch effects can introduce biases, despite stringent quality control measures and batch effect correction.

Future research should focus on corroborating these insights in human transplant samples and exploring the therapeutic potential of targeting identified pathways to modulate macrophage function and improve transplant efficacy. Exploring immune regulatory strategies that specifically target the proinflammatory macrophage response while avoiding broad immunosuppression represents a promising research direction. Finally, experimental validation of computational predictions, such as key pathway activation and cytokine expression, is essential to corroborate our results and translate them into clinical applications. Addressing these limitations in future studies will be critical for advancing our understanding of macrophage dynamics in islet transplantation and improving clinical outcomes.

In conclusion, our study significantly advances the knowledge of macrophage roles within the context of islet transplantation. By meticulously dissecting the interactions between immune pathways and cellular fate processes, we provide a detailed understanding of the immune response and identify potential targets for therapeutic intervention. These findings lay a foundation for innovative research pathways and therapeutic strategies aimed at improving transplantation therapies and achieving long-term success in treating type 1 diabetes. Our work underscores the necessity of further exploration to enhance transplant viability and highlights the importance of understanding the immunological aspects of transplant acceptance and longevity.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

LM: Conceptualization, Funding acquisition, Methodology, Writing – original draft, Writing – review & editing. SC: Formal analysis, Writing – review & editing. YL: Formal analysis, Visualization, Writing – review & editing. ZW: Formal analysis, Visualization, Writing – review & editing. ZC: Formal analysis, Funding acquisition, Writing – review & editing. ZP: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

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Conflict of interest

Author ZC was employed by company BGI Medical Group.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2024.1407118/full#supplementary-material

SUPPLEMENTARY TABLE 1
Total DEGs in Mø-C1.

SUPPLEMENTARY TABLE 2
Total DEGs in Mø-C2.

SUPPLEMENTARY TABLE 3
Total DEGs in Mø-C3.

SUPPLEMENTARY TABLE 4
Up-regulated DEGs in allograft in related pathways.

SUPPLEMENTARY TABLE 5
Down-regulated DEGs in allograft in related pathways.

SUPPLEMENTARY TABLE 6
Cytokine Signature Enrichment in Mø-C1.

SUPPLEMENTARY TABLE 7
Cytokine Signature Enrichment in Mø-C2.

SUPPLEMENTARY TABLE 8
Cytokine Signature Enrichment in Mø-C3.

References

1. Bornstein SR, Ludwig B, Steenblock C. Progress in islet transplantation is more important than ever. *Nat Rev Endocrinol*. (2022) 18:389–90. doi: 10.1038/s41574-022-00689-0

2. Harris E. FDA greenlights first cell therapy for adults with type 1 diabetes. *JAMA*. (2023) 330(5):402. doi: 10.1001/jama.2023.12542

3. Mullard A. FDA approves first cell therapy for type 1 diabetes. *Nat Rev Drug Discovery*. (2023) 22:611. doi: 10.1038/d41573-023-00113-w

4. FDA. FDA approves first cellular therapy to treat patients with type 1 diabetes (2023). Available online at: https://www.fda.gov/news-events/press-announcements/fda-approves-first-cellular-therapy-treat-patients-type-1-diabetes (Accessed February 5, 2024).

5. Marfil-Garza BA, Imes S, Verhoeff K, Hefler J, Lam A, Dajani K, et al. Pancreatic islet transplantation in type 1 diabetes: 20-year experience from a single-centre cohort in Canada. *Lancet Diabetes Endocrinol*. (2022) 10:519–32. doi: 10.1016/S2213-8587(22)00114-0

6. Chen Q-D, Liu L, Zhao X-H, Liang J-B, Li S-W. Challenges and opportunities in the islet transplantation microenvironment: a comprehensive summary of inflammatory cytokine, immune cells, and vascular endothelial cells. *Front Immunol*. (2023) 14:1293762. doi: 10.3389/fimmu.2023.1293762

7. Li Q, Lan P. Activation of immune signals during organ transplantation. *Signal Transduct Target Ther*. (2023) 8:110. doi: 10.1038/s41392-023-01377-9

8. Wang X, Brown NK, Wang B, Shariati K, Wang K, Fuchs S, et al. Local immunomodulatory strategies to prevent allo-rejection in transplantation of insulin-producing cells. *Adv Sci*. (2021) 8:2003708. doi: 10.1002/advs.202003708

9. Weir GC, Bonner-Weir S. Scientific and political impediments to successful islet transplantation. *Diabetes*. (1997) 46:1247–56. doi: 10.2337/diab.46.8.1247

10. Zahr E, Molano RD, Pileggi A, Ichii H, Jose SS, Bocca N, et al. Rapamycin impairs in *vivo* proliferation of islet beta-cells. *Transplantation*. (2007) 84:1576–83. doi: 10.1097/01.tp.0000296035.48728.28

11. Beilke JN, Kuhl NR, Kaer LV, Gill RG, Beilke JN, Kuhl NR, et al. NK cells promote islet allograft tolerance *via* a perforin-dependent mechanism. *Nat Med*. (2005) 11:1059–65. doi: 10.1038/nm1296

12. Yan L, Ye L, Chen Y, He S, Zhang C, Mao X, et al. The influence of microenvironment on survival of intraportal transplanted islets. *Front Immunol*. (2022) 13:849580. doi: 10.3389/fimmu.2022.849580

13. Li X, Meng Q, Zhang L. The fate of allogeneic pancreatic islets following intraportal transplantation: challenges and solutions. *J Immunol Res*. (2018) 2018:2424586. doi: 10.1155/2018/2424586

14. Na YR, Kim SW, Seok SH. A new era of macrophage-based cell therapy. *Exp Mol Med*. (2023) 55:1945–54. doi: 10.1038/s12276-023-01068-z

15. Gou W, Wang J, Song L, Kim D-S, Cui W, Strange C, et al. Alpha-1 antitrypsin suppresses macrophage activation and promotes islet graft survival after intrahepatic islet transplantation. *Am J Transplant*. (2021) 21:1713–24. doi: 10.1111/ajt.16342

16. Zhao Y-Z, Huang Z-W, Zhai Y-Y, Shi Y, Du C-C, Zhai J, et al. Polylysine-bilirubin conjugates maintain functional islets and promote M2 macrophage polarization. *Acta Biomater*. (2021) 122:172–85. doi: 10.1016/j.actbio.2020.12.047

17. Li Y, Ding X, Tian X, Zheng J, Ding C, Li X, et al. Islet transplantation modulates macrophage to induce immune tolerance and angiogenesis of islet tissue in type I diabetes mice model. *Aging (Albany NY)*. (2020) 12:24023–32. doi: 10.18632/aging.104085

18. Kumar M, Gupta P, Bhattacharjee S, Nandi SK, Mandal BB. Immunomodulatory injectable silk hydrogels maintaining functional islets and promoting anti-inflammatory M2 macrophage polarization. *Biomaterials*. (2018) 187:1–17. doi: 10.1016/j.biomaterials.2018.09.037

19. Chen P, Yao F, Lu Y, Peng Y, Zhu S, Deng J, et al. Single-cell landscape of mouse islet allograft and syngeneic graft. *Front Immunol*. (2022) 13:853349. doi: 10.3389/fimmu.2022.853349

20. Hao Y, Hao S, Andersen-Nissen E, Mauck WM, Zheng S, Butler A, et al. Integrated analysis of multimodal single-cell data. *Cell*. (2021) 184:3573–3587.e29. doi: 10.1016/j.cell.2021.04.048

21. McInnes L, Healy J, Saul N, Großberger L. UMAP: Uniform manifold approximation and projection. *JOSS*. (2018) 3:861. doi: 10.21105/joss.00861

22. Korsunsky I, Millard N, Fan J, Slowikowski K, Zhang F, Wei K, et al. Fast, sensitive and accurate integration of single-cell data with Harmony. *Nat Methods*. (2019) 16:1289–96. doi: 10.1038/s41592-019-0619-0

23. Hänzelmann S, Castelo R, Guinney J, Hänzelmann S, Castelo R, Guinney J, et al. GSVA: gene set variation analysis for microarray and RNA-Seq data. *BMC Bioinf*. (2013) 14:7. doi: 10.1186/1471-2105-14-7

24. Qiu X, Mao Q, Tang Y, Wang L, Chawla R, Pliner HA, et al. Reversed graph embedding resolves complex single-cell trajectories. *Nat Methods*. (2017) 14:979–82. doi: 10.1038/nmeth.4402

25. Cui A, Huang T, Li S, Ma A, Pérez JL, Sander C, et al. Dictionary of immune responses to cytokines at single-cell resolution. *Nature*. (2024) 625:377–84. doi: 10.1038/s41586-023-06816-9

26. Irac SE, Soon MSF, Borcherding N, Tuong ZK, Irac SE, Soon MSF, et al. Single-cell immune repertoire analysis. *Nat Methods*. (2024) 21:777–92. doi: 10.1038/s41592-024-02243-4

27. Wang Y, Wang J-Y, Schnieke A, Fischer K, Wang Y, Wang J-Y, et al. Advances in single-cell sequencing: insights from organ transplantation. *Military Med Res*. (2021) 8:45. doi: 10.1186/s40779-021-00336-1

28. Mass E, Nimmerjahn F, Kierdorf K, Schlitzer A, Mass E, Nimmerjahn F, et al. Tissue-specific macrophages: how they develop and choreograph tissue biology. *Nat Rev Immunol*. (2023) 23:563–79. doi: 10.1038/s41577-023-00848-y

29. Kloc M, Kubiak JZ. Monocyte and macrophage function diversity. *IJMS*. (2022) 23:12404. doi: 10.3390/ijms232012404

30. Lendeckel U, Venz S, Wolke C, Lendeckel U, Venz S, Wolke C. Macrophages: shapes and functions. *ChemTexts*. (2022) 8:12. doi: 10.1007/s40828-022-00163-4

31. Chung WY, Pollard CA, Kumar R, Drogemuller CJ, Naziruddin B, Stover C, et al. A comparison of the inflammatory response following autologous compared with allogenic islet cell transplantation. *Ann Transl Med*. (2021) 9:98–8. doi: 10.21037/atm-20-3519

32. Ravindranath MH, El Hilali F, Filippone EJ. The impact of inflammation on the immune responses to transplantation: tolerance or rejection? *Front Immunol*. (2021) 12:667834. doi: 10.3389/fimmu.2021.667834

33. Kloc M, Ghobrial RM. Chronic allograft rejection: A significant hurdle to transplant success. *Burns Trauma*. (2014) 2:3–10. doi: 10.4103/2321-3868.121646

34. Chen Y-P, Yin J-H, Li W-F, Li H-J, Chen D-P, Zhang C-J, et al. Single-cell transcriptomics reveals regulators underlying immune cell diversity and immune subtypes associated with prognosis in nasopharyngeal carcinoma. *Cell Res*. (2020) 30:1024–42. doi: 10.1038/s41422-020-0374-x

35. Wang S, Leng L, Wang Q, Gu Y, Li J, An Y, et al. A single-cell transcriptome atlas of human euploid and aneuploid blastocysts. *Front Immunol*. (2024) 15:1468–81. doi: 10.1038/s41588-024-01788-6

36. Huang SSY, Toufiq M, Eghtesady P, Van Panhuys N, Garand M, Huang SSY, et al. The molecular landscape of sepsis severity in infants: enhanced coagulation, innate immunity, and T cell repression. *Front Immunol*. (2024) 15:1281111. doi: 10.3389/fimmu.2024.1281111

37. Su EY, Fread K, Goggin S, Zunder ER, Cahan P. Direct comparison of mass cytometry and single-cell RNA sequencing of human peripheral blood mononuclear cells. *Sci Data*. (2024) 11:559. doi: 10.1038/s41597-024-03399-6

38. Walzer KA, Tandel J, Byerly JH, Daniels AM, Gullicksrud JA, Whelan EC, et al. Transcriptional control of the Cryptosporidium life cycle. *Nature*. (2024) 630:174–80. doi: 10.1038/s41586-024-07466-1

39. Wu J, Li W, Su J, Zheng J, Liang Y, Lin J, et al. Integration of single-cell sequencing and bulk RNA-seq to identify and develop a prognostic signature related to colorectal cancer stem cells. *Sci Rep*. (2024) 14:12270. doi: 10.1038/s41598-024-62913-3

40. Merli P, Caruana I, De Vito R, Strocchio L, Weber G, Bufalo FD, et al. Role of interferon-γ in immune-mediated graft failure after allogeneic hematopoietic stem cell transplantation. *Haematologica*. (2019) 104:2314–23. doi: 10.3324/haematol.2019.216101

41. Halawi A, El Kurdi AB, Vernon KA, Solhjou Z, Choi JY, Saad AJ, et al. Uncovering a novel role of focal adhesion and interferon-gamma in cellular rejection of kidney allografts at single cell resolution. *Front Immunol*. (2023) 14:1139358. doi: 10.3389/fimmu.2023.1139358

42. Fueyo-González F, McGinty M, Ningoo M, Anderson L, Cantarelli C, Angeletti A, et al. Interferon-β acts directly on T cells to prolong allograft survival by enhancing regulatory T cell induction through Foxp3 acetylation. *Immunity*. (2022) 55:459–474.e7. doi: 10.1016/j.immuni.2022.01.011

43. Boieri M, Ulvmoen A, Sudworth A, Lendrem C, Collin M, Dickinson AM, et al. IL-12, IL-15, and IL-18 pre-activated NK cells target resistant T cell acute lymphoblastic leukemia and delay leukemia development. *vivo OncoImmunol*. (2017) 6:e1274478. doi: 10.1080/2162402X.2016.1274478

44. Wu S, Ma R, Zhong Y, Chen Z, Zhou H, Zhou M, et al. Deficiency of IL-27 signaling exacerbates experimental autoimmune uveitis with elevated uveitogenic th1 and th17 responses. *IJMS*. (2021) 22:7517. doi: 10.3390/ijms22147517

45. Ashour D, Arampatzi P, Pavlovic V, Förstner KU, Kaisho T, Beilhack A, et al. IL-12 from endogenous cDC1, and not vaccine DC, is required for Th1 induction. *JCI Insight*. (2020) 5:e135143. doi: 10.1172/jci.insight.135143

46. Zhou H, Pu Z, Lu Y, Zheng P, Yu H, Mou L. Elucidating T cell dynamics and molecular mechanisms in syngeneic and allogeneic islet transplantation through single-cell RNA sequencing. *Front Immunol*. (2024) 15:1429205. doi: 10.3389/fimmu.2024.1429205

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Cutting edge of immune response and immunosuppressants in allogeneic and xenogeneic islet transplantation

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As an effective treatment for diabetes, islet transplantation has garnered significant attention and research in recent years. However, immune rejection and the toxicity of immunosuppressive drugs remain critical factors influencing the success of islet transplantation. While immunosuppressants are essential in reducing immune rejection reactions and can significantly improve the survival rate of islet transplants, improper use of these drugs can markedly increase mortality rates following transplantation. Additionally, the current availability of islet organ donations fails to meet the demand for organ transplants, making xenotransplantation a crucial method for addressing organ shortages. This review will cover the following three aspects: 1) the immune responses occurring during allogeneic islet transplantation, including three stages: inflammation and IBMIR, allogeneic immune response, and autoimmune recurrence; 2) commonly used immunosuppressants in allogeneic islet transplantation, including calcineurin inhibitors (Cyclosporine A, Tacrolimus), mycophenolate mofetil, glucocorticoids, and Bortezomib; and 3) early and late immune responses in xenogeneic islet transplantation and the immune effects of triple therapy (ECDI-fixed donor spleen cells (ECDI-SP) + anti-CD20 + Sirolimus) on xenotransplantation.

KEYWORDS

islet transplantation, immune response, immunosuppressants, xenotransplantation, allogenic and xenogenic islet transplantation

1 Introduction

Diabetes is a chronic metabolic disease characterized by high blood glucose levels, affecting over 500 million people worldwide. Type 1 diabetes (T1D) results from an autoimmune response that destroys the insulin-producing β-cells in the body, resulting in the inability to produce insulin to regulate blood glucose levels (1). Since the discovery of insulin in 1922, insulin therapy has been used to treat patients with T1D. This disease requires minute-to-minute regulation of blood glucose levels, and measures such as exogenous insulin supplementation and continuous glucose monitoring (CGM) can have a certain delay in detecting and controlling blood glucose levels, which insulin injections cannot achieve (2, 3). Only by transplanting insulin-producing cells from donors can we precisely measure and deliver the appropriate doses of insulin (4). Additionally, although intensive insulin therapy can improve glycated hemoglobin levels, it does not prevent diabetic complications (5). When patients face severe metabolic complications, failure of exogenous insulin treatment, or when insulin use fails to prevent acute complications, islet transplantation becomes a necessary treatment measure (6). The transplantation of pancreatic tissue, whether whole pancreas or islets, is a clinical option for the treatment of labile type 1 diabetes. Pancreas transplantation is usually performed as a multi-organ transplant procedure; most of these (72%) are combined pancreatorenal procedures. Therefore, it is particularly suitable for patients with type 1 diabetes combined with end-stage renal disease. Open surgery is required to transplant the entire pancreas into the abdominal cavity of the recipient and connect the blood vessels and digestive tract. The operation is complicated and traumatic, and the recovery time is long. Whole organ pancreas transplants restore euglycemia almost immediately following transplantation, and long-term graft survival rates are excellent. Despite the need for immunosuppression, recipient morbidity and mortality decreased significantly, as did the risk of complications associated with poor glycemic control and a better quality of life (7, 8). Islet transplantation refers to the isolation, purification and transplantation of islets from the pancreas of the donor into the recipient (detailed procedures are described below). Islet transplantation is suitable for type 1 diabetes patients who have experienced severe hypoglycemic events. Following Edmonton protocol, the islets are injected directly into the recipient's liver portal vein under the ultrasound observation, and the operation is less traumatic, the anesthesia time is shorter, the invasion is less, and the recovery time is fast. Although many patients experience significant improvements in blood sugar control after transplantation, exogenous insulin may still be required, and long-term success rates are relatively low.

As an alternative therapy, islet transplantation can sustainably reverse T1D. Successful islet transplantation eliminates the need for stringent blood glucose monitoring and prevents the progression of diabetic complications. However, a significant challenge faced by islet transplantation is the immune response of the body to the foreign islets. When donor islets are exposed to the recipient's immune system, the implants can trigger a rapid immune response (9, 10). Therefore, the survival rate of islets after isolation and transplantation becomes a major issue. Immunosuppressive therapy is currently the most popular immunomodulation method to ensure the survival of islet grafts. Clinical islet transplantation began in the 1970s (11), but due to various reasons, its clinical efficacy was not ideal. It was not until 1999 that Shapiro et al. (12) proposed and established a standard set, including donor selection, islet equivalent transplantation, and post-operative immunosuppressive regimens. They used a large number of isolated islet cells for transplantation and implemented a new regimen post-operatively, using a steroid-free regimen and reduced doses of calcineurin inhibitors (sirolimus, low-dose tacrolimus, and daclizumab), known as the "Edmonton Protocol" (12). Once this protocol was promoted, clinical results improved significantly, marking an important milestone in clinical IT. With the promotion of the Edmonton clinical protocol and the continuous improvement of islet cell isolation techniques, the survival rate of islet transplantation has significantly improved but is still relatively low compared to other organs. Moreover, it is known that the traditional methods of using immunosuppressive drugs during and after islet transplantation can cause many side effects, such as mouth ulcers, peripheral edema, anemia, weight loss, and paroxysmal diarrhea (9, 13). Therefore, to improve the survival rate after islet transplantation, many issues must be addressed, including islet viability, effective implantation, and the application of immunosuppressants that lead to islet damage (14). Therefore, the purpose of this article is to summarize the immune responses and mechanisms of action of immunosuppressants that occur after islet transplantation to better guide islet transplantation and improve islet survival rates.

