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Monitoring regulatory T cells as a prognostic marker in lung transplantation

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Lung transplantation is the major surgical procedure, which restores normal lung functioning and provides years of life for patients suffering from major lung diseases. Lung transplant recipients are at high risk of primary graft dysfunction, and chronic lung allograft dysfunction (CLAD) in the form of bronchiolitis obliterative syndrome (BOS). Regulatory T cell (Treg) suppresses effector cells and clinical studies have demonstrated that Treg levels are altered in transplanted lung during BOS progression as compared to normal lung. Here, we discuss levels of Tregs/FOXP3 gene expression as a crucial prognostic biomarker of lung functions during CLAD progression in clinical lung transplant recipients. The review will also discuss Treg mediated immune tolerance, tissue repair, and therapeutic strategies for achieving *in-vivo* Treg expansion, which will be a potential therapeutic option to reduce inflammation-mediated graft injuries, taper the toxic side effects of ongoing immunosuppressants, and improve lung transplant survival rates.

KEYWORDS

regulatory T cell, chronic lung allograft dysfunction, bronchiolitis obliterans syndrome, immune tolerance, tissue repair

Introduction

Lung transplantation is a life-saving surgical procedure for patients with end-stage lung diseases. Unfortunately, this treatment strategy is limited by the occurrence of CLAD which occurs when the patient's immune system relentlessly attacks the transplanted organ, disrupts the microvascular flow, and ultimately leads to irreversible small airway fibrosis. CLAD is a major cause of mortality in the first ten years and there are no current immunosuppressive regimens that can sufficiently rescue the restoration of functional microvascular flow during rejection (Figure 1) (1, 2). For recipients, lung transplantation has led to improved quality of life and longevity but outcomes among transplant recipients are quite heterogeneous with under 60% transplant survival at 5 years and under 20% transplant survival at 10 years post-transplantation (3, 4). This concept of regulation is likely not the result of actions of a particular cellular subset, but rather the collective effect of signaling between Tregs, antigen presenting cells (APC), and metabolites which have

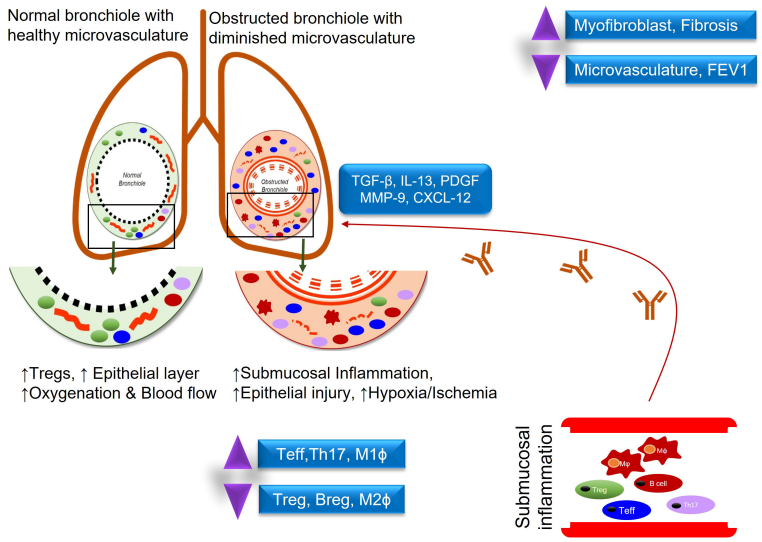

FIGURE 1
Demonstrates an inflammatory response in normal vs obstructed bronchioles after lung transplantation.

additional regulatory logistics such as the release of IL-10 or TGF-β, and a balance between Th17(IL-6, CXCL10) –Tregs (CCL22, IL-10) may foresee the risk of CLAD progression (5, 6). Once the mechanisms underlying regulation are better delineated, perhaps these processes can be augmented in all lung transplant recipients as part of a broader novel immunologic approach to transplantation.