2 Immune response in allogeneic islet Transplantation

2.1 Inflammatory response

Clinical islet transplantation requires four steps: perfusion of the donor pancreas, digestion of the pancreas to separate the islets from the exocrine tissue, purification of the islets, and transplantation via the portal vein infusion of islet into the recipient (15). When the prepared islets are infused into the patient's body through the portal vein, it triggers an inflammatory response. Early inflammatory response leads to the early loss of islet viability, posing a significant challenge to the long-term survival rate of islet transplantation. This early inflammatory reaction significantly affects islet viability, with estimates indicating that up to 50% of transplanted islets may be lost during this initial phase (16). Post-pancreas transplantation, ischemia-reperfusion creates an inflammatory environment, where the Instant Blood-Mediated Inflammatory Reaction (IBMIR) plays a crucial role. Injecting purified islets into the recipient's portal vein promotes an innate immune-dependent inflammatory response, known as IBMIR.

IBMIR is initiated by the intense activation of the coagulation cascade, where the negatively charged surface of the islets activates the intrinsic coagulation pathway (17), and the tissue factor (TF) expressed by the islets induces the extrinsic coagulation pathway (18). Simultaneously, islets secrete inflammatory factors such as IL-8 and MCP-1, which have chemotactic and pro-inflammatory effects on macrophages and neutrophils (19, 20). Activated platelets can adhere by binding to the extracellular matrix (ECM) and collagen on the

surface of islets. Additionally, due to the rapid transient expression of p-selectin on the membranes of activated platelet alpha granules and vascular endothelial Weibel-Palade bodies, the p-selectin lectin-like domain present on neutrophils and monocytes binds with sialyl Lewis x and p-selectin glycoprotein ligand-1, mediating the rolling of neutrophils and monocytes on endothelial cells and their adhesion to platelets (21, 22). On the other hand, vascular endothelial cells secrete IL-6 and IL-8, promoting the aggregation of neutrophils and macrophages (19). Complement activation is triggered by natural immune antibodies IgG and IgM. When isolated islets are exposed to blood, the complement system is rapidly activated, leading to the lysis of islet cells. Simultaneously, the production of anaphylatoxins C3a and C5a further induces the aggregation of macrophages and neutrophils, promoting the release of cytokines such as IL-1, IL-6, IL-8, and TNF-a by monocytes (23). Granulocytes appear 8 hours after islet transplantation, with extensive infiltration into the grafts after 12 hours. Neutrophils are the main members of the granulocyte family and the first line of defense in innate immunity. They contain various cytokines that, when activated, are released and cause damage to islets; neutrophils significantly contribute to the activation and recruitment of macrophages at acute inflammation sites. Once activated, they produce various chemokines to attract monocytes and macrophages. Additionally, neutrophil infiltration leads to the release of cytokines such as TNF-a and macrophage inflammatory protein-1a by T cells and macrophages, which can expand IBMIR and induce subsequent adaptive immunity, triggering and enhancing cellular rejection (23, 24) (Figure 1). Aggregated macrophages continuously secrete cytokines such as IL-6 and IL-8 to sustain the inflammatory response and release pro-inflammatory factors such as IL-1b, IFN-g, and TNF-a. The IL1b secreted by macrophages and neutrophils binds to IL-1b receptors on the surface of islet cells, activating IL-1 receptor-associated kinases and TNF receptor-associated factor 6, leading to the phosphorylation and degradation of IkB, releasing NF-kB, which then enters the nucleus to regulate the transcription of multiple genes, including IL-1, IL-6, TNF-a, and iNOS. TNF-a produced by macrophages and islet cells binds to TNF receptors, activating the NF-kB and MAPK pathways and inducing apoptosis. Apoptosis is mediated by caspase-3 activation through the MAPK pathway or by activating effector caspases, including FADD-mediated caspase-3 activation. IFN-g produced by macrophages binds to IFN-g receptors on islet cells, activating JAK1 and JAK2. Activated JAK2 then activates Signal Transducer and Activator of Transcription 1 (STAT1). STAT1 is then transferred to the nucleus for gene regulation, ultimately leading to islet cell apoptosis. The pro-apoptotic effect of STAT1 may be partially mediated by the activation of caspase-2, caspase-3, and caspase-7 (25). Under the combined action of cytokines IL-1b, TNF-a, and IFN-g, the overexpression of iNOS in b-cells and macrophages leads to excessive synthesis of NO. Subsequently, NO loses electrons and combines with superoxide radicals to form highly reactive peroxynitrite (ONOO-). The cytotoxicity of ONOO subsequently induces islet cell apoptosis. On the other hand, macrophages play an antigen-presenting role, promoting the activation of T cells into CD8+ T cells and CD4+ T cells. Activated T cells produce cytokines such as IFN-g, TNF-a, and lymphotoxin, thereby inducing b-cell apoptosis. (Figure 1). Lisa Özmen et al. (26) exposed human islets to ABO-compatible blood and found that administering Melaglavin dose-dependently eliminated IBMIR. In the absence of or at concentrations below 0.4

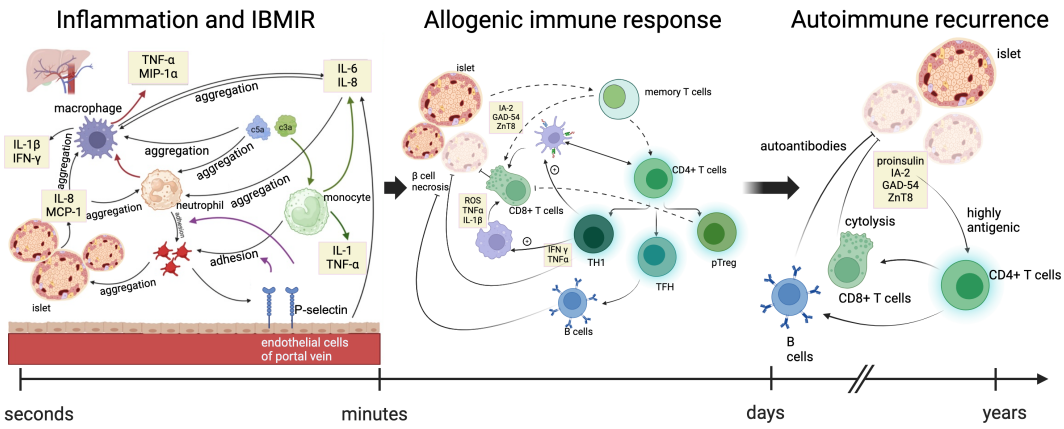

FIGURE 1
Immune mechanisms at three stages of allogeneic islet transplantation. The immune responses occurring during allogeneic islet transplantation included three stages: inflammation and IBMIR, allogeneic immune response, and autoimmune recurrence. In the early stages of transplantation, islets secrete pro-inflammatory factors and activate the complement system, promoting the recruitment of platelets, neutrophils, and monocyte macrophages to the graft. Vascular endothelial cells secrete cytokines and release P-selectin, promoting the adhesion between monocytes/neutrophils and platelets. Accumulated monocyte macrophages and neutrophils further enhance the recruitment of macrophages and the secretion of cytokines. Green arrows indicate the process by which the complement system promotes cytokine secretion by monocytes. Red arrows indicate the process by which neutrophils enhance cytokine secretion by macrophages. Purple arrows represent the process by which P-selectin promotes the adhesion of monocytes and neutrophils to platelets. Within days after transplantation, the release of inflammatory signals leads to increased cytokine production, with neutrophils signaling macrophages and dendritic cells to the site of islet phagocytosis, presenting antigens on their surface and recruiting adaptive immune cells. The infiltration of helper and cytotoxic T cells further damages the islets, recruiting B cells that produce antibodies against the allogeneic islets and differentiating T cells into memory T cells, ultimately leading to the rejection of the overall allogeneic transplant. TNFα, tumor necrosis factor alpha; MIP1α, macrophage inflammatory protein-1 alpha; IFN-γ, interferon; MCP-1, monocyte chemoattractant protein-1; GAD65, glutamic acid decarboxylase 65; IA-2, insulinoma-associated protein 2; ZnT8, transporter 8. ROS, reactive oxygen species; TH1, T helper 1 cells; TFH, follicular helper T cells; pTreg, peripheral regulatory T cells.

μmol/l of Melaglavin, the integrity of islets exposed to blood was lost. However, at concentrations of 1-10 μmol/l, Melaglavin inhibited coagulation and complement activation, leading to reduced platelet and leukocyte activation and consumption. This protective effect indicates that thrombin plays a crucial role in IBMIR and suggests that thrombin inhibition could improve the outcomes of clinical islet transplantation (26). L. Moberg et al. perfused human islets with fresh ABO-compatible blood for 30 minutes. In control samples (containing either only islets or blood with non-inhibitory anti-TF [4503]), coagulation occurred within 15 minutes. However, blood containing inhibitory anti-TF [4509] inhibited coagulation throughout the observation period. The study found that IBMIR is initiated by TF and consistently occurs during clinical islet transplantation, even in the absence of clinical symptoms like portal vein thrombosis. Inhibiting this process may increase the success rate of clinical islet transplantation and reduce the number of donors required per patient (18).

IBMIR, characteristic of innate inflammatory responses and thrombotic pathway, is driven by the activation of the coagulation cascade, with negatively charged islet surfaces activating the intrinsic coagulation pathway, and tissue factor (TF) expressed by the islets triggering the extrinsic pathway.

The innate immune system is the body's rapid response to an initial infection or injury. In IBMIR, the following components are mainly involved: Neutrophils: They are the first cells to arrive at the transplant site, release inflammatory mediators and oxygen free radicals, mediate local tissue damage and remove pathogens. Monocytes and macrophages: Monocytes are recruited and converted into macrophages, which further release cytokines (such as TNF-α and IL-1) that intensify the inflammatory response and enhance recruitment of immune cells. Cytokines released by neutrophils and macrophages in IBMIR not only promote local inflammatory responses, but may also affect T cell activation and subsequent adaptive immune responses (27).The activation of innate immune cells can lead to apoptosis or necrosis of the transplanted islet cells, thus reducing the survival rate of the grafts. The inflammatory response triggered by IBMIR may cause more immune cells to aggregate, forming positive feedback and further aggravating the damage. There is a close interaction between IBMIR and congenital leukocyte response, which together affect the success rate of islet transplantation.

2.2 Allogeneic immune response

The allogeneic immune response, which is adaptive immunity, occurs later but leads to long-term functional reduction of β-cells, resulting in a significant portion of islets losing their insulin independence. Analysis of pancreatic sections from T1D patients reveals significant immune infiltration within individual islets, confirming the crucial role of CD4 and CD8 T cells in β-cell destruction (14, 28). Despite high levels of systemic inflammation markers in T2D patients, their islets do not exhibit similar T cell infiltration, in stark contrast to the pancreatic sections of T1D patients, making islet autoantibodies a differential diagnostic marker between T1D and T2D (4). The presence or development of alloreactivity (against human leukocyte antigens, HLA) and its impact on allogeneic graft survival is well-defined in the solid organ transplantation literature. Donor-specific antibodies (DSA) binding to endothelial cells or islets (which constitutively express Class I HLA and aberrantly upregulate Class II HLA) can activate the classical complement pathway. Even in the absence of complement, some DSAs can promote antibody-dependent cellular cytotoxicity, where innate immune cells bind to Fc fragments, triggering the release of cytolytic enzymes by neutrophils and NK cells. C4d is a degradation product of the classical complement pathway, covalently bonded to the endothelium, serving as a marker for antibody-mediated immunity (4). Transplanting allogeneic islets or pancreas to T1D recipients expressing major and minor histocompatibility antigens on endogenous islets and pancreas can elicit complex adaptive B cell and T cell responses, leading to classical allogeneic graft rejection.

Key effector immune cells include cytotoxic T cells ($CD8^+$ T cells), macrophages, plasma cells, and $CD4^+$ T helper cells. In human T1D, existing evidence from single-islet studies from the Network for Pancreatic Organ Donors with Diabetes suggests that β-cell destruction is largely mediated by direct contact between CD8 T cells and β-cells, as well as CD4 T cell-mediated M1 macrophage polarization (29–31).

CD8+ T cells eliminate cells presenting non-self antigens by inducing apoptosis through the release of cytotoxic molecules (such as granzymes and perforin) or through cell-surface interactions (such as the binding of Fas ligand (also known as CD95L) on T cells to Fas receptors on the target cells) (32). Activated CD8+ T cells infiltrating the graft also induce macrophage activation, particularly through the expression of pro-inflammatory cytokines such as IFN-γ (33).

Macrophages typically exhibit pro-inflammatory characteristics and display M1 polarization during acute rejection, producing pro-inflammatory cytokines that lead to direct cellular damage and coordinate pro-inflammatory immune responses (34). Their primary function is phagocytosis, recognizing damaged allogeneic graft tissue through pattern recognition receptors such as Toll-like receptors. As antigen-presenting cells, macrophages can present allogeneic antigens on MHC class II molecules, thereby promoting the adaptive immune response (35).

Plasma cells are another type of effector immune cell derived from B cells and form the cornerstone of humoral immunity. They enable the body to combat foreign invaders not only by neutralizing pathogens but also by performing various effector functions, including regulating hypersensitivity reactions, activating the complement cascade, and modulating the mucosal microbiome. However, their activity can be problematic in solid organ transplantation (36). In transplantation, plasma cells can produce donor-specific antibodies (DSAs), which lead to acute and chronic rejection by activating the complement system, resulting in vascular injury and graft loss. The impact of DSAs has been extensively evaluated in various solid organ transplants (37–39).

$CD4^+$ T helper cells play a critical role in immune rejection. They coordinate the activation of other immune cells, such as B cells and cytotoxic T cells, to enhance the immune response against allogeneic material. These $CD4^+$ T cells are capable of producing and releasing various cytokines, including interferon-gamma (IFN-

γ) and interleukin-2 (IL-2). Additionally, $CD4^+$ T cells actively interact with B cells, promoting antibody production and thereby strengthening humoral immunity (40, 41). CD4 T cells can provide "help" to B cells and stimulate antibody production (as described above), as well as promote effector CD8 T cell responses and stimulate resident macrophages in the islets (42, 43).

Auto-reactive CD4 T cells interact with dendritic cells presenting islet antigens (44) and can differentiate into T helper 1 (TH1) cells, follicular helper T cells (TFH), peripheral regulatory T cells (pTreg), or anergic cells. TFH cells help B cells produce high-affinity islet-specific antibodies (29). TH1 cells activate dendritic cells and enhance antigen presentation to islet-specific CD8 T cells (45), thereby inducing the proliferation of effector CD8 T cells (45). TH1 cells migrate to the pancreas (46), secrete pro-inflammatory cytokines interferon-γ (IFNγ) and TNFα, and induce β-cell death (47). TH1-derived IFNγ and TNFα stimulate M1 macrophages in the islets to produce reactive oxygen species (ROS), TNFα, and IL-1β (48), further amplifying the cycle of β-cell death (30)The resulting inflammation leads to increased infiltration of CD8 T cells, which directly kill β-cells via perforin and granzyme B (49), while natural and peripheral regulatory T cells (nTreg and pTreg) attempt to suppress this response through TGFβ and IL-10 (50).

2.3 Autoimmune recurrence

Patients with T1D and concurrent autoantibodies have a lower success rate for islet transplantation due to the presence of memory CD4+ and CD8+ T cells, which rapidly reactivate to target islet antigens (IA-2, GAD-54, and ZnT8) and destroy the transplanted islets (42, 43). Patients with T1D who have long-term β-cell transplants still have the ability to destroy islets. Reviewing the case of David Sutherland's identical twin transplant surgery, where the pancreas of an unaffected twin was transplanted into the twin with long-term T1D without immunosuppression, resulted in the loss of transplanted β-cell function and pancreatitis (51, 52). This is because most individuals' immune systems develop the ability to distinguish self from non-self. In T1D, the loss of the ability to recognize insulin-producing islet β-cells as self leads to an autoimmune response, which destroys β-cells in the natural pancreas (53, 54). This autoimmune response is primarily mediated by T cells, which are the main effector cells in the β-cell destruction process. Moreover, there is ample evidence that isolated allogeneic islet transplants may cause autoimmune recurrence in a small but significant proportion of patients. In the autoimmune process, when islets or pancreas are transplanted into recipients with T1D, donor β-cells express β-cell-specific antigens that are attacked by T cells and B cells (55–58). These include insulin (proinsulin), glutamic acid decarboxylase 65 (GAD65), insulinoma-associated protein 2 (IA2), and zinc transporter 8 (ZnT8), which are highly antigenic to both B cells and T cells in humans (59). This explains why islet autoantibodies sometimes rise sharply within weeks after transplantation. This increase usually occurs without any signs of allogeneic immunity (60). Therefore, transplanting islets or pancreas into T1D recipients is a renewed challenge to the autoreactive memory response and may lead to the recurrence of autoimmune function post-transplant.

In summary, when allogeneic islets are transplanted into T1D patients, a comprehensive immune response is elicited against the foreign tissue. Besides the classic rejection of the allogeneic graft, the outcomes of islet or pancreatic transplantation may be severely impacted by early intense inflammatory responses and the reactivation of autoimmunity. In simple terms, the three stages of immune response experienced are inflammation and IBMIR, allogeneic immune response, and autoimmune recurrence. Within days after transplantation, the release of inflammatory signals leads to increased cytokine production, with neutrophils signaling macrophages and dendritic cells to the site of islet phagocytosis, presenting antigens on their surface and recruiting adaptive immune cells. The infiltration of helper and cytotoxic T cells further damages the islets, recruiting B cells that produce antibodies against the allogeneic islets and differentiating T cells into memory T cells, ultimately leading to the rejection of the overall allogeneic transplant. The entire process of allogeneic transplant rejection may be amplified in T1D patients because they have effectively primed T cells specifically targeting β-cells.

3 Immunosuppressants in allogeneic islet transplantation

A major issue in islet transplantation is transplant rejection. To prevent this complication, immunosuppressive drugs such as cyclosporine, tacrolimus, mycophenolate mofetil, and corticosteroids must be used (61). However, immunosuppressants have severe side effects, including inducing diabetes, nephrotoxicity, and carcinogenic effects (62–65).

3.1 Calcineurin inhibitors

There are many types of calcineurin inhibitors (CNIs), such as the commonly used cyclosporine and rapamycin. The potent immunosuppressive properties of cyclosporine were discovered in 1976. Cyclosporine blocks the clonal expansion of resting T cells by inhibiting the transcription of genes encoding IL-2 and the high-affinity IL-2 receptor, which is crucial for T cell activation (66).