Immunotolerance phase

The immune system guards the host against a broad range of foreign pathogenic microorganisms and tissue antigens, which involve an organized display of cellular and molecular interactions to counter-attack foreign entities through discrete recognition of antigenic peptides and thereby establish a powerful effector response, and long-term immunologic memory (7). However, this effector response remains tightly regulated, but critical tissue injuries and organ malfunctions of the host may develop during abnormal immune reactions, which involve autoimmunity, hyper-responsiveness, and organ rejections (8–12). Although, this is a very critical issue and a major challenge to drug discovery programs to establish a constant phase of immunological tolerance to avoid injuries to the host tissues, and thus key cellular and molecular signaling of immunological self-tolerance will highlight the crucial immune checkpoints to regulate powerful immune responses to transplant recipients. Treg is one of the essential immune cells, which suppress immune responses, maintain self-immunotolerance, and contribute vitally to tissue and vascular repair (13, 14). Treg can inhibit the proliferation of T cells via direct cell-cell contact, through granzyme B and perforin-mediated; or through reducing costimulatory signals and inhibiting antigen presentation (15, 16). Tregs routinely play a major role in maintaining immunological tolerance to self-antigens and suppress immune responses injurious to the transplant recipients (17–20).

Functionally mature T cell subsets-Tregs in the thymus are a unique $CD4^+$ T-cell subpopulation, which in mice is characterized by the surface expression of CD25, nuclear expression of FOXP3, and secrete IL-10, TGF-β to suppress heightened immune responses, and also trigger inducible Treg expansion (21–23). Unlike mouse, Human Treg population is highly heterogeneous, and different markers including CD3, CD4, CD25, and FOXP3 have been minimally required to define human Treg cells (24). Besides, staining for Ki67 and CD45RA showed to provide additional information on the activation status of Tregs (25). Demethylation of FOXP3 determines stable FOXP3 expression in clinical transplants, which has been widely recognized as an essential transcription factor in Tregs (26, 27). During an alloimmune inflammation, Hypoxia Inducible Factor 1 Subunit Alpha (HIF-1α) expression upregulates Th17 cells while downregulates Tregs through the binding to FOXP3 (28, 29). IL-2 plays an important role in stabilizing FOXP3 gene expression, and a high expression of the IL-2 receptor correspond dictate the effective immunosuppressive functions of Tregs (30). In response to IL-2 receptor signaling, Janus kinases (JAKs) initiate phosphorylation of Signal transducer and activator of transcription 5 (STAT5) and an activated STAT5 binds to the FOXP3 promoter and conserved non-coding sequence 2 (CNS2), signaling Treg activation (31). In addition, IL-6 induces CNS2 methylation to suppress FOXP3 expression, and IL-21 activates STAT3 to suppress FOXP3 expression, whereas TNF-a dephosphorylates & restores Treg function (32, 33) (Figure 2).

Immunosuppression

An immunosuppressive regimen remains essential for lung transplantation success, and a wide variety of immunosuppressive agents, as well as combinations of them, are available for use after lung

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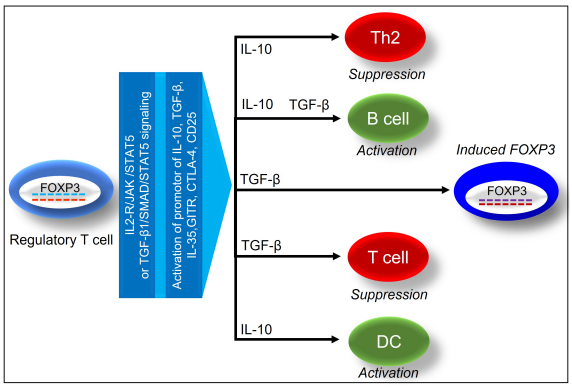

FIGURE 2
Demonstrates the immunosuppressive effects of FOXP3^{+} regulatory T cells on tissue and vascular repair.

transplantation, giving patients more personal choice (34, 35). Although these drugs are effective, their side effects can be severe, reducing a patient's life expectancy. Consequently, new immunosuppressive therapies are required that promote immune tolerance without the side effects currently observed. An effective immunosuppression can be achieved by combining various signaling pathways that work through the immunomodulation functions of various immune cells, and the selective inhibition of effector and memory T cells through these pathways could theoretically be used to decrease the amount of immunosuppressive drugs and promote the induction of tolerance (36). In addition, Treg-based immunomodulation may reduce the toxic effects associated with current immunosuppressive treatments (37–39). The utilization of this approach could be a game-changer when it comes to managing transplanted patients, improving outcomes, and reducing toxic treatments. The current immunosuppressive agents used in clinics modulate Treg activity through a variety of signaling pathways (40). Such agents are effective in controlling inflammatory conditions; however, their use is associated with several adverse effects. An immunosuppressive drug commonly used in transplantation is calcineurin inhibitors (CNIs), mammalian Target of Rapamycin inhibitors (mTOR), corticosteroids, mycophenolate preparations, anti-thymocyte globulin (ATG), anti-CD25 antibody, anti-CD52 antibody, Lymphocyte function-associated antigen-3 (LFA-3) fusion protein antibody, anti-IL-6R antibody, anti-CD28 antibody, and Cytotoxic T-lymphocyte-associated protein 4 (CTLA4) antibody (36, 41, 42). In general, immunosuppression affects immune cells of the graft, thereby playing a crucial role in tissue repair, fibrosis progression, and lung function following lung transplantation (Table 1).

Tregs and tissue repair

The cytokines and growth factors play important roles in cell proliferation, migration, and matrix synthesis, which make them critical to fundamental homeostatic and pathophysiological

TABLE 1 Various Immunosuppressants drugs and Treg levels during transplantation.

	Immunosuppressants	Target pathways	Tregs
1	Calcineurin inhibitors	↓ Calcineurin & NFAT, FOXP3	↓
	CTLA4-Ig	Blocks CD28 signaling	↓
	anti-IL-2R	Blocks IL-2 receptor signaling	↓
2	m*TOR* inhibitors	↓ mTORc1	↑
	histone deacetylases inhibitor	↓ Histone deacetylases and 1FOXP3	↑
	Low-dose IL-2	↑ IL-2R signaling on Tregs.	↑
	Rabbit anti-thymocyte globulin	T cells markers.	↑
	Anti-IL-6R	prevents IL-6/IL-6R binding	↑
3	Complement inhibitors	Blocks C5 cleavage	-
	Steroids	↓ pro-inflammatory cytokines	-
	Antiproliferative agents	Inhibit purine synthesis	-

↓ (Downregulation); ↑ Upregulation; - (No effects).