Tacrolimus (FK506) was the first macrolide antibiotic explored for its effective immunosuppressive properties in 1987 (67, 68). The mechanism of toxicity of tacrolimus is as follows: tacrolimus binds to the immunophilin FK506-binding protein 12 (FKBP12) to form a complex that binds and inhibits the mammalian target of rapamycin (mTOR) kinase, thereby exerting immunosuppressive activity (69, 70). This kinase is a key regulator of cell metabolism, growth, and proliferation. Importantly, inhibition of mTOR by tacrolimus causes cell cycle arrest in the mid-to-late G1 phase, thus potentially inhibiting tumor cell growth and, importantly, its immunosuppressive function by inhibiting T cell and B cell proliferation (71). However, FKBP12 and mTOR are ubiquitously expressed. Therefore, there is a possibility of "off-target" effects on cells other than tumor and immune regulatory cells.

mTOR kinase exists in two distinct complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). They have different substrates and are regulated differently (Figure 2). Although they share some core components, such as mTOR, mLST8, and DEPTOR, they also contain other unique proteins. For example, a unique component of mTORC1 is RAPTOR (regulatory associated protein of mTOR), which acts as a bridge to bind mTOR to its downstream effectors (72, 73). An important component of mTORC2 is the protein Rictor (rapamycin-insensitive companion of mTOR), which is necessary for the formation of the mTORC2 complex and its kinase activity (74, 75). Importantly, mTORC1 is highly sensitive to inhibition by rapamycin, whereas mTORC2 was initially thought to be resistant to rapamycin (74, 75), but in fact, it is sensitive to long-term rapamycin treatment in some cell types (76–78). Therefore, both complexes may play a role in the immunosuppressive and toxic effects of rapamycin. Consistent with its role as a key regulator of cell metabolism, proliferation, and growth, mTORC1 activity is regulated by nutrients, growth factors, and cellular energy levels (Figure 2). The best-characterized targets of mTORC1 are eIF4E-binding protein (4E-BP) and S6 kinase protein (S6K), both of which play important roles in the regulation of protein synthesis.

The role of mTORC1 in B cell function is as follows. An important aspect of maintaining glucose homeostasis is the maintenance of pancreatic B cell mass and the ability of B cell mass to increase in insulin-resistant states such as obesity. The increase in B cell mass is due to increased neogenesis (progenitor cell generation) and proliferation (hyperplasia), hypertrophy, and reduced apoptosis. There is substantial evidence indicating that rapamycin significantly reduces the proliferation of B cells and progenitor cells, thereby affecting the maintenance of B cell mass. The most compelling evidence for the role of mTORC1 in regulating B cell mass comes from *in vivo* transgenic mouse models (79). Overactivation of mTORC1 by selectively overexpressing Rheb (80) or deleting TSC1 (81) or TSC2 (81, 82) in B cells leads to increased B cell size and mass, along with improved insulin secretion and glucose tolerance. These effects may be partially mediated by S6K, as mice lacking S6K1 or rpS6 exhibit hypoinsulinemia and glucose intolerance with reduced B cell size (83, 84). Additionally, transgenic mice overexpressing

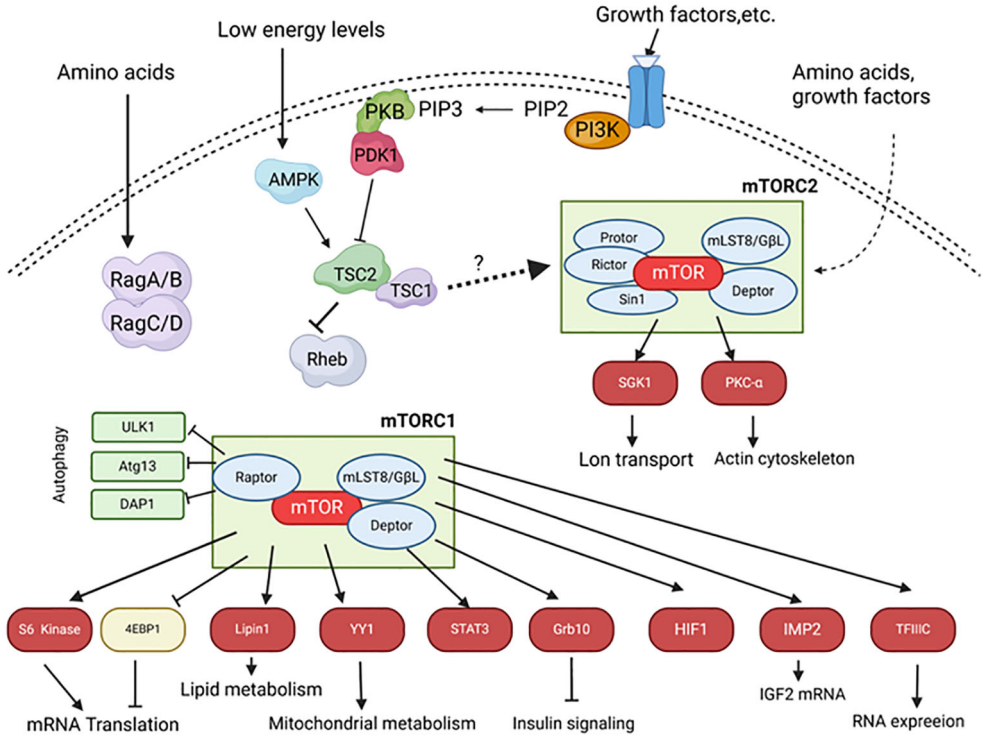

FIGURE 2
The mTOR signaling pathway in islets. Upon stimulation by insulin and other growth factors, phosphoinositide 3-kinase (PI3K) converts phosphatidylinositol 4,5-bisphosphate (PIP2) into phosphatidylinositol 3,4,5-trisphosphate (PIP3), which localizes PKB to the membrane and activates it through PDK1 and mTORC2. Activated PKB phosphorylates and inhibits TSC1/2. Rheb, a small GTPase inhibited by TSC2, positively regulates mTORC1 activity. mTORC1 phosphorylates S6 kinase 1/2 and 4EBP1, leading to increased mRNA translation. Amino acids activate mTORC1 through Rag A/B and C/D. Under low energy conditions, the ratio of AMP to ATP increases, activating AMP-activated kinase (AMPK), which phosphorylates and activates the TSC1/2 complex, thereby inhibiting mTORC1. mTORC2 activity is primarily mediated through unknown pathways. mTORC2 phosphorylates and activates PKB, serum- and glucocorticoid-induced kinase 1 (SGK1), and PKC. Arrows indicate stimulatory effects; block ends indicate inhibitory effects; solid lines represent direct effects, and dashed lines represent indirect effects. Atg13, Autophagy-related protein 13; DAP1, Death-associated protein 1; Deptor, DEP domain-containing mTOR-interacting protein; 4EBP, eIF4E-binding protein; GbL-G, Protein Gβ-like; HIF1, Hypoxia-inducible factor 1; IMP2, Insulin-like growth factor 2 mRNA-binding protein; mLST8, Mammalian lethal with Sec13 protein 8; PDK1, Phosphoinositide-dependent protein kinase 1; Protor, Protein observed with Rictor; Raptor, Regulatory associated protein of mTOR; Rictor, Rapamycin-insensitive companion of mTOR; Sin1, Stress-activated protein kinase-interacting protein 1; TFIIIC, Transcription factor 3C; ULK1, Unc-51 like kinase 1.

constitutively active S6K exhibit improved glucose tolerance and enhanced insulin secretion with increased B cell size (85). Although these studies strongly suggest the critical role of mTORC1, manipulation of mTOR upstream regulators (e.g., Rheb) may affect pathways beyond mTORC1, so causality cannot be definitively established. There is extensive work investigating the role of mTOR in regulating cell proliferation in certain cell types, but little is known about the exact mechanisms by which mTORC1 signaling regulates B cell cycle progression. However, it is known that mTORC1 can regulate the synthesis and stability of cyclins D2 and D3 in B cells (86). These cyclins form complexes with cyclin-dependent kinase 4, controlling cell cycle progression. In rat islets treated with rapamycin, reduced levels of cyclins D1 and D2 were observed, accompanied by decreased β-cell proliferation (87).

mTORC1 also appears to play a role in insulin secretion by pancreatic B cells. Knockdown of TSC1 in mice results in significantly increased insulin production, independent of B cell mass (81). Additionally, long-term rapamycin treatment inhibits glucose-stimulated insulin secretion (GSIS) in cloned B cell lines as well as rodent and human islets. However, it is unclear whether this effect is mediated by mTORC1 or mTORC2. The control of insulin secretion in B cells involves many complex signaling pathways, and the mechanism by which rapamycin regulates insulin secretion remains unknown. One proposed mechanism is that inhibition of mTORC1 reduces mitochondrial function, particularly the activity of α-ketoglutarate dehydrogenase. This leads to reduced carbohydrate metabolism, thereby decreasing mitochondrial ATP production (88), which is known to regulate insulin secretion in B cells (89). Another explanation is that rapamycin promotes autophagy, a process primarily controlled by mTORC1 rather than mTORC2, or intracellular degradation of cytoplasmic proteins involved in insulin production, leading to inhibition of insulin secretion (89).

It is not completely clear how the activity of mTORC2 is regulated, but there is evidence that it can be stimulated by amino acids and growth factors (90, 91). Downstream targets of mTORC2 include protein kinase C (PKC)-α (85–87) and protein kinase B (PKB) (92), two serine/threonine kinases that play roles in the regulation of key cellular processes such as apoptosis, proliferation, motility, and differentiation, as well as serum- and glucocorticoid-induced kinase 1 (93), which plays a role in the control of ion transport (94) (Figure 2).

In mice, B cell-specific deletion of the Rictor gene (an important component of mTORC2) is associated with reduced plasma insulin levels due to decreased insulin secretion from islets, leading to hyperglycemia (95). This is related to reduced B cell mass and proliferation but does not increase B cell apoptosis. Research by Adam D. Barlow et al. has demonstrated that knocking down Rictor in rat islets using small interfering RNA results in increased B cell apoptosis and reduced GSIS (76). These studies specifically demonstrate that mTORC2 activity plays a dominant role in B cell survival and function. Importantly, prolonged rapamycin treatment (24 hours) of MIN6 cells, rat islets, or human islets leads to dissociation of mTORC2, thereby inhibiting its expression. This precedes the toxic effects of rapamycin on function and activity, occurring simultaneously with reduced PKB phosphorylation and downstream signaling. Interestingly, the expression of constitutively active PKB in MIN6 cells and rat islets can mitigate the harmful effects of rapamycin on GSIS and cell viability (76). Overall, this suggests that rapamycin B cell toxicity is primarily mediated through inhibition of mTORC2 and its subsequent impact on PKB signaling. However, this is based on *in vitro* experiments with B cells and needs to be further confirmed *in vivo*.

Extensive research indicates that PKB, as a key downstream effector of mTORC2, plays an important role in B cell survival and function. These studies further reveal the potential role of mTORC2 in B cell homeostasis. For instance, transgenic mice expressing constitutively active PKB in B cells show a significant increase in B cell mass due to increased B cell number and size (96, 97). This is manifested by significantly elevated plasma insulin levels, improved glucose tolerance, and resistance to streptozotocin-induced diabetes. In INS-1 cells, rat B cell lines, and primary rat B cells, expression of constitutively active PKB has also been shown to protect against lipotoxicity (98), cytokine-induced cytotoxicity (99), and AMPK-mediated cytotoxicity (100). Conversely, studies in transgenic mice lacking PKB show significantly elevated blood glucose levels, reduced insulin levels, and impaired glucose tolerance.

Rapamycin is a key immunosuppressant, particularly in islet cell and kidney transplantation. However, extensive *in vitro* and *in vivo* evidence strongly suggests that rapamycin has harmful effects on pancreatic B cells and peripheral insulin sensitivity. This toxicity is mainly because rapamycin inhibits mTOR, which is part of complex signaling pathways controlling many important cellular functions (including mRNA translation, cell proliferation, cell growth, differentiation, protein synthesis, angiogenesis, and apoptosis) through mTORC1 and mTORC2 (71). In summary, rapamycin-induced B cell toxicity and insulin resistance are likely mediated primarily through mTORC2 rather than mTORC1 (76, 95).

In addition to the above mechanisms, although rapamycin is structurally unrelated to cyclosporine, it shares many intracellular pathways that inhibit calcineurin and subsequently block IL-2 production. It acts by limiting the dephosphorylation and translocation of nuclear factor of activated T cells (NFAT). NFATs play a critical role in T cell activation. When T cells are stimulated by antigens, intracellular calcium levels rise rapidly, activating calcineurin. Activated calcineurin dephosphorylates NFATs, exposing nuclear localization signals and causing NFATs to translocate from the cytoplasm to the nucleus. In the nucleus, NFATs bind to specific DNA sequences, regulating the transcription of related genes and participating in T cell proliferation, differentiation, and cytokine production. Calcineurin signaling is essential for insulin secretion and β-cell proliferation (101), and specific inactivation of calcineurin in β-cells is associated with age-related hyperglycemia (102). Apoptosis of islet cells related to calcineurin inhibition is also thought to occur through the limitation of cAMP response element-binding protein (CREB), which reduces the expression of insulin receptor substrate-2 (IRS-2), limits Akt phosphorylation, and affects insulin secretion (103, 104). CNIs also reduce the expression of cell surface glucose transporter 4 (GLUT4) and decrease insulin-stimulated glucose uptake in adipocytes (105), potentially leading to peripheral insulin resistance.

Moreover, rapamycin promotes reduced mitochondrial Ca2+ uptake, which has been shown to impair respiration and ATP production, leading to impaired glucose-stimulated insulin secretion (GSIS) (106). CNIs, particularly rapamycin, enhance the deleterious effects of glucolipotoxicity on β-cells, inducing the expression of forkhead box protein O1 (FoxO1), thereby limiting proliferation (107), and reducing insulin content and secretion (108). Rapamycin causes reversible graft dysfunction, characterized by amyloid deposition and macrophage infiltration in transplanted islets (101), with no clear evidence of β-cell death. Ultrastructural examination of the grafts shows reduced insulin granules, accompanied by increased transcripts associated with extracellular matrix deposition and inflammation. Heparin is primarily used to reduce IBMIR-mediated cell destruction of islets, promoting the fibrillation of human islet amyloid polypeptide (IAPP) and has been shown to simultaneously promote amyloid deposition and reduce β-cell apoptosis (109). Rapamycin exerts its antifibrotic function by inhibiting JAK2/STAT3 signaling activation through targeting JAK2, thereby inhibiting M2 macrophage polarization (110). After transplanting human islets into NSG mice, rapamycin inhibits β-cell function by activating islet-resident macrophages through inhibition of the NFAT pathway and by stimulating macrophages to produce IL-1β through increased amyloid deposition in the transplanted islets (111). Heparinase treatment significantly reduces amyloid deposition and subsequent β-cell toxicity (112).

As two types of CNIs, cyclosporine and rapamycin have similar mechanisms of action, but rapamycin has been shown to be 10-100 times more potent than cyclosporine in inhibiting mixed lymphocyte cultures and the generation of cytotoxic T cells *in vitro* (66).

3.2 Mycophenolate Mofetil

In 1993, Mycophenolate Mofetil (MMF), the 2-4 morpholinoethyl ester of the biologically active compound mycophenolic acid, was introduced as a new immunosuppressant (113). MMF reversibly inhibits inosine monophosphate dehydrogenase (IMPDH), a key enzyme in the *de novo* synthesis of the purine nucleotides in DNA (i.e., guanine and adenine) (114). Lymphocytes play a crucial role in graft rejection, and without IMPDH, they cannot produce sufficient amounts of purines (115). Consequently, MMF can prevent the proliferation of T cells and B cells, thereby inhibiting antibody production. Additionally, by lowering intracellular GTP levels in lymphocytes, MMF inhibits glycosylation and the expression of certain adhesion molecules, thus reducing lymphocyte migration to the graft (116). However, its effect on T cell proliferation has garnered more attention due to the critical role of T cells in the allogeneic response (117). MMF is considered a safe drug, with the most commonly reported side effects being mild and primarily involving the gastrointestinal system (diarrhea, abdominal pain, nausea, and vomiting) (118–120). Its main advantage is the lack of nephrotoxicity and diabetogenic effects, making MMF an important drug in kidney and islet transplantation.

3.3 Glucocorticoids

T1D is secondary to the initial autoimmunity of islets, resulting from the inflammatory destruction of β-cells (74). Inflammatory macrophages are key in maintaining islet injury (121). Pro-inflammatory cytokines, partly derived from macrophages and damaged β-cells, further inhibit β-cell function by inducing nitric oxide production (122, 123). As T1D progresses, pro-inflammatory cytokines inhibit β-cell regeneration, stimulate peripheral insulin resistance, and maintain insulin inflammation (124). Glucocorticoids (GC) are used clinically for their powerful anti-inflammatory and immunosuppressive effects (125), but high doses of glucocorticoids promote peripheral insulin resistance and inhibit β-cell function (62, 63, 126), thus discouraging their use in T1D treatment and transplantation protocols (12). However, the general notion that GC's effects on β-cells are purely harmful has been increasingly challenged (127–131). It has now been demonstrated that selective GC regeneration within β-cells can prevent inflammatory β-cell destruction, suggesting that GC-targeting therapy with 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) may improve the course of T1D and islet transplantation aggravated by high-dose hormones.

3.4 Bortezomib

In addition to the health risks posed by infections and cancer due to broad immunosuppression, numerous studies report that widely used immunosuppressants such as glucocorticoids or calcineurin inhibitors are cytotoxic to islet β-cells (71, 132). Thus, there has been a need to develop tolerance-promoting regimens that can retain the viability and function of islets post-transplantation. Bortezomib, a selective inhibitor of the 26S proteasome, has been FDA-approved for treating relapsed multiple myeloma (133, 134). Bortezomib's mechanism of action involves inhibiting the proteasomal degradation of IκB, thereby inhibiting the activation of nuclear factor κB (NF-κB) (135, 136). Since NF-κB is a key transcription factor involved in the expression of various genes related to immune responses, numerous studies have demonstrated the immunosuppressive effects of bortezomib. It selectively depletes alloreactive T cells *in vitro* and reduces the secretion of T helper 1 (Th1) cell cytokines (137). Additionally, bortezomib can modulate the function of dendritic cells (DCs): treatment with bortezomib induces a skewed phenotypic maturation of DCs in response to lipopolysaccharides (LPS) and other endogenous stimuli while reducing cytokine production (138). Other studies have also reported that bortezomib can prevent graft-versus-host disease (GVHD) and allograft rejection in mouse models of allogeneic stem cell and cardiac transplantation (139, 140). Furthermore, bortezomib can inhibit the activation of rapamycin-resistant memory T cells without affecting the viability of regulatory T cells (Tregs) in non-human primates (141). Overall, these immunomodulatory effects suggest that bortezomib has the potential to be a promising immunosuppressant candidate in islet transplantation.

So-Hee Hong et al. (142) conducted a related study using BALB/c spleen cells to pre-sensitize C57 BL/6 mice, administering low-dose bortezomib (0.1 mg/kg) for 4 consecutive days to observe its immunosuppressive effects *in vivo*. Since NF-κB is the primary transcription factor for DC maturation, DC maturation status was detected by measuring the expression levels of MHC class II molecules and other co-stimulatory molecules in CD11c+ DCs. The conclusions suggested that low-dose bortezomib only reduced the expression of MHC class II molecules without affecting other co-stimulatory molecules expressed on DCs. Unlike other studies showing bortezomib's inhibitory effect on alloreactive T cells with high-dose treatment, short-term low-dose bortezomib treatment did not significantly affect the percentage of splenic effector memory cells (CD4+CD44 and CD8+CD44) and the number of T cells producing allogeneic antigen-specific interferon-γ. Based on these results, it was speculated that low-dose bortezomib might inhibit DCs *in vivo* by altering their MHC class II expression.

Additionally, some studies suggest that high-dose rapamycin treatment impairs β-cell regeneration and reduces islet engraftment, adversely affecting islet transplantation (143, 144). Therefore, So-Hee Hong et al. (142) developed a new combination therapy based on low-dose bortezomib and rapamycin, which is highly tolerable and minimally cytotoxic to β-cells, as a potential alternative and tolerance-promoting immunosuppressive regimen in allogeneic islet transplantation. They tested the efficacy of low-dose bortezomib alone or in combination with rapamycin in an islet transplantation model. Low-dose (0.1 mg/kg) bortezomib treatment groups showed longer graft survival rates compared to control groups (0.05 mg/kg group: P=0.1, 0.1 mg/kg group: P=0.0036). Low-dose (1 mg/kg) rapamycin was added to the same transplantation environment. Compared to the control group, the 0.05 mg/kg bortezomib + rapamycin group (P=0.0011) and the 0.1 mg/kg bortezomib + rapamycin group showed significantly prolonged islet graft survival (P=0.001). Although not statistically significant, the combination of rapamycin and bortezomib increased graft survival compared to the bortezomib-only treatment group. In the 0.1 mg/kg bortezomib plus rapamycin treatment group, 4 out of 6 mice maintained normoglycemia for 100 days, while 2 out of 6 mice in the 0.1 mg/kg bortezomib-only treatment group maintained normoglycemia for 100 days. Additionally, the mean graft survival period increased from 24 days to 58 days after adding rapamycin to the 0.05 mg/kg bortezomib treatment group. To determine whether low-dose bortezomib + rapamycin treatment induces immune tolerance, grafts were removed from recipient mice that maintained normoglycemia for over 100 days, and a second graft (islets from BALB/c donors) was transplanted into the contralateral kidney. Interestingly, mice with the second graft maintained normoglycemia for 50 days without any immunosuppression. To determine whether this tolerance was systemic, BALB/c and C3H (third-party) as well as C57 BL/6 (control) skin grafts were transplanted into the flank of the second transplant recipients. The C3H skin grafts were rejected on day 14 post-transplant (DPT). The rejection of BALB/c skin grafts was somewhat delayed but ultimately rejected on DPT 18. Unexpectedly, the rejection of BALB/c skin appeared to result in the rejection of the second islet graft, as blood glucose levels returned to hyperglycemia 20 days after the skin rejection reaction. Thus, it was concluded that this combination therapy induced tolerance to islet-specific antigens, and its inhibitory effect was insufficient to prevent strong skin graft rejection. Many studies have shown that Th1 cells are major participants in graft rejection in various transplant models, with interferon-γ playing a key role by activating cytotoxic CD8+ T cells (145, 146). So-Hee Hong et al. investigated whether bortezomib alone or in combination with rapamycin could reduce Th1 and interferon-γ-producing cells. Splenocytes from allogeneic islet transplant mice that maintained normoglycemia for over 60 days were stimulated *in vitro* with irradiated BALB/c splenocytes, followed by ELISPOT analysis. The combination therapy group showed almost no detectable interferon-γ-producing cells. Although a reduction in interferon-γ-producing cells was also observed in the bortezomib-only group, it was not as pronounced as in the combination therapy group. Moreover, no significant changes were observed in other cytokine-producing cells in the combination therapy group. MLR assays were used to detect BALB/c-specific T cell responses in recipient mice treated with bortezomib + rapamycin. The results indicated that the mice's T cells had a lower proliferative response to BALB/c antigens but not to third-party C3H antigens. Therefore, these results suggest that low-dose, short-term combination therapy with bortezomib and rapamycin significantly increases graft survival and induces tolerance to islet antigens while inducing severe BALB/c-specific T cell hyporesponsiveness, increased Tregs, and reduced inflammatory cytokines (142).

4 Xenotransplantation

The increasing number of patients in need of organ transplants has made xenotransplantation of islets a potential future treatment option for diabetic patients due to the shortage of organ donors. According to recent advances in preclinical studies on non-human primates, porcine islets may be the ideal choice among various animal organs and tissues for xenotransplantation (147), mainly due to the biochemical compatibility of porcine and human insulin and the potential to obtain a large number of donor pigs through relatively short turnover breeding strategies. Additionally, another theoretical advantage of porcine islets is their potential resistance to autoimmune recurrence against human β-cells (148). The main barrier to interspecies transplantation is the preformed xenogeneic antibodies that cause hyperacute rejection. Hyperacute rejection (HAR) is a rapidly occurring rejection in islet transplantation and other organ transplants, usually occurring within minutes to hours after transplantation. This condition arises from the interaction between pre-existing antibodies from humans or non-human primates (NHP) and the antigens present in the graft (149).Among these antibodies, the most common are IgMs and IgGs that identify galactose-α1,3-galactose (α-Gal) residues, which are attached to glycoproteins and glycolipids by the α1,3 galactosyltransferase (α1,3GT) found in non-primate genomes. Humans and apes do not have α-Gal epitopes (150). Furthermore, approximately 70–90% of these antibodies

specifically target α-Gal epitopes (151). As a result, when an organ from a pig is transplanted into a human or a non-human primate (NHP), the existing anti-Gal antibodies attach to the α-Gal epitopes found on the graft's vascular endothelium. This interaction triggers the production of complement component 3b (C3b), activates the complement system (152), and leads to the formation of a membrane attacking complex (MAC).These responses result in the lysis of endothelial cells, damage to the vasculature, and ultimately, rejection of the graft (153, 154). Additionally, the disruption of endothelial vascular integrity leads to interstitial hemorrhage, tissue ischemia, and necrosis (155, 156). Additionally, the failure of the graft is exacerbated by thrombotic occlusion of capillaries, fibrinoid necrosis in arterial walls, and the accumulation of neutrophils (157). The histopathological characteristics of hyperacute rejection (HAR) include compromised vascular integrity, edema, thrombi rich in fibrin and platelets, as well as interstitial hemorrhage accompanied by extensive deposition of immunoglobulins and terminal complement products on the walls of vessels (157). In order to reduce the occurrence of hyperacute rejection, the following measures can be taken: first, immunosuppressants mentioned in this paper are the main measures; knocking out the a1,3GT gene in pigs (GTKO pigs) (158).With the identification of carbohydrate xenoantigens (159) and advances in genetic engineering, it is possible to eliminate these xenoantigens (160) to prevent hyperacute rejection. However, T-cell-mediated xenogeneic immune responses are very intense and more challenging to control compared to immune responses against allogeneic antigens (161).

The xenogeneic T-cell response to porcine islets can be triggered through both direct and indirect antigen presentation (162). Once activated, T cells can mediate graft destruction through direct cytotoxicity (163) or by differentiating into cytokine-producing helper T cells that assist B cells in class switching and antibody production, or by activating innate cells such as macrophages and NK cells involved in xenotransplant rejection (164, 165). Th1 and Th2 cytokines, such as IFN-γ and IL-4, play significant roles in this process (166–168). It has been experimentally demonstrated that the infusion of carbodiimide-fixed donor splenocytes (ECDI-SP) can exert effective immunoregulatory effects through the silent clearance of apoptotic cells, effectively inducing donor-specific tolerance (169–172).

4.1 Early acute inflammatory response

Islet xenotransplantation represents a promising therapeutic alternative for treating type 1 diabetes. However, shortly after transplanting donor islets into the recipient, a robust innate immune response is triggered, including an IBMIR, which adversely affects the functionality of the islet transplant (153).

IBMIR is triggered by the xenogeneic contact between blood and islets, involving the activation of coagulation and complement systems, as well as complex interactions between leukocytes and platelets, which significantly impact the function and survival of xenografts, thereby adversely affecting the outcomes of islet xenotransplantation (17, 173) (Figure 3). Therefore, the following section explains the mechanisms of IBMIR components.

Research for IBMIR found that platelet-independent complement activation was observed 30 minutes after porcine islets were exposed to plasma, and the formation of membrane attack complexes could be observed in porcine islet tissue pathology sections 60 minutes later, with up to 40% of islets losing their function (174). Complement system activation occurs through three different pathways (known as the classical pathway, lectin pathway, and alternative pathway), depending on the nature of the initial trigger. Regardless of the activation pathway, all pathways converge at the cleavage of C3 by C3 convertase. C3 convertase cleaves the central component C3 into the anaphylatoxins C3a and C3b (175), with the primary function of C3b and its cleavage product iC3b being opsonization for phagocytosis. Additionally, iC3b can bind to complement receptors CR3 and CR4, leading to immune cell adhesion and activation (176, 177). Since complement activation is associated with the proteolytic cleavage of its components, proteases represent another "non-traditional" pathway of complement activation (178, 179).

The classical pathway (CP) is triggered by antigen-antibody complexes recognized by C1q. A major process in this pathway is the production of CP C3 convertase C4b2b, generated by the cleavage of C4 into C4a and C4b, followed by the splitting of C2 into C2a and C2b (180). Activation of the lectin pathway (LP) is initiated by the binding of mannose-binding lectin (MBL) or ficolins to pathogen surfaces, involving the participation of MBL-associated serine proteases MASP-1 and MASP-2, which is significantly similar to CP activation (181).

The spontaneous hydrolysis of C3 to C3(H2O) accounts for the constitutive and continuous low-level activation of the alternative pathway (AP) (182). The generated C3b assembles the APC3 convertase C3bBb together with factor B and factor D (183). The APC3 convertase complex is stabilized by the binding of properdin (184–186).

In all three pathways, the cleavage of C3 to produce C3b is a major component of C5 convertase, which cleaves C5 into the anaphylatoxins C5a and C5b (187). C5b participates in the formation of the membrane attack complex (MAC) by recruiting complement components C6, C7, C8, and C9, with the primary function of mediating the lysis of pathogens or target cells (188).

On the other hand, C3a and C5a anaphylatoxins, by interacting with G-protein-coupled C3a and C5a receptors, are highly effective chemoattractants, promoting the recruitment of inflammatory cells to sites of injury or infection. Furthermore, C3a and C5a can activate immune cells, upregulating the expression and release of inflammatory cytokines and mediators (175, 189).

The coagulation cascade is involved in both hemostasis and thrombosis (190). The tissue factor of the so-called extrinsic pathway is a core participant in coagulation (191), involved in the pathology of thrombosis, including cardiovascular diseases (192, 193) and biomaterial-related processes (194). Inflammatory stimuli or endothelial cell activation produce the extrinsic factor X complex composed of TF and activated coagulation factor VII (FVIIa) (195). The extrinsic factor X complex, in turn, promotes the activation of factor X (FX), which, together with activated FVa and Ca2+, forms

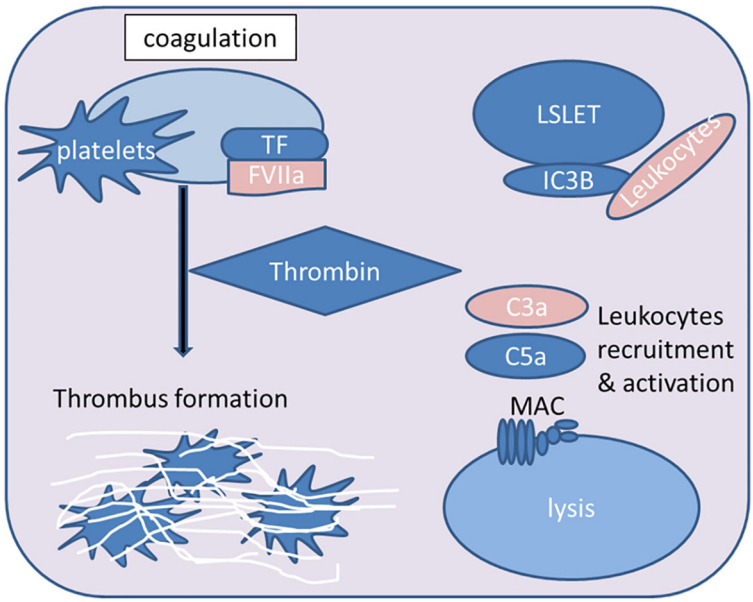

FIGURE 3
Overview of key steps in the IBMIR process during islet xenotransplantation. The contact between xenogeneic blood and islets triggers the activation of the extrinsic coagulation pathway mediated by tissue factor (TF). Consequently, downstream effector thrombin is produced, leading to fibrin deposition and thrombosis. The attachment of platelets to the islets further supports the pro-coagulant effect. Activated complement fragments (iC3b) deposit on the islet surface, and the anaphylatoxins C3a and C5a activate and attract leukocytes. The formation of the membrane attack complex (MAC) mediates islet lysis (FVIIa, activated coagulation factor VII; MAC, membrane attack complex).

the prothrombinase complex that mediates the conversion of prothrombin to thrombin (196). Thrombin can activate platelets, cleave prothrombin into thrombin, leading to the formation of insoluble thrombin (197).

Coagulation and thrombosis are involved in acute reactions to both allogeneic and xenogeneic islet transplantation (18) (198). Notably, exposure of human or porcine islets to human blood results in rapid activation of coagulation, evidenced by upregulated TF levels (199) and significant thrombin generation (26). Moreover, allogeneic islet transplantation is associated with thrombotic manifestations, such as fibrin deposition and the localization of transplanted islets within thrombi (198). Therefore, endogenous antithrombotic agents are significant as potential beneficial modulators of IBMIR. The fine-tuning of the coagulation cascade (200) is mediated by antithrombin III (ATIII), which inactivates thrombin, FXa, and FIXa (201); activated protein C (APC), which, along with protein S, blocks FVa and FVIIIa (202); tissue factor pathway inhibitor (TFPI); and thrombomodulin (TM). TFPI binds and inhibits FXa or the TF/FVIIa complex (203). TM's anticoagulant activity is mediated by binding to thrombin. The TM-thrombin complex further promotes the generation of APC (204). However, thrombin bound to TM can cleave and activate thrombin-activatable fibrinolysis inhibitor (TAFI) (205), conferring procoagulant properties by blocking fibrinolysis. In the context of xenogeneic islet transplantation, transgenic pigs overexpressing hemostasis-regulating molecules have been generated. For this purpose, the expression of hTFPI protected the xenografts, promoting the achievement of normoglycemia after xenotransplantation. Porcine TM has been shown to be a poor cofactor for human thrombin, resulting in the loss of its protective function and increased coagulation (206). Thus, transgenic overexpression of hTM in pigs can avoid the thrombotic manifestations observed after xenotransplantation of porcine islets (207).

The contact of host blood with transplanted islets rapidly triggers a series of thrombo-inflammatory responses, including upregulation of TF expression (199) and thrombin generation (26). Additionally, the induction of TAFI further propagates the procoagulant effect (208). Intravascular coagulation is induced (209), forming thrombi that capture the islets (198). Concurrently, activation of CP and AP of the complement system occurs, generating anaphylatoxins that lead to the recruitment of inflammatory cells to the graft. Moreover, active complement fragments deposit on the graft, promoting complement-dependent islet lysis (210). Platelets and leukocytes infiltrate the transplantation site and adhere to the islet surface (26, 211). Consequently, the integrity of the islet grafts is compromised, leading to substantial early loss of transplanted islets (212, 213). The acute destruction of a significant proportion of transplanted islets by IBMIR is the primary reason why a high number of islets are required for effective islet transplantation (214). Interestingly, the degree of islet damage increases with the decreasing compatibility between donor and recipient species. Therefore, in the case of xenogeneic islet transplantation, IBMIR becomes more relevant because the recipient cannot control IBMIR induced by xenogeneic islet transplantation due to incompatibility between regulators and effectors, respectively, for the IBMIR of xenografts and recipient cells (215). Furthermore, regulatory proteins are considerably lacking in porcine islet preparations (216). Thus, developing effective treatment regimens targeting the regulatory parameters of IBMIR is imperative (173) (Figure 3).

In further studies targeting IBMIR, Bennet et al. cultured isolated islets in whole blood in the presence of soluble CR1 (sCR1). They demonstrated that sCR1 treatment blocked complement activation associated with IBMIR and protected the islets from damage. Simultaneous inhibition with sCR1 and heparin eliminated the adverse effects of IBMIR by reducing the activation of coagulation, complement, and leukocytes. Interestingly, *in vivo* experiments confirmed the protective effect of sCR1, as the use of this inhibitor supported islet integrity, which could be evaluated by the reduction in insulin release shortly after transplantation (198).

Notably, isolated islets can act as a source of procoagulant factors. TF, the main trigger of coagulation *in vivo*, has been found in isolated islets (18, 199), and its knockout (217, 218) or inhibition with specific antibodies (219) has been shown to be beneficial in blocking IBMIR. Interestingly, nicotinamide (a vitamin B derivative) has been used to reduce the expression levels of TF and coagulation, thereby improving IBMIR (20), and leading to improved islet function after transplantation (220).

Islet xenografts can be considered as foreign biological surfaces, and exposure to recipient blood triggers a strong innate immune response. Therefore, an emerging strategy to eliminate the adverse effects of IBMIR is to coat the surface of isolated islets with inhibitory molecules, thereby locally inhibiting the coagulation and complement systems at the transplant site. A 14-patient Phase 1/2a study in New Zealand showed that neonatal porcine islets encapsulated with alginate-poly-L-ornithine-alginate (APA) were safe and reduced unawareness of hypoglycemia in patients with type 1 diabetes (221). Strategies such as donor-specific hematopoietic progenitor cell transplantation (mixed chimerism) and concomitant donor-specific thymus transplantation showed great promise for improving immune tolerance (221, 222).

4.2 Early acute rejection

Studies have shown that during early acute rejection of porcine islet xenografts, the rejecting host graft exhibits direct and indirect anti-donor T cell IL-17 responses and produces strong anti-pig antibodies with severe B cell infiltration (148). IL-17 produced by the early donor stimulus dominates the early acute rejection response rather than IFN-γ production. Treatment with porcine ECDI-SP inhibits the host anti-pig IL-17 response, and when combined with transient B cell depletion (such as anti-CD20 monoclonal antibody) and short-course sirolimus, this triple therapy significantly and durably suppresses the host anti-pig IL-17 response and significantly prolongs the survival time of porcine islet xenografts (223). During early acute rejection, B cells may help induce the differentiation of IL-17-producing T cells and the production of xenogeneic antibodies by plasma cells. Studies have shown that B cell antigens presented by B1 B cells can effectively promote Th17 differentiation (224–226). Conversely, Th17 cells are effective B cell helper cells that can induce B cell proliferation *in vitro* and trigger their class switching *in vivo* (227). It can be imagined that the induced xenogeneic IL-17 response feeds back to promote B cell proliferation and differentiation, establishing a positive feedback loop between B cells and Th17 cells, effectively promoting early acute rejection of islet xenografts.

4.3 Late rejection

In the context of late rejection initially protected by porcine ECDI-SP + anti-CD20 + sirolimus triple therapy, it was found that late rejection appeared to be entirely cell-mediated, as xenogeneic antibodies could not be detected after the rejection of the islet xenografts. Secondly, the phenomenon of late rejection seemed to always be associated with highly aggressive B cell infiltration in the graft. Thirdly, indirect xenogeneic IFN-γ responses appeared before the late rejection after B cell reconstitution (148). It can be imagined that newly emerged B cells directly acquire xenogeneic antigens in the graft and induce indirect anti-donor IFN-γ responses. Graft-infiltrating B cells may also directly initiate cytotoxic T lymphocytes within the graft, leading to the *in situ* destruction of the graft (228).