processes such as wound healing, inflammation, tissue repair and fibrosis (43). Depending on the cytokine and its role, it may be appropriate to either enhance (recombinant cytokine, gene transfer) or inhibit (cytokine or receptor antibodies, soluble receptors, signal transduction inhibitors, antisense) the cytokine to achieve the desired outcome. Consequently, cytokines, which are central to this constellation of events for coordinating multiple cell types, have become targets for therapeutic intervention to modulate the wound healing process, which is crucial to transplant survival. In wound healing, several immune cells participate in the process, including platelets, neutrophils, macrophages, fibroblasts, lymphocytes, epithelial and endothelial cells. However, Tregs, as well as their associated regulatory mediators, help to protect the tissue from inflammation (44–48). During hemostasis, platelets release transforming growth factor-β1 (TGF-β1), Platelet-derived growth factor (PDGF), fibroblast growth factor (FGF-2), and Vascular endothelial growth factor (VEGF) to recruit neutrophils and macrophages, while neutrophils release reactive oxygen species (ROS), nitric oxide (NO), proteases, VEGF, and IL-17 to destroy pathogens (49–51). Besides, NK cells secrete IFN-γ, TNF-α and also release perforins and granzymes that are cytotoxic to infected cells (52). Moreover, neutrophils release TNF-α, IL-1β, IL-6, and MCP-1, which attract monocytes and dendritic cells and activate T cells that cause Th1 pro-inflammatory responses (53). In the inflammatory phase of acute wound healing, macrophages secrete IL-1, VEGF, FGF-2, TNF- α, IL-6, IFN-γ, TGF-β, and PDGF, which promote the proliferation of fibroblasts, keratinocytes, and epithelial cells, whereas in the remodeling phase IL-4, IL-10, and IL-13 induce the transition of M1 to M2 macrophages (50, 53). Besides, other cells, such as mesenchymal stem cells (MSCs) and fibroblasts, secrete Tumor necrosis factor- (TNF) stimulated gene-6 (TSG-6), which promotes wound healing by limiting macrophage activation, inflammation, and fibrosis (54, 55). M2 macrophages generally inhibit inflammation and promote tissue repair through IL-10 and TGF-β, which stimulate extra cellular matrix (ECM) synthesis, angiogenesis, and fibroblast proliferation (44). During inflammation, lymphocytes are also recruited to the wound and release IFN- γ, TGF-β, IL-10, IL-2, IL-17, and IL-22 (56). Later, angiogenesis replaces damaged vessels with granulation tissue, in which epidermal cells, fibroblasts, vascular endothelial cells, and macrophages produce β-FGF, TGF-β, and VEGF to bolster angiogenesis (57). VEGF induces angiogenesis through adenosine, which in turn stimulates hypoxia-induced proliferation, therefore A_{2A} receptors, is now considered a potent regulator of the early stages of tissue repair caused due to overactivation of various inflammatory mediators (9, 58–60). Tregs promote tissue repair through various regulatory cytokines, which include IL-10, TGF-β, IL-33, IL-35 and amphiregulin (61–63) (Figure 2). IL-10, an anti-inflammatory cytokine, favors tissue repair, and regulate FOXP3 (64). IL-10 is a potent antifibrotic, reparative, as well as vasculo-protective cytokine that assists in the repair of tissue following a sporadic alloimmune response during transplantation (46, 65–71). The anti-inflammatory properties of IL-10 help to suppress the production of pro-inflammatory cytokines such as IFN- γ, IL-2, IL-3, and TNF- α by Th1 cells, mast cells, NK cells, endothelial cells, eosinophils, and macrophages (72–78).

In addition to limiting collateral tissue damage caused by uncontrolled immune responses, IL-10 helps maintain the regulatory microenvironment by upregulating TSG-6, M2 macrophages, and, tolerogenic dendritic cells (DC-10), antigen-specific T regulatory type 1 (Tr1), while suppressing Th1/Th17 effector immunity (65, 67, 68, 71, 73, 79, 80). Through the surface expression of TSG-6, FOXJ1, Fascin-1, and β-catenin proteins, IL-10 enhances microvascular supply, tissue oxygenation, and airway epithelium regeneration in allografts, further supporting the therapeutic benefits during wound healing and tissue repair (46, 65, 66, 81). The relationship between inflammation and fibrogenesis has led to IL-10 being identified as a potential antifibrotic target as well as a gatekeeper of fibrotic/antifibrotic signaling, so immune and cell-based therapies aiming to capitalize on IL-10 as a target could be effective in treating lung transplanted patients suffering from delayed would healing. These studies supported that IL-10 is vital for regenerative functions, and associated with a proportional increase in another anti-inflammatory protein TSG-6, and further upregulation of $CD4^+FOXP3^+$ Tregs, which thereby support the reestablishment of microvascular supply, tissue oxygenation, airway epithelial repair, and suppression of collagen deposition in allografts (17, 19, 46, 64, 82, 83). TSG-6 has been established to regulate pro-inflammatory cytokines and augment tissue repair in various animal models (84, 85) while suppressing inflammatory reactions triggered by ischemia in the heart and thereby limiting the destruction of cardiomyocytes (86). However, TSG-6 gene inactivation has been associated with the upregulation of inflammatory immune response, while over-expression of the TSG-6 gene has been associated with the downregulation of inflammatory responses (87–92). TSG-6 is a crucial regulatory mediator secreted by fibroblasts, monocytes, and mesenchymal stem cells to facilitate healing in an inflamed or metabolically active tissue microenvironment (92). TSG-6 is rapidly upregulated in response to inflammatory cytokines to protect tissues from inflammation, and is also involved in anti-inflammatory, antifibrotic, proangiogenic, and analgesic functions during inflammation (93). TSG-6 can modulate matrix structure and organization by upregulating several regulatory cells, such as Tregs, M2 macrophages, Matrix metalloproteinases (MMPs), and associated anti-inflammatory cytokines, thereby suppressing proinflammatory cytokines (IL-1β, IL-6, and TNF-α) and oxidative stress to prevent extensive tissue damage during inflammation (84, 94–96). IL-10 enhances microvascular supply, tissue oxygenation, and airway epithelium regeneration in allografts through the surface expression of TSG-6, further supporting the therapeutic benefits during wound healing and tissue repair (46, 65, 66, 81). The relationship between inflammation and fibrogenesis has led to IL-10 being identified as a potential antifibrotic target as well as a gatekeeper of fibrotic/antifibrotic signaling, so immune and cell-based therapies aiming to capitalize on IL-10 as a target could be effective in treating lung transplanted patients suffering from delayed would healing.