5 Strategies for treating the immune response to xenogeneic islet transplantation

5.1 Islet encapsulation

Islet encapsulation is an advanced method of islet transplantation, where isolated islets from humans or pigs can be transplanted without the need for toxic immunosuppression. This proves particularly beneficial for porcine islet xenotransplantation. Encapsulating islets with a semipermeable barrier allows for the exchange of nutrients and hormones, including insulin, while maintaining immune isolation, thus overcoming one of the major obstacles of xenotransplantation. Although clinical trials of porcine islets have achieved some success in New Zealand and Argentina, more research may be needed to develop optimal encapsulation methods and materials before this technology is ready for larger clinical trials in the United States. Key factors influencing encapsulation technology include:

1. Capsule size and material: Traditionally, smaller capsules are believed to be more effective due to easier material exchange through the capsule (229). However, recent studies suggest that spherical materials with diameters ≥1.5 mm exhibit significantly better biocompatibility compared to smaller or differently shaped counterparts (230). An *in vivo* study demonstrated that 1.5 mm alginate-encapsulated rat islets could restore blood glucose control in streptozotocin-induced diabetic C57 BL/6 mice for up to 180 days. This indicates that biocompatibility might be more crucial than material exchange efficiency in terms of effectiveness. Alginate consists of linear binary copolymers of β-D-mannuronic acid and α-L-guluronic acid. The length and sequence of mannuronic and guluronic acid chains in alginate hydrogels, as well as the mannuronic to guluronic acid ratio (M

ratio), determine alginate's mechanical strength, elasticity, durability, permeability, and swelling properties. The use of

multivalent cations (Ca^{2+}, Ba^{2+}) and polycations (poly-L-lysine or poly-L-ornithine) during alginate synthesis alters its properties (231). For example, alginate-poly-L-ornithine capsules provide high biocompatibility, better stability, and improved mechanical strength but induce excessive pericapsular cell overgrowth and macrophage activation, leading to capsule fibrosis. Multivalent cations like barium can avoid such fibrosis but reduce molar selectivity.

2. Transplantation site: Since the early 1970s, the liver infused via the portal vein has been widely accepted as the optimal site for islet transplantation in rodents. This principle, due to the prevalence and importance of rodent studies, has been extended to most animal models and nearly become the preferred site for microencapsulated islet transplantation (232). However, subsequent studies have identified several reasons why the liver is not the best site, including: (1) interaction with blood flow causing IBMIR, reducing islet mass by up to 50%; (2) the possibility of thrombosis during infusion; (3) relatively lower oxygen tension compared to the pancreas (233, 234). Intraperitoneal transplantation is also common, mainly due to its low volume limitation on grafts. However, this site has several drawbacks, including lack of close contact with blood flow, uncertain distribution of encapsulated islets, and the tendency for capsules to stack in the pelvic cavity of bipedal animals, making them difficult to retrieve. Subcapsular kidney transplantation is often used in animal models, considering the large number of encapsulated islets used for clinical transplantation and the lack of aggregation at this site. The subcutaneous space can be used for large numbers of encapsulated islets; however, this site is notoriously poor for blood access. Prevascularized subcutaneous spaces seem promising for both device and device-free methods (235), though this area requires two surgeries for prevascularization and actual transplantation. The omental pouch can also be used without two surgeries, potentially making it an ideal site for encapsulated islet implantation. In fact, studies using immunocompetent diabetic rat models have shown long-term function of encapsulated islets in the omental pouch (236). Researchers have also explored potential sites such as the gastric submucosa, peritoneal space, spleen, bone, and muscle. Animal models have identified specific advantages of several alternative sites, such as low blood contact to reduce IBMIR or the ability to biopsy the site after islet delivery, but to date, these positive results have been offset by equally compelling negative factors such as insufficient oxygen supply, surgical difficulty, or the need for more islets to correct blood glucose imbalance. Ongoing research may eventually yield a better site for islet infusion, though the liver remains the best choice for clinical islet transplantation despite its recognized limitations.

Porcine and microencapsulated islets have both been used clinically without significant side effects. However, compared to allogeneic naked islet transplantation, this method's effectiveness is still suboptimal. Improving islet quality, enhancing capsule biocompatibility, and determining suitable implantation sites are crucial for the implementation of this therapy (237). Further research should make this method as effective as allogeneic naked islet transplantation, representing a real breakthrough in overcoming donor shortages and avoiding or mitigating the side effects associated with immunosuppressive drugs.

5.2 Immunosuppressants for xenotransplantation

In 1994, CG Groth reported the first xenotransplantation of porcine fetal islet-like cell clusters in a T1D patient. This study demonstrated the feasibility of porcine islet transplantation but did not show improvement in the patient's condition (238). Over the following decades, porcine islet xenotransplantation has been more thoroughly explored in preclinical trials with non-human primates (239, 240). Humoral rejection is the main obstacle to the success of xenotransplantation. The α1,3Gal epitope, present on the surface of almost all animals except humans and some primates, is the primary antigen causing hyperacute rejection in pig-to-human and pig-to-non-human primate islet xenotransplantation. The University of Pittsburgh and Revivicor, Inc. designed Gal knockout (GTKO) pigs that do not express Gal (158). This proved to be a significant milestone in the development of xenotransplantation. Although other xenogeneic antigens were later discovered, Gal remains the most relevant, and GTKO pigs are considered a potential choice for eventual clinical translation. However, Gal knockout does not prevent islet rejection, and other genetic manipulations have been explored. In 2009, the Pittsburgh islet team first demonstrated long-term function (up to one year) of islet grafts in streptozotocin-induced diabetic non-human primates transplanted with porcine islets genetically modified to express human complement regulatory protein (hCD46). hCD46 expressed on porcine islets limited antibody-mediated rejection, allowing for the reduction of immunosuppression to maintain sufficient islet mass for long-term normal function. However, it did not reduce the initial islet loss associated with IBMIR as expected (241). This led to the further development of multigene pig islet donors capable of providing multifaceted protection to enhance islet transplantation. Five years later, the same group achieved similar success, long-term transplantation of islets from multigene pigs for the first time. A pig with four modified genes, (i) GTKO, and (ii) hCD46, (iii) human tissue factor pathway inhibitor (hTFPI) for antithrombotic and anti-inflammatory effects, and (iv) CTLA4-Ig to inhibit cellular immune responses, demonstrated improved success rates in retaining islet mass early postoperatively and maintaining islet implantation and function for up to one year during transplantation (242). This study also provided preliminary insights into glucose metabolism in pigs expressing human genes regulated by the insulin promoter, demonstrating that multiple islet-targeting transgenes inserted into pigs were not harmful to islet function and opened the door to further experiments and genetic manipulation for islet xenotransplantation (243). Multigene donor pigs have been shown to be a reproducibly effective source of islets for pig-to-non-human primate xenotransplantation (209). CG Park and colleagues at Seoul National University in Korea are conducting ongoing research of great significance for islet xenotransplantation, successfully maintaining normal blood glucose levels in diabetic primates within 600 days post porcine islet transplantation (244). A common feature of these successful long-term porcine-to-non-human primate islet studies is the use of CD154 monoclonal antibody (mAb)-based immunosuppression to prevent rejection.

Although there is evidence that anti-CD154 mAb is effective and safe in pig-to-non-human primate islet transplantation models (245), it is associated with thromboembolic complications in humans and is not clinically translatable.

Despite promising data on the use of anti-CD40 antibody (co-stimulatory blockade) in organ xenotransplantation (246, 247), the islet xenotransplantation community is still searching for a clinically translatable immunosuppressant that can successfully prevent rejection without causing excessive side effects. New techniques for targeted genomic editing, particularly clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein-9 nuclease (Cas9), offer hope that further genetic manipulation of porcine islets can improve compatibility between host and donor, thus allowing successful control of rejection with previously unfeasible immunosuppression. The field of islet xenotransplantation is steadily advancing and may soon approach clinical-grade experience and technology to begin clinical trials (248).

6 Conclusion and perspectives

6.1 Affirming islet transplantation

Islet transplantation holds great promise for the treatment of T1DM, as it offers the potential to restore euglycemia in a reliable manner, protects against hypoglycemia and glycemic liability in a way that exogenous insulin administration has thus far been unable to achieve. It has reduced many complications of diabetes and greatly improved patient healing.

6.2 Role and limitations of immunosuppressants

Limited islet survival after implantation hinders the success of IT due to innate immune attack through IBMIR, recurrent autoimmune islet destruction, or alloimmune rejection. The need for lifelong immunosuppressive therapy and the attendant risks of infection, cancer, and nephrotoxicity pose their own unique additional challenges, making this treatment unattractive to all but those at risk of severe brittle hypoglycemia. Optimizing new blood vessel formation by better controlling angiogenesis, suppressing inflammation, and reducing oxidative stress can all further improve outcomes.

6.3 Challenges and difficulties

The number of islets available for transplantation is a major limitation for both autoislet and alloislet approaches to β-cell replacement therapy. Therefore, the establishment of an unlimited source of islet tissue for transplantation has been a long-sought-after goal.

6.4 Future research directions

Stem cells: Significant progress has been made in the science and application of pluripotent stem cells, which are now entering early-stage pilot clinical trials. The possibility that cell transplantation can be accomplished with less need for immunosuppressants remains a real possibility, and progress is being made in immunomodulatory control through Treg infusion, MSC co-transplantation, and other innovative approaches.

Porcine islet xenotransplantation: Porcine islets have the advantage of targeting normal insulin similar to that present in humans, as well as the physiological ability to handle the heavy demands of insulin secretion. Importantly, porcine IAPP contains amino acid substitutions in the region corresponding to residues 20 to 29 that prevent the formation of fibrils (249, 250). Disadvantages include the larger immunologic barrier of xenogeneic than allogeneic tissue that presents an additional risk for hyperacute rejection and requires more intensive immunosuppression (239, 240),

Islet encapsulation technology: Islet encapsulation provides a barrier to protect transplanted islets, mainly by preventing excessive fibrosis, promoting local vascularization, and preventing future chronic immunosuppressive rejection. The latest data from NOD mice appear to confirm that agarose microencapsulated islets protect against autoimmune reactions (251). A recent paper published in PNAS shows that large encapsulated islets placed in the omentum protect grafts from immune attack and improve glucose metabolic control (252). These data demonstrate the potential of this technique as a safe method for successful islet transplantation.

Author contributions

LY: Writing – original draft. JL: Writing – original draft. MY: Writing – original draft. SS: Writing – review & editing, Validation. XZ: Writing – review & editing, Supervision, Funding acquisition. YW: Writing – review & editing, Validation, Supervision, Funding acquisition, Conceptualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Daneman D. Type 1 diabetes. *Lancet*. (2006) 367:847–58. doi: 10.1016/S0140-6736(06)68341-4

2. Batra L, Shrestha P, Zhao H, Woodward KB, Togay A, Tan M, et al. Localized immunomodulation with PD-L1 results in sustained survival and function of allogeneic islets without chronic immunosuppression. *J Immunol*. (2020) 204:2840–51. doi: 10.4049/jimmunol.2000055

3. DeWitt DE, Hirsch IB. Outpatient insulin therapy in type 1 and type 2 diabetes mellitus: scientific review. *Jama*. (2003) 289:2254–64. doi: 10.1001/jama.289.17.2254

4. Chen QD, Liu L, Zhao XH, Liang JB, Li SW. Challenges and opportunities in the islet transplantation microenvironment: a comprehensive summary of inflammatory cytokine, immune cells, and vascular endothelial cells. *Front Immunol*. (2023) 14:1293762. doi: 10.3389/fimmu.2023.1293762

5. Pambianco G, Costacou T, Ellis D, Becker DJ, Klein R, Orchard TJ. The 30-year natural history of type 1 diabetes complications: the Pittsburgh Epidemiology of Diabetes Complications Study experience. *Diabetes*. (2006) 55:1463–9. doi: 10.2337/db05-1423

6. Kandaswamy R, Skeans MA, Gustafson SK, Carrico RJ, Prentice MA, Israni AK, et al. Pancreas. *Am J Transplant*. (2016) 16 (Suppl 2):47–68. doi: 10.1111/ajt.13667

7. Sutherland DE, Gruessner RW, Dunn DL, Matas AJ, Humar A, Kandaswamy R, et al. Lessons learned from more than 1,000 pancreas transplants at a single institution. *Ann Surg*. (2001) 233:463–501. doi: 10.1097/00000658-200104000-00003

8. Meloche RM. Transplantation for the treatment of type 1 diabetes. *World J Gastroenterol*. (2007) 13:6347–55. doi: 10.3748/wjg.v13.i47.6347

9. Gibly RF, Graham JG, Luo X, Lowe WL Jr., Hering BJ, Shea LD. Advancing islet transplantation: from engraftment to the immune response. *Diabetologia*. (2011) 54:2494–505. doi: 10.1007/s00125-011-2243-0

10. Kanak MA, Takita M, Kunnathodi F, Lawrence MC, Levy MF, Naziruddin B. Inflammatory response in islet transplantation. *Int J Endocrinol*. (2014) 2014:451035. doi: 10.1155/2014/451035

11. Sutherland DE, Gores PF, Farney AC, Wahoff DC, Matas AJ, Dunn DL, et al. Evolution of kidney, pancreas, and islet transplantation for patients with diabetes at the University of Minnesota. *Am J Surg*. (1993) 166:456–91. doi: 10.1016/S0002-9610(05)81142-0

12. Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med*. (2000) 343:230–8. doi: 10.1056/NEJM200007273430401

13. Hirshberg B, Rother KI, Digon BJ 3rd, Lee J, Gaglia JL, Hines K, et al. Benefits and risks of solitary islet transplantation for type 1 diabetes using steroid-sparing immunosuppression: the National Institutes of Health experience. *Diabetes Care*. (2003) 26:3288–95. doi: 10.2337/diacare.26.12.3288

14. Rother KI, Harlan DM. Challenges facing islet transplantation for the treatment of type 1 diabetes mellitus. *J Clin Invest*. (2004) 114:877–83. doi: 10.1172/JCI200423235

15. Shapiro AM, Pokrywczynska M, Ricordi C. Clinical pancreatic islet transplantation. *Nat Rev Endocrinol*. (2017) 13:268–77. doi: 10.1038/nrendo.2016.178

16. Ricordi C. Islet transplantation: a brave new world. *Diabetes*. (2003) 52:1595–603. doi: 10.2337/diabetes.52.7.1595

17. Bennet W, Groth CG, Larsson R, Nilsson B, Korsgren O. Isolated human islets trigger an instant blood mediated inflammatory reaction: implications for intraportal islet transplantation as a treatment for patients with type 1 diabetes. *Ups J Med Sci*. (2000) 105:125–33. doi: 10.1517/03009734000000059

18. Moberg L, Johansson H, Lukinius A, Berne C, Foss A, Källen R, et al. Production of tissue factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation. *Lancet*. (2002) 360:2039–45. doi: 10.1016/S0140-6736(02)12020-4

19. Delaune V, Berney T, Lacotte S, Toso C. Intraportal islet transplantation: the impact of the liver microenvironment. *Transpl Int*. (2017) 30:227–38. doi: 10.1111/tri.2017.30.issue-3

20. Moberg L, Olsson A, Berne C, Felldin M, Foss A, Källen R, et al. Nicotinamide inhibits tissue factor expression in isolated human pancreatic islets: implications for clinical islet transplantation. *Transplantation*. (2003) 76:1285–8. doi: 10.1097/01.TP.0000098905.86445.0F

21. Langer HF, Chavakis T. Leukocyte-endothelial interactions in inflammation. *J Cell Mol Med*. (2009) 13:1211–20. doi: 10.1111/j.1582-4934.2009.00811.x

22. Purdy M, Obi A, Myers D, Wakefield T. P- and E- selectin in venous thrombosis and non-venous pathologies. *J Thromb Haemost*. (2022) 20:1056–66. doi: 10.1111/jth.15689

23. Yan LL, Ye LP, Chen YH, He SQ, Zhang CY, Mao XL, et al. The influence of microenvironment on survival of intraportal transplanted islets. *Front Immunol*. (2022) 13:849580. doi: 10.3389/fimmu.2022.849580

24. Vivot K, Benahmed MA, Seyfritz E, Bietiger W, Elbayed K, Ruhland E, et al. A metabolomic approach ((1)H HRMAS NMR spectroscopy) supported by histology to study early post-transplantation responses in islet-transplanted livers. *Int J Biol Sci*. (2016) 12:1168–80. doi: 10.7150/ijbs.15189

25. Sironi JJ, Ouchi T. STAT1-induced apoptosis is mediated by caspases 2, 3, and 7. *J Biol Chem*. (2004) 279:4066–74. doi: 10.1074/jbc.M307774200

26. Ozmen L, Ekdahl KN, Elgue G, Larsson R, Korsgren O, Nilsson B. Inhibition of thrombin abrogates the instant blood-mediated inflammatory reaction triggered by isolated human islets: possible application of the thrombin inhibitor melagatran in clinical islet transplantation. *Diabetes*. (2002) 51:1779–84. doi: 10.2337/diabetes.51.6.1779

27. Chen D, Zhang N, Fu S, Schröppel B, Guo Q, Garin A, et al. CD4+ CD25+ regulatory T-cells inhibit the islet innate immune response and promote islet engraftment. *Diabetes*. (2006) 55:1011–21. doi: 10.2337/diabetes.55.04.06.db05-1048

28. Marfil-Garza BA, Hefler J, Verhoeff K, Lam A, Dajani K, Anderson B, et al. Pancreas and islet transplantation: comparative outcome analysis of a single-centre cohort over 20-years. *Ann Surg*. (2023) 277:672–80. doi: 10.1097/SLA.0000000000005783

29. Rodriguez-Calvo T, Ekwall O, Amirian N, Zapardiel-Gonzalo J, von Herrath MG. Increased immune cell infiltration of the exocrine pancreas: a possible contribution to the pathogenesis of type 1 diabetes. *Diabetes*. (2014) 63:3880–90. doi: 10.2337/db14-0549

30. Coppieters KT, Dotta F, Amirian N, Campbell PD, Kay TW, Atkinson MA, et al. Demonstration of islet-autoreactive CD8 T cells in insulitic lesions from recent onset and long-term type 1 diabetes patients. *J Exp Med*. (2012) 209:51–60. doi: 10.1084/jem.20111187

31. Babon JA, DeNicola ME, Blodgett DM, Crèvecoeur I, Buttrick TS, Maehr R, et al. Analysis of self-antigen specificity of islet-infiltrating T cells from human donors with type 1 diabetes. *Nat Med*. (2016) 22:1482–7. doi: 10.1038/nm.4203

32. Duneton C, Winterberg PD, Ford ML. Activation and regulation of alloreactive T cell immunity in solid organ transplantation. *Nat Rev Nephrol*. (2022) 18:663–76. doi: 10.1038/s41581-022-00600-0

33. Halloran PF, Einecke G, Sikosana MLN, Madill-Thomsen K. The biology and molecular basis of organ transplant rejection. *Handb Exp Pharmacol*. (2022) 272:1–26. doi: 10.1007/164_2021_557

34. Li J, Li C, Zhuang Q, Peng B, Zhu Y, Ye Q, et al. The evolving roles of macrophages in organ transplantation. *J Immunol Res*. (2019) 2019:5763430. doi: 10.1155/2019/5763430

35. Ronca V, Wootton G, Milani C, Cain O. The immunological basis of liver allograft rejection. *Front Immunol*. (2020) 11:2155. doi: 10.3389/fimmu.2020.02155

36. Agarwal D, Allman D, Naji A. Novel therapeutic opportunities afforded by plasma cell biology in transplantation. *Am J Transplant*. (2020) 20:1984–91. doi: 10.1111/ajt.15813

37. Ho EK, Vlad G, Vasilescu ER, de la Torre L, Colovai AI, Burke E, et al. Pre- and posttransplantation allosensitization in heart allograft recipients: major impact of de novo alloantibody production on allograft survival. *Hum Immunol*. (2011) 72:5–10. doi: 10.1016/j.humimm.2010.10.013

38. Loupy A, Hill GS, Jordan SC. The impact of donor-specific anti-HLA antibodies on late kidney allograft failure. *Nat Rev Nephrol*. (2012) 8:348–57. doi: 10.1038/nrneph.2012.81

39. Morrell MR, Pilewski JM, Gries CJ, Pipeling MR, Crespo MM, Ensor CR, et al. *De novo* donor-specific HLA antibodies are associated with early and high-grade bronchiolitis obliterans syndrome and death after lung transplantation. *J Heart Lung Transplant*. (2014) 33:1288–94. doi: 10.1016/j.healun.2014.07.018

40. Le Moine A, Goldman M. Non-classical pathways of cell-mediated allograft rejection: new challenges for tolerance induction? *Am J Transplant*. (2003) 3:101–6. doi: 10.1034/j.1600-6143.2002.00026.x

41. Plenter RJ, Grazia TJ, Doan AN, Gill RG, Pietra BA. CD4 T cells mediate cardiac xenograft rejection via host MHC Class II. *J Heart Lung Transplant*. (2012) 31:1018–24. doi: 10.1016/j.healun.2012.05.018

42. Reffet S, Thivolet C. Immunology of pancreatic islet transplantation. *Diabetes Metab*. (2006) 32:523–6. doi: 10.1016/S1262-3636(06)72805-1

43. Regnell SE, Lernmark Å. Early prediction of autoimmune (type 1) diabetes. *Diabetologia*. (2017) 60:1370–81. doi: 10.1007/s00125-017-4308-1

44. Sun JK, Keenan HA, Cavallerano JD, Asztalos BF, Schaefer EJ, Sell DR, et al. Protection from retinopathy and other complications in patients with type 1 diabetes of extreme duration: the joslin 50-year medalist study. *Diabetes Care*. (2011) 34:968–74. doi: 10.2337/dc10-1675

45. Soeldner JS, Tuttleman M, Srikanta S, Ganda OP, Eisenbarth GS. Insulin-dependent diabetes mellitus and autoimmunity: islet-cell autoantibodies, insulin autoantibodies, and beta-cell failure. *N Engl J Med*. (1985) 313:893–4. doi: 10.1056/NEJM198510033131417

46. Eisenbarth GS. Type I diabetes mellitus. A chronic autoimmune disease. *N Engl J Med*. (1986) 314:1360–8. doi: 10.1056/NEJM198605223142106

47. Zhang L, Gianani R, Nakayama M, Liu E, Kobayashi M, Baschal E, et al. Type 1 diabetes: chronic progressive autoimmune disease. *Novartis Found Symp*. (2008) 292:85–94. doi: 10.1002/9780470697405.ch7

48. Sutherland DE, Goetz FC, Sibley RK. Recurrence of disease in pancreas transplants. *Diabetes*. (1989) 38 (Suppl 1):85–7. doi: 10.2337/diab.38.1.s85

49. Sarikonda G, Pettus J, Phatak S, Sachithanantham S, Miller JF, Wesley JD, et al. CD8 T-cell reactivity to islet antigens is unique to type 1 while CD4 T-cell reactivity exists in both type 1 and type 2 diabetes. *J Autoimmun*. (2014) 50:77–82. doi: 10.1016/j.jaut.2013.12.003

50. Rodriguez-Calvo T, Suwandi JS, Amirian N, Zapardiel-Gonzalo J, Anquetil F, Sabouri S, et al. Heterogeneity and lobularity of pancreatic pathology in type 1 diabetes during the prediabetic phase. *J Histochem Cytochem*. (2015) 63:626–36. doi: 10.1369/0022155415576543

51. Sibley RK, Sutherland DE, Goetz F, Michael AF. Recurrent diabetes mellitus in the pancreas iso- and allograft. A light and electron microscopic and immunohistochemical analysis of four cases. *Lab Invest*. (1985) 53:132–44.

52. Sutherland DE, Sibley R, Xu XZ, Michael A, Srikanta AM, Taub F, et al. Twin-to-twin pancreas transplantation: reversal and reenactment of the pathogenesis of type I diabetes. *Trans Assoc Am Physicians*. (1984) 97:80–7.

53. Baxter AG. Immunogenetics and the cause of autoimmune disease. *Autoimmunity*. (1997) 25:177–89. doi: 10.3109/08916939709008024

54. Bach JF, Chatenoud L. Tolerance to islet autoantigens in type 1 diabetes. *Annu Rev Immunol*. (2001) 19:131–61. doi: 10.1146/annurev.immunol.19.1.131

55. Thivolet C, Abou-Amara S, Martin X, Lefrancois N, Petruzzo P, McGregor B, et al. Serological markers of recurrent beta cell destruction in diabetic patients undergoing pancreatic transplantation. *Transplantation*. (2000) 69:99–103. doi: 10.1097/00007890-200001150-00018

56. Pugliese A, Reijonen HK, Nepom J, Burke GW 3rd. Recurrence of autoimmunity in pancreas transplant patients: research update. *Diabetes Manag (Lond)*. (2011) 1:229–38. doi: 10.2217/dmt.10.21

57. Laughlin E, Burke G, Pugliese A, Falk B, Nepom G. Recurrence of autoreactive antigen-specific CD4+ T cells in autoimmune diabetes after pancreas transplantation. *Clin Immunol*. (2008) 128:23–30. doi: 10.1016/j.clim.2008.03.459

58. Vendrame F, Pileggi A, Laughlin E, Allende G, Martin-Pagola A, Molano RD, et al. Recurrence of type 1 diabetes after simultaneous pancreas-kidney transplantation, despite immunosuppression, is associated with autoantibodies and pathogenic autoreactive CD4 T-cells. *Diabetes*. (2010) 59:947–57. doi: 10.2337/db09-0498

59. Di Lorenzo TP, Peakman M, Roep BO. Translational mini-review series on type 1 diabetes: Systematic analysis of T cell epitopes in autoimmune diabetes. *Clin Exp Immunol*. (2007) 148:1–16. doi: 10.1111/j.1365-2249.2006.03244.x

60. Braghi S, Bonifacio E, Secchi A, Di Carlo V, Pozza G, Bosi E. Modulation of humoral islet autoimmunity by pancreas allotransplantation influences allograft outcome in patients with type 1 diabetes. *Diabetes*. (2000) 49:218–24. doi: 10.2337/diabetes.49.2.218

61. Alejandro R, Lehmann R, Ricordi C, Kenyon NS, Angelico MC, Burke G, et al. Long-term function (6 years) of islet allografts in type 1 diabetes. *Diabetes*. (1997) 46:1983–9. doi: 10.2337/diab.46.12.1983

62. Delaunay F, Khan A, Cintra A, Davani B, Ling ZC, Andersson A, et al. Pancreatic beta cells are important targets for the diabetogenic effects of glucocorticoids. *J Clin Invest*. (1997) 100:2094–8. doi: 10.1172/JCI119743

63. Drachenberg CB, Klassen DK, Weir MR, Wiland A, Fink JC, Bartlett ST, et al. Islet cell damage associated with tacrolimus and cyclosporine: morphological features in pancreas allograft biopsies and clinical correlation. *Transplantation*. (1999) 68:396–402. doi: 10.1097/00007890-199908150-00012

64. Kahan BD, Koch SM. Current immunosuppressant regimens: considerations for critical care. *Curr Opin Crit Care*. (2001) 7:242–50. doi: 10.1097/00075198-200108000-00006

65. Yale JF, Roy RD, Grose M, Seemayer TA, Murphy GF, Marliss EB. Effects of cyclosporine on glucose tolerance in the rat. *Diabetes*. (1985) 34:1309–13. doi: 10.2337/diab.34.12.1309

66. Nanji SA, Shapiro AM. Islet transplantation in patients with diabetes mellitus: choice of immunosuppression. *BioDrugs*. (2004) 18:315–28. doi: 10.2165/00063030-200418050-00004

67. Ochiai T, Nagata M, Nakajima K, Suzuki T, Sakamoto K, Enomoto K, et al. Studies of the effects of FK506 on renal allografting in the beagle dog. *Transplantation*. (1987) 44:729–33. doi: 10.1097/00007890-198712000-00001

68. Ochiai T, Nakajima K, Nagata M, Suzuki T, Asano T, Uematsu T, et al. Effect of a new immunosuppressive agent, FK 506, on heterotopic cardiac allotransplantation in the rat. *Transplant Proc*. (1987) 19:1284–6.

69. Sabatini DM, Erdjument-Bromage H, Lui M, Tempst P, Snyder SH. RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. *Cell*. (1994) 78:35–43. doi: 10.1016/0092-8674(94)90570-3

70. Thomson AW, Turnquist HR, Raimondi G. Immunoregulatory functions of mTOR inhibition. *Nat Rev Immunol*. (2009) 9:324–37. doi: 10.1038/nri2546

71. Barlow AD, Nicholson ML, Herbert TP. Evidence for rapamycin toxicity in pancreatic β-cells and a review of the underlying molecular mechanisms. *Diabetes*. (2013) 62:2674–82. doi: 10.2337/db13-0106

72. Hara K, Maruki Y, Long X, Yoshino K, Oshiro N, Hidayat S, et al. Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. *Cell*. (2002) 110:177–89. doi: 10.1016/S0092-8674(02)00833-4

73. Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, et al. mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell*. (2002) 110:163–75. doi: 10.1016/S0092-8674(02)00808-5

74. Jacinto E, Loewith R, Schmidt A, Lin S, Rüegg MA, Hall A, et al. Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat Cell Biol*. (2004) 6:1122–8. doi: 10.1038/ncb1183

75. Sarbassov DD, Ali SM, Kim DH, Guertin DA, Latek RR, Erdjument-Bromage H, et al. Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr Biol*. (2004) 14:1296–302. doi: 10.1016/j.cub.2004.06.054

76. Barlow AD, Xie J, Moore CE, Campbell SC, Shaw JA, Nicholson ML, et al. Rapamycin toxicity in MIN6 cells and rat and human islets is mediated by the inhibition of mTOR complex 2 (mTORC2). *Diabetologia*. (2012) 55:1355–65. doi: 10.1007/s00125-012-2475-7

77. Zeng Z, Sarbassov dos D, Samudio IJ, Yee KW, Munsell MF, Ellen Jackson C, et al. Rapamycin derivatives reduce mTORC2 signaling and inhibit AKT activation in AML. *Blood*. (2007) 109:3509–12. doi: 10.1182/blood-2006-06-030833

78. Sarbassov DD, Ali SM, Sengupta S, Sheen JH, Hsu PP, Bagley AF, et al. Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Mol Cell*. (2006) 22:159–68. doi: 10.1016/j.molcel.2006.03.029

79. Xie J, Herbert TP. The role of mammalian target of rapamycin (mTOR) in the regulation of pancreatic β-cell mass: implications in the development of type-2 diabetes. *Cell Mol Life Sci*. (2012) 69:1289–304. doi: 10.1007/s00018-011-0874-4

80. Hamada S, Hara K, Hamada T, Yasuda H, Moriyama H, Nakayama R, et al. Upregulation of the mammalian target of rapamycin complex 1 pathway by Ras homolog enriched in brain in pancreatic beta-cells leads to increased beta-cell mass and prevention of hyperglycemia. *Diabetes*. (2009) 58:1321–32. doi: 10.2337/db08-0519

81. Mori H, Inoki K, Opland D, Münzberg H, Villanueva EC, Faouzi M, et al. Critical roles for the TSC-mTOR pathway in β-cell function. *Am J Physiol Endocrinol Metab*. (2009) 297:E1013–22. doi: 10.1152/ajpendo.00262.2009

82. Shigeyama Y, Kobayashi T, Kido Y, Hashimoto N, Asahara S, Matsuda T, et al. Biphasic response of pancreatic beta-cell mass to ablation of tuberous sclerosis complex 2 in mice. *Mol Cell Biol*. (2008) 28:2971–9. doi: 10.1128/MCB.01695-07

83. Pende M, Kozma SC, Jaquet M, Oorschot V, Burcelin R, Le Marchand-Brustel Y, et al. Hypoinsulinaemia, glucose intolerance and diminished beta-cell size in S6K1-deficient mice. *Nature*. (2000) 408:994–7. doi: 10.1038/35050135

84. Ruvinsky I, Sharon N, Lerer T, Cohen H, Stolovich-Rain M, Nir T, et al. Ribosomal protein S6 phosphorylation is a determinant of cell size and glucose homeostasis. *Genes Dev*. (2005) 19:2199–211. doi: 10.1101/gad.351605

85. Elghazi L, Balcazar N, Blandino-Rosano M, Cras-Méneur C, Fatrai S, Gould AP, et al. Decreased IRS signaling impairs beta-cell cycle progression and survival in transgenic mice overexpressing S6K in beta-cells. *Diabetes*. (2010) 59:2390–9. doi: 10.2337/db09-0851

86. Balcazar N, Sathyamurthy A, Elghazi L, Gould A, Weiss A, Shiojima I, et al. mTORC1 activation regulates beta-cell mass and proliferation by modulation of cyclin D2 synthesis and stability. *J Biol Chem*. (2009) 284:7832–42. doi: 10.1074/jbc.M807458200

87. Niclauss N, Bosco D, Morel P, Giovannoni L, Berney T, Parnaud G. Rapamycin impairs proliferation of transplanted islet β cells. *Transplantation*. (2011) 91:714–22. doi: 10.1097/TP.0b013e31820c10c8

88. Shimodahira M, Fujimoto S, Mukai E, Nakamura Y, Nishi Y, Sasaki M, et al. Rapamycin impairs metabolism-secretion coupling in rat pancreatic islets by suppressing carbohydrate metabolism. *J Endocrinol*. (2010) 204:37–46. doi: 10.1677/JOE-09-0216

89. Maechler P, Wollheim CB. Mitochondrial function in normal and diabetic beta-cells. *Nature*. (2001) 414:807–12. doi: 10.1038/414807a

90. Gan X, Wang J, Su B, Wu D. Evidence for direct activation of mTORC2 kinase activity by phosphatidylinositol 3,4,5-trisphosphate. *J Biol Chem*. (2011) 286:10998–1002. doi: 10.1074/jbc.M110.195016

91. Tato I, Bartrons R, Ventura F, Rosa JL. Amino acids activate mammalian target of rapamycin complex 2 (mTORC2) via PI3K/Akt signaling. *J Biol Chem*. (2011) 286:6128–42. doi: 10.1074/jbc.M110.166991

92. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science*. (2005) 307:1098–101. doi: 10.1126/science.1106148

93. García-Martínez JM, Alessi DR. mTOR complex 2 (mTORC2) controls hydrophobic motif phosphorylation and activation of serum- and glucocorticoid-induced protein kinase 1 (SGK1). *Biochem J*. (2008) 416:375–85. doi: 10.1042/BJ20081668

94. Diakov A, Korbmacher C. A novel pathway of epithelial sodium channel activation involves a serum- and glucocorticoid-inducible kinase consensus motif in the C terminus of the channel's alpha-subunit. *J Biol Chem*. (2004) 279:38134–42. doi: 10.1074/jbc.M403260200

95. Gu Y, Lindner J, Kumar A, Yuan W, Magnuson MA. Rictor/mTORC2 is essential for maintaining a balance between beta-cell proliferation and cell size. *Diabetes*. (2011) 60:827–37. doi: 10.2337/db10-1194

96. Bernal-Mizrachi E, Wen W, Stahlhut S, Welling CM, Permutt MA. Islet beta cell expression of constitutively active Akt1/PKB alpha induces striking hypertrophy, hyperplasia, and hyperinsulinemia. *J Clin Invest*. (2001) 108:1631–8. doi: 10.1172/JCI200113785

97. Tuttle RL, Gill NS, Pugh W, Lee JP, Koeberlein B, Furth EE, et al. Regulation of pancreatic beta-cell growth and survival by the serine/threonine protein kinase Akt1/PKBalpha. *Nat Med*. (2001) 7:1133–7. doi: 10.1038/nm1001-1133

98. Wrede CE, Dickson LM, Lingohr MK, Briaud I, Rhodes CJ. Protein kinase B/Akt prevents fatty acid-induced apoptosis in pancreatic beta-cells (INS-1). *J Biol Chem*. (2002) 277:49676–84. doi: 10.1074/jbc.M208756200

99. Collier JJ, Fueger PT, Hohmeier HE, Newgard CB. Pro- and antiapoptotic proteins regulate apoptosis but do not protect against cytokine-mediated cytotoxicity in rat islets and beta-cell lines. *Diabetes*. (2006) 55:1398–406. doi: 10.2337/db05-1000

100. Cai Y, Wang Q, Ling Z, Pipeleers D, McDermott P, Pende M, et al. Akt activation protects pancreatic beta cells from AMPK-mediated death through stimulation of mTOR. *Biochem Pharmacol*. (2008) 75:1981–93. doi: 10.1016/j.bcp.2008.02.019

101. Xu C, Niu YJ, Liu XJ, Teng YQ, Li CF, Wang HY, et al. Tacrolimus reversibly reduces insulin secretion, induces insulin resistance, and causes islet cell damage in rats. *Int J Clin Pharmacol Ther*. (2014) 52:620–7. doi: 10.5414/CP202090

102. Dai C, Walker JT, Shostak A, Padgett A, Spears E, Wisniewski S, et al. Tacrolimus- and sirolimus-induced human β cell dysfunction is reversible and preventable. *JCI Insight*. (2020) 5(1):e130770. doi: 10.1172/jci.insight.130770

103. Pereira MJ, Palming J, Rizell M, Aureliano M, Carvalho E, Svensson MK, et al. and tacrolimus reduce the amount of GLUT4 at the cell surface in human adipocytes: increased endocytosis as a potential mechanism for the diabetogenic effects of immunosuppressive agents. *J Clin Endocrinol Metab*. (2014) 99:E1885–94. doi: 10.1210/jc.2014-1266

104. Lombardi A, Trimarco B, Iaccarino G, Santulli G. Impaired mitochondrial calcium uptake caused by tacrolimus underlies beta-cell failure. *Cell Commun Signal*. (2017) 15:47. doi: 10.1186/s12964-017-0203-0

105. Okamoto H, Hribal ML, Lin HV, Bennett WR, Ward A, Accili D. Role of the forkhead protein FoxO1 in beta cell compensation to insulin resistance. *J Clin Invest*. (2006) 116:775–82. doi: 10.1172/JCI24967

106. Triñanes J, Rodriguez-Rodriguez AE, Brito-Casillas Y, Wagner A, De Vries APJ, Cuesto G, et al. Deciphering tacrolimus-induced toxicity in pancreatic β Cells. *Am J Transplant*. (2017) 17:2829–40. doi: 10.1111/ajt.14323

107. Ekberg H, Tedesco-Silva H, Demirbas A, Vítko S, Nashan B, Gürkan A, et al. Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med*. (2007) 357:2562–75. doi: 10.1056/NEJMoa067411

108. Vincenti F, Friman S, Scheuermann E, Rostaing L, Jenssen T, Campistol JM, et al. Results of an international, randomized trial comparing glucose metabolism disorders and outcome with cyclosporine versus tacrolimus. *Am J Transplant*. (2007) 7:1506–14. doi: 10.1111/j.1600-6143.2007.01749.x

109. Kale A, Rogers NM. No time to die-how islets meet their demise in transplantation. *Cells*. (2023) 12(5):796. doi: 10.3390/cells12050796

110. Liu B, Jiang Q, Chen R, Gao S, Xia Q, Zhu J, et al. Tacrolimus ameliorates bleomycin-induced pulmonary fibrosis by inhibiting M2 macrophage polarization via JAK2/STAT3 signaling. *Int Immunopharmacol*. (2022) 113:109424. doi: 10.1016/j.intimp.2022.109424

111. Duan K, Liu J, Zhang J, Chu T, Liu H, Lou F, et al. Advancements in innate immune regulation strategies in islet transplantation. *Front Immunol*. (2023) 14:1341314. doi: 10.3389/fimmu.2023.1341314

112. Henderson JR, Moss MC. A morphometric study of the endocrine and exocrine capillaries of the pancreas. *Q J Exp Physiol*. (1985) 70:347–56. doi: 10.1113/expphysiol.1985.sp002920

113. Allison AC, Eugui EM. The design and development of an immunosuppressive drug, mycophenolate mofetil. *Springer Semin Immunopathol*. (1993) 14:353–80. doi: 10.1007/BF00192309

114. Allison AC, Eugui EM. Preferential suppression of lymphocyte proliferation by mycophenolic acid and predicted long-term effects of mycophenolate mofetil in transplantation. *Transplant Proc*. (1994) 26:3205–10.