Tregs monitoring in clinical lung transplantation

However, numerous clinical studies have been investigating various biomarkers of CLAD to further improve diagnosis, and to characterize early biological processes that lead to the progression of CLAD. Presence of Tregs post lung transplantation have been documented both in peripheral blood and Bronchoalveolar lavage (BAL) samples with varying frequencies, which affected several clinical variables (97, 98). There is little information available about the long-term evolution of peripheral Tregs after lung transplantation. The aim of this review is to discuss the Treg long-term kinetics in lung transplant recipients and their relationship with several clinical variables. As reported, patients with chronic rejection had a significantly lower abundance of peripheral Tregs, while patients without chronic rejection had a significantly higher level of peripheral Tregs (99). Furthermore, these peripheral Tregs were capable of suppressing T cell proliferation and releasing IL-10 *in vitro* (100). In an investigation of peripheral blood mononuclear cells from lung transplant patients, Bharat and colleagues showed that chronic rejection was associated with a decrease in peripheral Tregs and $CD4^+$ T cells producing IL-10. In addition, Tregs were found to induce these IL-10^+ T cells *in vitro* (101). In a prospective study, the Hannover group reported the results that Tregs were associated with freedom from chronic lung allograft dysfunction at both early and late time points after transplantation (102). In a study conducted 3 weeks after transplantation, the $CD4^+CD25^{high}CD127^{Lo}$ Treg phenotype was found to inhibit chronic lung allograft dysfunction (102). Moreover, peripheral blood monocyte-derived dendritic cells of lung transplant patients without BOS expressed higher levels of (indolamine oxidase) IDO than those with BOS. It may be of even greater importance to point out that these IDO-expressing dendritic cells were capable of expanding regulatory T cells (103). Clinical studies also investigated the BAL and concluded that Treg abundance varied considerably between individuals with acute rejection and those without, and conflicting associations were identified (97, 104). It has been found, however, that decreased Treg counts in the BAL specimens are associated with BOS in a very small percentage of patients (99).

An analysis of the long-term peripheral kinetics of Tregs was performed by Piloni et al. to determine the association between Tregs and different clinical variables following lung transplantation (98). In previous studies, peripheral Tregs were found to be an important regulatory subset of lung transplant recipients. A recent study confirmed the role of Tregs in lung graft acceptance and rejection. There was a significant decline in peripheral Treg counts in CLAD patients, which demonstrated a significant correlation between the degree of this decrease and the severity of BOS (98). Lung transplants (with BOS) had significantly lower peripheral Tregs than clinically stable lung recipients, and peripheral Tregs early after Lung transplantations are responsible for a protective effect against CLAD, which is associated with a drop in Tregs, $T_{IL\text{-}10}$ cells, and an upregulation of $T_{IFN\text{-}\gamma}$ cells in Lung transplant patients (98, 101, 105). In clinical lung transplantation, the assessment of Tregs has emerged first as a tool to predict the progression of CLAD (100, 106–108), FOXP3 activation, and subsequent increase in IL-10 production has been reported in patients with stable lung functions compared to CLAD patients (103). Besides, stable patients also showed increased expression of IDO, which converts tryptophan-kynurenine, and this IDO activity has been therapeutically associated with tolerance in part through direct inhibition of T cell proliferation (109). Conversely, high plasma levels of kynurenine-tryptophan—reflecting high IDO expression—were reported in BOS patients compared to stable patients (110).