115. Allison AC, Hovi T, Watts RW, Webster AD. The role of *de novo* purine synthesis in lymphocyte transformation. *Ciba Found Symp*. (1977) 48):207–24.

116. Allison AC, Eugui EM. Mycophenolate mofetil and its mechanisms of action. *Immunopharmacology*. (2000) 47:85–118. doi: 10.1016/S0162-3109(00)00188-0

117. Rosenberg AS, Singer A. Cellular basis of skin allograft rejection: an *in vivo* model of immune-mediated tissue destruction. *Annu Rev Immunol*. (1992) 10:333–58. doi: 10.1146/annurev.iy.10.040192.002001

118. Mycophenolate mofetil for the treatment of a first acute renal allograft rejection: three-year follow-up. The Mycophenolate Mofetil Acute Renal Rejection Study Group. *Transplantation*. (2001) 71:1091–7. doi: 10.1097/00007890-200104270-00014

119. Mathew TH. A blinded, long-term, randomized multicenter study of mycophenolate mofetil in cadaveric renal transplantation: results at three years. Tricontinental Mycophenolate Mofetil Renal Transplantation Study Group. *Transplantation*. (1998) 65:1450–4. doi: 10.1097/00007890-199806150-00007

120. Pescovitz MD, Conti D, Dunn J, Gonwa T, Halloran P, Sollinger H, et al. Intravenous mycophenolate mofetil: safety, tolerability, and pharmacokinetics. *Clin Transplant*. (2000) 14:179–88. doi: 10.1034/j.1399-0012.2000.140301.x

121. Hutchings P, Rosen H, O'Reilly L, Simpson E, Gordon S, Cooke A. Transfer of diabetes in mice prevented by blockade of adhesion-promoting receptor on macrophages. *Nature*. (1990) 348:639–42. doi: 10.1038/348639a0

122. Corbett JA, Mikhael A, Shimizu J, Frederick K, Misko TP, McDaniel ML, et al. Nitric oxide production in islets from nonobese diabetic mice: aminoguanidine-sensitive and -resistant stages in the immunological diabetic process. *Proc Natl Acad Sci U.S.A*. (1993) 90:8992–5.

123. Flodström M, Tyrberg B, Eizirik DL, Sandler S. Reduced sensitivity of inducible nitric oxide synthase-deficient mice to multiple low-dose streptozotocin-induced diabetes. *Diabetes*. (1999) 48:706–13. doi: 10.2337/diabetes.48.4.706

124. Eizirik DL, Colli ML, Ortis F. The role of inflammation in insulitis and beta-cell loss in type 1 diabetes. *Nat Rev Endocrinol*. (2009) 5:219–26. doi: 10.1038/nrendo.2009.21

125. Silverman MN, Sternberg EM. Glucocorticoid regulation of inflammation and its functional correlates: from HPA axis to glucocorticoid receptor dysfunction. *Ann N Y Acad Sci*. (2012) 1261:55–63. doi: 10.1111/j.1749-6632.2012.06633.x

126. Lambillotte C, Gilon P, Henquin JC. Direct glucocorticoid inhibition of insulin secretion. An *in vitro* study of dexamethasone effects in mouse islets. *J Clin Invest*. (1997) 99:414–23. doi: 10.1172/JCI119175

127. Buchwald P, Bocca N, Marzorati S, Hochhaus G, Bodor N, Stabler C, et al. Feasibility of localized immunosuppression: 1. Exploratory studies with glucocorticoids in a biohybrid device designed for cell transplantation. *Pharmazie*. (2010) 65:421–8.

128. Hult M, Ortsäter H, Schuster G, Graedler F, Beckers J, Adamski J, et al. Short-term glucocorticoid treatment increases insulin secretion in islets derived from lean mice through multiple pathways and mechanisms. *Mol Cell Endocrinol*. (2009) 301:109–16. doi: 10.1016/j.mce.2008.09.038

129. Lund T, Fosby B, Korsgren O, Scholz H, Foss A. Glucocorticoids reduce pro-inflammatory cytokines and tissue factor *in vitro* and improve function of transplanted human islets *in vivo*. *Transpl Int*. (2008) 21:669–78. doi: 10.1111/tri.2008.21.issue-7

130. Rafacho A, Marroquí L, Taboga SR, Abrantes JL, Silveira LR, Boschero AC, et al. Glucocorticoids *in vivo* induce both insulin hypersecretion and enhanced glucose sensitivity of stimulus-secretion coupling in isolated rat islets. *Endocrinology*. (2010) 151:85–95. doi: 10.1210/en.2009-0704

131. Turban S, Liu X, Ramage L, Webster SP, Walker BR, Dunbar DR, et al. Optimal elevation of β-cell 11β-hydroxysteroid dehydrogenase type 1 is a compensatory mechanism that prevents high-fat diet-induced β-cell failure. *Diabetes*. (2012) 61:642–52. doi: 10.2337/db11-1054

132. Balamurugan AN, Bottino R, Giannoukakis N, Smetanka C. Prospective and challenges of islet transplantation for the therapy of autoimmune diabetes. *Pancreas*. (2006) 32:231–43. doi: 10.1097/01.mpa.0000203961.16630.2f

133. Colson K, Doss DS, Swift R, Tariman J, Thomas TE. Bortezomib, a newly approved proteasome inhibitor for the treatment of multiple myeloma: nursing implications. *Clin J Oncol Nurs*. (2004) 8:473–80. doi: 10.1188/04.CJON.473-480

134. Field-Smith A, Morgan GJ, Davies FE. Bortezomib (Velcadetrade mark) in the treatment of multiple myeloma. *Ther Clin Risk Manag*. (2006) 2:271–9. doi: 10.2147/tcrm.2006.2.3.271

135. Brignole C, Marimpietri D, Pastorino F, Nico B, Di Paolo D, Cioni M, et al. Effect of bortezomib on human neuroblastoma cell growth, apoptosis, and angiogenesis. *J Natl Cancer Inst*. (2006) 98:1142–57. doi: 10.1093/jnci/djj309

136. Vodanovic-Jankovic S, Hari P, Jacobs P, Komorowski R, Drobyski WR. NF-kappaB as a target for the prevention of graft-versus-host disease: comparative efficacy of bortezomib and PS-1145. *Blood*. (2006) 107:827–34. doi: 10.1182/blood-2005-05-1820

137. Blanco B, Pérez-Simón JA, Sánchez-Abarca LI, Carvajal-Vergara X, Mateos J, Vidriales B, et al. Bortezomib induces selective depletion of alloreactive T lymphocytes and decreases the production of Th1 cytokines. *Blood*. (2006) 107:3575–83. doi: 10.1182/blood-2005-05-2118

138. Nencioni A, Schwarzenberg K, Brauer KM, Schmidt SM, Ballestrero A, Grünebach F, et al. Proteasome inhibitor bortezomib modulates TLR4-induced dendritic cell activation. *Blood*. (2006) 108:551–8. doi: 10.1182/blood-2005-08-3494

139. Sun K, Welniak LA, Panoskaltsis-Mortari A, O'Shaughnessy MJ, Liu H, Barao I, et al. Inhibition of acute graft-versus-host disease with retention of graft-versus-tumor effects by the proteasome inhibitor bortezomib. *Proc Natl Acad Sci U.S.A.* (2004) 101:8120–5. doi: 10.1073/pnas.0401563101

140. Sun K, Wilkins DE, Anver MR, Sayers TJ, Panoskaltsis-Mortari A, Blazar BR, et al. Differential effects of proteasome inhibition by bortezomib on murine acute graft-versus-host disease (GVHD): delayed administration of bortezomib results in increased GVHD-dependent gastrointestinal toxicity. *Blood*. (2005) 106:3293–9. doi: 10.1182/blood-2004-11-4526

141. Kim JS, Lee JI, Shin JY, Kim SY, Shin JS, Lim JH, et al. Bortezomib can suppress activation of rapamycin-resistant memory T cells without affecting regulatory T-cell viability in non-human primates. *Transplantation*. (2009) 88:1349–59. doi: 10.1097/TP.0b013e3181bd7b3a

142. Hong SH, Kim K, Shin JS, Chung H, Park CG. A combination regimen of low-dose bortezomib and rapamycin prolonged the graft survival in a murine allogeneic islet transplantation model. *Immunol Lett*. (2019) 216:21–7. doi: 10.1016/j.imlet.2019.10.005

143. Berney T, Secchi A. Rapamycin in islet transplantation: friend or foe? *Transpl Int*. (2009) 22:153–61. doi: 10.1111/j.1432-2277.2008.00743.x

144. Zhang N, Su D, Qu S, Tse T, Bottino R, Balamurugan AN, et al. Sirolimus is associated with reduced islet engraftment and impaired beta-cell function. *Diabetes*. (2006) 55:2429–36. doi: 10.2337/db06-0173

145. Diamond AS, Gill RG. An essential contribution by IFN-gamma to CD8+ T cell-mediated rejection of pancreatic islet allografts. *J Immunol*. (2000) 165:247–55. doi: 10.4049/jimmunol.165.1.247

146. Simeonovic CJ, Townsend MJ, Morris CF, Hapel AJ, Fung MC, Mann DA, et al. Immune mechanisms associated with the rejection of fetal murine proislet allografts and pig proislet xenografts: comparison of intragraft cytokine mRNA profiles. *Transplantation*. (1999) 67:963–71. doi: 10.1097/00007890-199904150-00006

147. Kim HJ, Byun N, Kwon O, Park CG. Cross-sensitization between xeno- and allo-antigens on subsequent allogeneic and xenogeneic pancreatic islet transplantation in a murine model. *Biochem Biophys Res Commun*. (2016) 480:474–8. doi: 10.1016/j.bbrc.2016.10.076

148. Kang HK, Wang S, Dangi A, Zhang X, Singh A, Zhang L, et al. Differential role of B cells and IL-17 versus IFN-γ During early and late rejection of pig islet xenografts in mice. *Transplantation*. (2017) 101:1801–10. doi: 10.1097/TP.0000000000001489

149. Cooper DK, Human PA, Lexer G, Rose AG, Rees J, Keraan M, et al. Effects of cyclosporine and antibody adsorption on pig cardiac xenograft survival in the baboon. *J Heart Transplant*. (1988) 7:238–46.

150. Cooper DKC, Ekser B, Tector AJ. Immunobiological barriers to xenotransplantation. *Int J Surg*. (2015) 23:211–6. doi: 10.1016/j.ijsu.2015.06.068

151. Galili U. Natural anti-carbohydrate antibodies contributing to evolutionary survival of primates in viral epidemics? *Glycobiology*. (2016) 26:1140–50. doi: 10.1093/glycob/cww088

152. Zeyland J, Lipiński D, Słomski R. The current state of xenotransplantation. *J Appl Genet*. (2015) 56:211–8. doi: 10.1007/s13353-014-0261-6

153. Yang YG, Sykes M. Xenotransplantation: current status and a perspective on the future. *Nat Rev Immunol*. (2007) 7:519–31. doi: 10.1038/nri2099

154. Kobayashi T, Cooper DK. Anti-Gal, alpha-Gal epitopes, and xenotransplantation. *Subcell Biochem*. (1999) 32:229–57. doi: 10.1007/978-1-4615-4771-6_10

155. Platt JL, Fischel RJ, Matas AJ, Reif SA, Bolman RM, Bach FH. Immunopathology of hyperacute xenograft rejection in a swine-to-primate model. *Transplantation*. (1991) 52:214–20. doi: 10.1097/00007890-199108000-00006

156. Schuurman HJ, Cheng J, Lam T. Pathology of xenograft rejection: a commentary. *Xenotransplantation*. (2003) 10:293–9. doi: 10.1034/j.1399-3089.2003.02092.x

157. Ierino FL, Sandrin MS. Spectrum of the early xenograft response: from hyperacute rejection to delayed xenograft injury. *Crit Rev Immunol*. (2007) 27:153–66. doi: 10.1615/CritRevImmunol.v27.i2

158. Phelps CJ, Koike C, Vaught TD, Boone J, Wells KD, Chen SH, et al. Production of alpha 1,3-galactosyltransferase-deficient pigs. *Science*. (2003) 299:411–4. doi: 10.1126/science.1078942

159. Park JY, Park MR, Kwon DN, Kang MH, Oh M, Han JW, et al. Alpha 1,3-galactosyltransferase deficiency in pigs increases sialyltransferase activities that potentially raise non-gal xenoantigenicity. *J Biomed Biotechnol*. (2011) 2011:560850. doi: 10.1155/2011/560850

160. Chen Y, Stewart JM, Gunthart M, Hawthorne WJ, Salvaris EJ, O'Connell PJ, et al. Xenoantibody response to porcine islet cell transplantation using GTKO, CD55, CD59, and fucosyltransferase multiple transgenic donors. *Xenotransplantation*. (2014) 21:244–53. doi: 10.1111/xen.12091

161. Hering BJ, Walawalkar N. Pig-to-nonhuman primate islet xenotransplantation. *Transpl Immunol*. (2009) 21:81–6. doi: 10.1016/j.trim.2009.05.001

162. Griesemer A, Yamada K, Sykes M. Xenotransplantation: immunological hurdles and progress toward tolerance. *Immunol Rev*. (2014) 258:241–58. doi: 10.1111/imr.12152

163. Yi S, Feng X, Wang Y, Kay TW, Wang Y, O'Connell PJ. CD4+ cells play a major role in xenogeneic human anti-pig cytotoxicity through the Fas/Fas ligand lytic pathway. *Transplantation*. (1999) 67:435–43. doi: 10.1097/00007890-199902150-00017

164. Lin ML, Zhan Y, Nutt SL, Brady J, Wojtasiak M, Brooks AG, et al. NK cells promote peritoneal xenograft rejection through an IFN-gamma-dependent mechanism. *Xenotransplantation*. (2006) 13:536–46. doi: 10.1111/j.1399-3089.2006.00348.x

165. Yi S, Feng X, Hawthorne WJ, Patel AT, Walters SN, O'Connell PJ. CD4+ T cells initiate pancreatic islet xenograft rejection via an interferon-gamma-dependent recruitment of macrophages and natural killer cells. *Transplantation*. (2002) 73:437–46. doi: 10.1097/00007890-200202150-00019

166. Lee RS, Yamada K, Womer KL, Pillsbury EP, Allison KS, Marolewski AE, et al. Blockade of CD28-B7, but not CD40-CD154, prevents costimulation of allogeneic porcine and xenogeneic human anti-porcine T cell responses. *J Immunol*. (2000) 164:3434–44. doi: 10.4049/jimmunol.164.6.3434

167. Mulley WR, Wee JL, Christiansen D, Milland J, Ierino FL, Sandrin MS. Lentiviral expression of CTLA4Ig inhibits primed xenogeneic lymphocyte proliferation and cytokine responses. *Xenotransplantation*. (2006) 13:248–52. doi: 10.1111/j.1399-3089.2006.00297.x

168. Singh NP, Guo L, Mhoyan A, Shirwan H. Predominant expression of Th2 cytokines and interferon-gamma in xenogeneic cardiac grafts undergoing acute vascular rejection. *Transplantation*. (2003) 75:586–90. doi: 10.1097/01.TP.0000052594.83318.68

169. Getts DR, Turley DM, Smith CE, Harp CT, McCarthy D, Feeney EM, et al. Tolerance induced by apoptotic antigen-coupled leukocytes is induced by PD-L1+ and IL-10-producing splenic macrophages and maintained by T regulatory cells. *J Immunol*. (2011) 187:2405–17. doi: 10.4049/jimmunol.1004175

170. Kheradmand T, Wang S, Bryant J, Tasch JJ, Lerret N, Pothoven KL, et al. Ethylenecarbodiimide-fixed donor splenocyte infusions differentially target direct and indirect pathways of allorecognition for induction of transplant tolerance. *J Immunol*. (2012) 189:804–12. doi: 10.4049/jimmunol.1103705

171. Martin AJ, McCarthy D, Waltenbaugh C, Goings G, Luo X, Miller SD. Ethylenecarbodiimide-treated splenocytes carrying male CD4 epitopes confer histocompatibility Y chromosome antigen transplant protection by inhibiting CD154 upregulation. *J Immunol*. (2010) 185:3326–36. doi: 10.4049/jimmunol.1000802

172. Turley DM, Miller SD. Peripheral tolerance induction using ethylenecarbodiimide-fixed APCs uses both direct and indirect mechanisms of antigen presentation for prevention of experimental autoimmune encephalomyelitis. *J Immunol*. (2007) 178:2212–20. doi: 10.4049/jimmunol.178.4.2212

173. Nilsson B, Ekdahl KN, Korsgren O. Control of instant blood-mediated inflammatory reaction to improve islets of Langerhans engraftment. *Curr Opin Organ Transplant*. (2011) 16:620–6. doi: 10.1097/MOT.0b013e32834c2393

174. Liuwantara D, Chew YV, Favaloro EJ, Hawkes JM, Burns HL, O'Connell PJ, et al. Characterizing the mechanistic pathways of the instant blood-mediated inflammatory reaction in xenogeneic neonatal islet cell transplantation. *Transplant Direct*. (2016) 2:e77. doi: 10.1097/TXD.0000000000000590

175. Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. *Nat Immunol*. (2010) 11:785–97. doi: 10.1038/ni.1923

176. Ehlers MR. CR3: a general purpose adhesion-recognition receptor essential for innate immunity. *Microbes Infect*. (2000) 2:289–94. doi: 10.1016/S1286-4579(00)00299-9

177. Lachmann PJ. The amplification loop of the complement pathways. *Adv Immunol*. (2009) 104:115–49. doi: 10.1016/S0065-2776(08)04004-2

178. Amara U, Flierl MA, Rittirsch D, Klos A, Chen H, Acker B, et al. Molecular intercommunication between the complement and coagulation systems. *J Immunol*. (2010) 185:5628–36. doi: 10.4049/jimmunol.0903678

179. Oikonomopoulou K, Ricklin D, Ward PA, Lambris JD. Interactions between coagulation and complement–their role in inflammation. *Semin Immunopathol*. (2012) 34:151–65. doi: 10.1007/s00281-011-0280-x

180. Gaboriaud C, Ling WL, Thielens NM, Bally I, Rossi V. Deciphering the fine details of c1 assembly and activation mechanisms: "mission impossible"? *Front Immunol*. (2014) 5:565. doi: 10.3389/fimmu.2014.00565

181. Gál P, Barna L, Kocsis A, Závodszky P. Serine proteases of the classical and lectin pathways: similarities and differences. *Immunobiology*. (2007) 212:267–77. doi: 10.1016/j.imbio.2006.11.002

182. Thurman JM, Holers VM. The central role of the alternative complement pathway in human disease. *J Immunol*. (2006) 176:1305–10. doi: 10.4049/jimmunol.176.3.1305

183. Ricklin D. Manipulating the mediator: modulation of the alternative complement pathway C3 convertase in health, disease and therapy. *Immunobiology*. (2012) 217:1057–66. doi: 10.1016/j.imbio.2012.07.016

184. Alcorlo M, Tortajada A, Rodríguez de Córdoba S, Llorca O. Structural basis for the stabilization of the complement alternative pathway C3 convertase by properdin. *Proc Natl Acad Sci U.S.A.* (2013) 110:13504–9. doi: 10.1073/pnas.1309618110

185. Hourcade DE. The role of properdin in the assembly of the alternative pathway C3 convertases of complement. *J Biol Chem*. (2006) 281:2128–32. doi: 10.1074/jbc.M508928200

186. Lesher AM, Nilsson B, Song WC. Properdin in complement activation and tissue injury. *Mol Immunol*. (2013) 56:191–8. doi: 10.1016/j.molimm.2013.06.002

187. Rawal N, Pangburn MK. Structure/function of C5 convertases of complement. *Int Immunopharmacol*. (2001) 1:415–22. doi: 10.1016/S1567-5769(00)00039-4

188. Bubeck D. The making of a macromolecular machine: assembly of the membrane attack complex. *Biochemistry*. (2014) 53:1908–15. doi: 10.1021/bi500157z

189. Klos A, Tenner AJ, Johswich KO, Ager RR, Reis ES, Köhl J. The role of the anaphylatoxins in health and disease. *Mol Immunol*. (2009) 46:2753–66. doi: 10.1016/j.molimm.2009.04.027

190. Williams JC, Mackman N. Tissue factor in health and disease. *Front Biosci (Elite Ed)*. (2012) 4:358–72. doi: 10.2741/e383

191. Owens AP 3rd, Mackman N. Tissue factor and thrombosis: The clot starts here. *Thromb Haemost*. (2010) 104:432–9. doi: 10.1160/TH09-11-0771

192. Saha D, S S, Sergeeva EG, Ionova ZI, Gorbach AV. Tissue factor and atherothrombosis. *Curr Pharm Des*. (2015) 21:1152–7. doi: 10.2174/1381612820666141013154946

193. Samad F, Ruf W. Inflammation, obesity, and thrombosis. *Blood*. (2013) 122:3415–22. doi: 10.1182/blood-2013-05-427708

194. Kourtzelis I, Markiewski MM, Doumas M, Rafail S, Kambas K, Mitroulis I, et al. Complement anaphylatoxin C5a contributes to hemodialysis-associated thrombosis. *Blood*. (2010) 116:631–9. doi: 10.1182/blood-2010-01-264051

195. Edgington TS, Mackman N, Brand K, Ruf W. The structural biology of expression and function of tissue factor. *Thromb Haemost*. (1991) 66:67–79.

196. Walker RK, Krishnaswamy S. The activation of prothrombin by the prothrombinase complex. The contribution of the substrate-membrane interaction to catalysis. *J Biol Chem*. (1994) 269:27441–50. doi: 10.1016/S0021-9258(18)47005-6

197. Mosesson MW. Fibrinogen and fibrin structure and functions. *J Thromb Haemost*. (2005) 3:1894–904. doi: 10.1111/j.1538-7836.2005.01365.x

198. Bennet W, Sundberg B, Lundgren T, Tibell A, Groth CG, Richards A, et al. Damage to porcine islets of Langerhans after exposure to human blood *in vitro*, or after intraportal transplantation to cynomologus monkeys: protective effects of sCR1 and heparin. *Transplantation*. (2000) 69:711–9. doi: 10.1097/00007890-200003150-00007

199. Johansson H, Lukinius A, Moberg L, Lundgren T, Berne C, Foss A, et al. Tissue factor produced by the endocrine cells of the islets of Langerhans is associated with a negative outcome of clinical islet transplantation. *Diabetes*. (2005) 54:1755–62. doi: 10.2337/diabetes.54.6.1755

200. Davie EW, Fujikawa K, Kisiel W. The coagulation cascade: initiation, maintenance, and regulation. *Biochemistry*. (1991) 30:10363–70. doi: 10.1021/bi00107a001

201. Hirsh J. Current anticoagulant therapy–unmet clinical needs. *Thromb Res*. (2003) 109 Suppl 1:S1–8. doi: 10.1016/S0049-3848(03)00250-0

202. Bouwens EA, Stavenuiter F, Mosnier LO. Mechanisms of anticoagulant and cytoprotective actions of the protein C pathway. *J Thromb Haemost*. (2013) 11 Suppl 1:242–53. doi: 10.1111/jth.12247

203. Wood JP, Ellery PE, Maroney SA, Mast AE. Biology of tissue factor pathway inhibitor. *Blood*. (2014) 123:2934–43. doi: 10.1182/blood-2013-11-512764

204. Sadler JE. Thrombomodulin structure and function. *Thromb Haemost*. (1997) 78:392–5.

205. Mosnier LO, Meijers JC, Bouma BN. Regulation of fibrinolysis in plasma by TAFI and protein C is dependent on the concentration of thrombomodulin. *Thromb Haemost*. (2001) 85:5–11.

206. Roussel JC, Moran CJ, Salvaris EJ, Nandurkar HH, d'Apice AJ, Cowan PJ. Pig thrombomodulin binds human thrombin but is a poor cofactor for activation of human protein C and TAFI. *Am J Transplant*. (2008) 8:1101–12. doi: 10.1111/j.1600-6143.2008.02210.x

207. Wuensch A, Baehr A, Bongoni AK, Kemter E, Blutke A, Baars W, et al. Regulatory sequences of the porcine THBD gene facilitate endothelial-specific expression of bioactive human thrombomodulin in single- and multitransgenic pigs. *Transplantation*. (2014) 97:138–47. doi: 10.1097/TP.0b013e3182a95cbc

208. Wang S, Zhao Z, Cong Z, Suo G. Thrombin-activatable fibrinolysis inhibitor is activated in an instant blood-mediated inflammatory reaction after intraportal islet transplant. *Exp Clin Transplant*. (2014) 12:62–6. doi: 10.6002/ect

209. Hawthorne WJ, Salvaris EJ, Phillips P, Hawkes J, Liuwantara D, Burns H, et al. Control of IBMIR in neonatal porcine islet xenotransplantation in baboons. *Am J Transplant*. (2014) 14:1300–9. doi: 10.1111/ajt.12722

210. Tjernberg J, Ekdahl KN, Lambris JD, Korsgren O, Nilsson B. Acute antibody-mediated complement activation mediates lysis of pancreatic islets cells and may cause tissue loss in clinical islet transplantation. *Transplantation*. (2008) 85:1193–9. doi: 10.1097/TP.0b013e31816b22f3

211. Goto M, Johansson H, Maeda A, Elgue G, Korsgren O, Nilsson B. Low molecular weight dextran sulfate prevents the instant blood-mediated inflammatory reaction induced by adult porcine islets. *Transplantation*. (2004) 77:741–7. doi: 10.1097/01.TP.0000114872.26990.4F

212. Biarnés M, Montolio M, Nacher V, Raurell M, Soler J, Montanya E. Beta-cell death and mass in syngeneically transplanted islets exposed to short- and long-term hyperglycemia. *Diabetes*. (2002) 51:66–72. doi: 10.2337/diabetes.51.1.66

213. van der Windt DJ, Bottino R, Casu A, Campanile N, Cooper DK. Rapid loss of intraportally transplanted islets: an overview of pathophysiology and preventive strategies. *Xenotransplantation*. (2007) 14:288–97. doi: 10.1111/j.1399-3089.2007.00419.x

214. Harlan DM, Kenyon NS, Korsgren O, Roep BO. Current advances and travails in islet transplantation. *Diabetes*. (2009) 58:2175–84. doi: 10.2337/db09-0476

215. van der Windt DJ, Bottino R, Kumar G, Wijkstrom M, Hara H, Ezzelarab M, et al. Clinical islet xenotransplantation: how close are we? *Diabetes*. (2012) 61:3046–55. doi: 10.2337/db12-0033

216. Tai JH, Sun H, Liu W, Melling CW, Hasilo C, White DJ. Isolating human islets of Langerhans causes loss of decay accelerating factor (CD55) on beta-cells. *Cell Transplant*. (2008) 17:1349–59. doi: 10.3727/096368908787648092

217. Ji M, Yi S, Smith-Hurst H, Phillips P, Wu J, Hawthorne W, et al. The importance of tissue factor expression by porcine NICC in triggering IBMIR in the xenograft setting. *Transplantation*. (2011) 91:841–6. doi: 10.1097/TP.0b013e3182106091

218. Ma X, Ye B, Gao F, Liang Q, Dong Q, Liu Y, et al. Tissue factor knockdown in porcine islets: an effective approach to suppressing the instant blood-mediated inflammatory reaction. *Cell Transplant*. (2012) 21:61–71. doi: 10.3727/096368911X580563

219. Berman DM, Cabrera O, Kenyon NM, Miller J, Tam SH, Khandekar VS, et al. Interference with tissue factor prolongs intrahepatic islet allograft survival in a nonhuman primate marginal mass model. *Transplantation*. (2007) 84:308–15. doi: 10.1097/01.tp.0000275401.80187.1e

220. Jung DY, Park JB, Joo SY, Joh JW, Kwon CH, Kwon GY, et al. Effect of nicotinamide on early graft failure following intraportal islet transplantation. *Exp Mol Med*. (2009) 41:782–92. doi: 10.3858/emm.2009.41.11.084

221. Matsumoto S, Tan P, Baker J, Durbin K, Tomiya M, Azuma K, et al. Clinical porcine islet xenotransplantation under comprehensive regulation. *Transplant Proc*. (2014) 46:1992–5. doi: 10.1016/j.transproceed.2014.06.008

222. Yamada K, Sykes M, Sachs DH. Tolerance in xenotransplantation. *Curr Opin Organ Transplant*. (2017) 22:522–8. doi: 10.1097/MOT.0000000000000466

223. Lee FT, Dangi A, Shah S, Burnette M, Yang YG, Kirk AD, et al. Rejection of xenogeneic porcine islets in humanized mice is characterized by graft-infiltrating Th17 cells and activated B cells. *Am J Transplant*. (2020) 20:1538–50. doi: 10.1111/ajt.15763

224. Weber MS, Prod'homme T, Patarroyo JC, Molnarfi N, Karnezis T, Lehmann-Horn K, et al. B-cell activation influences T-cell polarization and outcome of anti-CD20 B-cell depletion in central nervous system autoimmunity. *Ann Neurol*. (2010) 68:369–83. doi: 10.1002/ana.22081

225. Zhong X, Gao W, Degauque N, Bai C, Lu Y, Kenny J, et al. Reciprocal generation of Th1/Th17 and T(reg) cells by B1 and B2 B cells. *Eur J Immunol*. (2007) 37:2400–4. doi: 10.1002/eji.200737296

226. Zhong X, Lau S, Bai C, Degauque N, Holodick NE, Steven SJ, et al. A novel subpopulation of B-1 cells is enriched with autoreactivity in normal and lupus-prone mice. *Arthritis Rheum*. (2009) 60:3734–43. doi: 10.1002/art.25015

227. Mitsdoerffer M, Lee Y, Jäger A, Kim HJ, Korn T, Kolls JK, et al. Proinflammatory T helper type 17 cells are effective B-cell helpers. *Proc Natl Acad Sci U.S.A*. (2010) 107:14292–7. doi: 10.1073/pnas.1009234107

228. Graham KL, Krishnamurthy B, Fynch S, Ayala-Perez R, Slattery RM, Santamaria P, et al. Intra-islet proliferation of cytotoxic T lymphocytes contributes to insulitis progression. *Eur J Immunol*. (2012) 42:1717–22. doi: 10.1002/eji.201242435

229. Calafiore R, Basta G. Clinical application of microencapsulated islets: actual prospectives on progress and challenges. *Adv Drug Delivery Rev*. (2014) 67-68:84–92. doi: 10.1016/j.addr.2013.09.020

230. Veiseh O, Doloff JC, Ma M, Vegas AJ, Tam HH, Bader AR, et al. Size- and shape-dependent foreign body immune response to materials implanted in rodents and non-human primates. *Nat Mater*. (2015) 14:643–51. doi: 10.1038/nmat4290

231. Krishnan R, Alexander M, Robles L, Foster CE 3rd, Lakey JR. Islet and stem cell encapsulation for clinical transplantation. *Rev Diabetes Stud*. (2014) 11:84–101. doi: 10.1900/RDS.2014.11.84

232. Bottino R, Knoll MF, Knoll CA, Bertera S, Trucco MM. The future of islet transplantation is now. *Front Med (Lausanne)*. (2018) 5:202. doi: 10.3389/fmed.2018.00202

233. Merani S, Toso C, Emamaullee J, Shapiro AM. Optimal implantation site for pancreatic islet transplantation. *Br J Surg*. (2008) 95:1449–61. doi: 10.1002/bjs.6391

234. Van Der Windt DJ, Echeverri GJ, Ijzermans JNM, Cooper DKC. The choice of anatomical site for islet transplantation. *Cell Transplant*. (2008) 17:1005–14. doi: 10.3727/096368908786991515

235. Pepper AR, Gala-Lopez B, Pawlick R, Merani S, Kin T, Shapiro AM. A prevascularized subcutaneous device-less site for islet and cellular transplantation. *Nat Biotechnol*. (2015) 33:518–23. doi: 10.1038/nbt.3211

236. Pareta R, McQuilling JP, Sittadjody S, Jenkins R, Bowden S, Orlando G, et al. Long-term function of islets encapsulated in a redesigned alginate microcapsule construct in omentum pouches of immune-competent diabetic rats. *Pancreas*. (2014) 43:605–13. doi: 10.1097/MPA.0000000000000107

237. Shimoda M, Matsumoto S. Microencapsulation in clinical islet xenotransplantation. *Methods Mol Biol*. (2017) 1479:335–45. doi: 10.1007/978-1-4939-6364-5_25

238. Groth CG, Korsgren O, Tibell A, Tollemar J, Möller E, Bolinder J, et al. Transplantation of porcine fetal pancreas to diabetic patients. *Lancet*. (1994) 344:1402–4. doi: 10.1016/S0140-6736(94)90570-3

239. Cardona K, Korbutt GS, Milas Z, Lyon J, Cano J, Jiang W, et al. Long-term survival of neonatal porcine islets in nonhuman primates by targeting costimulation pathways. *Nat Med*. (2006) 12:304–6. doi: 10.1038/nm1375

240. Hering BJ, Wijkstrom M, Graham ML, Hårdstedt M, Aasheim TC, Jie T, et al. Prolonged diabetes reversal after intraportal xenotransplantation of wild-type porcine islets in immunosuppressed nonhuman primates. *Nat Med*. (2006) 12:301–3. doi: 10.1038/nm1369

241. van der Windt DJ, Bottino R, Casu A, Campanile N, Smetanka C, He J, et al. Long-term controlled normoglycemia in diabetic non-human primates after transplantation with hCD46 transgenic porcine islets. *Am J Transplant*. (2009) 9:2716–26. doi: 10.1111/j.1600-6143.2009.02850.x

242. Bottino R, Wijkstrom M, van der Windt DJ, Hara H, Ezzelarab M, Murase N, et al. Pig-to-monkey islet xenotransplantation using multi-transgenic pigs. *Am J Transplant*. (2014) 14:2275–87. doi: 10.1111/ajt.12868

243. Wijkstrom M, Bottino R, Iwase H, Hara H, Ekser B, van der Windt D, et al. Glucose metabolism in pigs expressing human genes under an insulin promoter. *Xenotransplantation*. (2015) 22:70–9. doi: 10.1111/xen.12145

244. Shin JS, Kim JM, Kim JS, Min BH, Kim YH, Kim HJ, et al. Long-term control of diabetes in immunosuppressed nonhuman primates (NHP) by the transplantation of adult porcine islets. *Am J Transplant*. (2015) 15:2837–50. doi: 10.1111/ajt.13345

245. Bottino R, Knoll MF, Graeme-Wilson J, Klein EC, Ayares D, Trucco M, et al. Safe use of anti-CD154 monoclonal antibody in pig islet xenotransplantation in monkeys. *Xenotransplantation*. (2017) 24(1). doi: 10.1111/xen.12283

246. Iwase H, Hara H, Ezzelarab M, Li T, Zhang Z, Gao B, et al. Immunological and physiological observations in baboons with life-supporting genetically engineered pig kidney grafts. *Xenotransplantation*. (2017) 24(2). doi: 10.1111/xen.12293

247. Mohiuddin MM, Singh AK, Corcoran PC, Thomas Iii ML, Clark T, Lewis BG, et al. Chimeric 2C10R4 anti-CD40 antibody therapy is critical for long-term survival of GTKO.hCD46.hTBM pig-to-primate cardiac xenograft. *Nat Commun*. (2016) 7:11138. doi: 10.1038/ncomms11138

248. Ellis CE, Korbutt GS. Justifying clinical trials for porcine islet xenotransplantation. *Xenotransplantation*. (2015) 22:336–44. doi: 10.1111/xen.12196

249. Potter KJ, Abedini A, Marek P, Klimek AM, Butterworth S, Driscoll M, et al. Islet amyloid deposition limits the viability of human islet grafts but not porcine islet grafts. *Proc Natl Acad Sci U.S.A*. (2010) 107:4305–10. doi: 10.1073/pnas.0909024107

250. Zhang X, Cheng B, Gong H, Li C, Chen H, Zheng L, et al. Porcine islet amyloid polypeptide fragments are refractory to amyloid formation. *FEBS Lett*. (2011) 585:71–7. doi: 10.1016/j.febslet.2010.11.050

251. Kobayashi T, Aomatsu Y, Iwata H, Kin T, Kanehiro H, Hisanga M, et al. Survival of microencapsulated islets at 400 days posttransplantation in the omental pouch of NOD mice. *Cell Transplant*. (2006) 15:359–65. doi: 10.3727/000000006783981954

252. Ludwig B, Reichel A, Steffen A, Zimerman B, Schally AV, Block NL, et al. Transplantation of human islets without immunosuppression. *Proc Natl Acad Sci U.S.A*. (2013) 110:19054–8. doi: 10.1073/pnas.1317561110

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