Other clinical studies also demonstrated T cell subsets within the lung and reported that patients with acute rejection had lower $CD3^+$ cells that expressed FOXP3 compared to non-rejectors (97, 104, 111, 112). At present, most clinical settings still use Donor-specific antibodies (DSA) as an only biomarker in clinical testing. A prospective cohort study of 138 patients performed Tregs analyses from peripheral blood before the transplant procedure and up to the two-year after transplantation. Treg ($CD4^+CD25^{high}$) data demonstrated that 23% of total recruited patients reflected CLAD symptoms within the two-year after transplantation, but there was no statistical difference was reported between the CLAD-free and CLAD developing patients. However, there was significant increase in the population of $CD127^{low}$, $FOXP3^+$, IL-2^+ and $CD152^+$ cells were recorded in the CLAD-free group within three-weeks post-lung transplantation. These findings suggested that increasing levels of $CD25^{high}CD127^{low}$, $CD25^{high}FOXP3^+$, and $CD25^{high}$IL-$2^{+\ of}$ $CD4^+$ T cell phenotypes with three-weeks after lung transplantation were recognized as a protective mechanism against the progression of CLAD (102).

Another clinical study reported a decrease in peripheral blood Tregs ($CD4^+CD25^{high}$) in BOS patients compared to patients with stable lung function (100). A subsequent study also demonstrated that drop in Treg ($CD4^+CD25^{high}CD127^-$) counts was directly correlated with an increased risk to CLAD progression, and frequency of Treg population was associated with the severity of BOS progression (98). Other clinical studies further demonstrated an increase in Tregs ($CD3^+CD4^+CD25^{high}CD69^-$) in peripheral blood and BAL compared to those in stable lung transplant recipients. This study concluded that stable and evolutive obstructive bronchitis (OB) were dominated by a Treg, Th1, and Th2 activation, however, compared to evolutive OB, Treg and Th2 cells predominated in stable OB conditions, which speculate that Treg could offset the Th activation seen in evolving OB and participate in maintenance of airway obstruction (113).

Similarly, another clinical study also reported the occurrence of low $CD4^+FOXP3^+$ cells in BAL samples collected from who later developed BOS (99). Besides, a higher level of regulatory $CCR7^+CD3^+CD4^+CD25^{high}FOXP3^+CD45RA^-$ T cells, which were found protective against the progression of BOS reported in lung transplant recipients (114).

In a recent investigation, CD15s was identified as a specific marker of $FOXP3^+$ effector Tregs, which suppress the immune system. An accumulation of $CD15s^+$11Tregs was reported in BAL following lung transplantation, and a comparison was made between the numbers of $CD15s^+$Tregs in BAL and those in blood. It was demonstrated that long-term lung transplant survivors accumulate a subset of Tregs expressing CD15s in the BAL, but not in the blood (115).

Tregs mediated immunotherapy

Treg is a potential therapeutic option for the targeted induction and preservation of immunotolerance (116), which is accomplished by the removal of alloreactive T effector cells, or by polarizing alloreactive effector T cells-Tregs ratio in favor of Tregs to suppress alloreactive T effector cells, and subdue graft associated injuries (17, 117, 118). Most of the current immunosuppressive options are inadequate to control early damage to microcirculation resulting in poor long-term outcomes, and therefore both preclinical and clinical data hold great promise to improve long-term outcomes post-transplantation. Several studies have shown the immunosuppressive and therapeutic efficacy of Tregs in various preclinical disease models to treat transplant-related complications (17–19, 66). The direct and indirect therapeutic benefits of Tregs have been investigated in clinical and preclinical studies (17, 18, 119), which echoed that Treg-mediated immunosuppression has been a promising area of cell-based immunotherapy for solid organ transplants (26, 37, 120, 121). Clinical studies adopting Treg mediated immunotherapy in various diseases including type 1 diabetes in children (37), and living donor liver and kidney transplantation have shown that selective augmentation of Tregs can be an effective strategy for promoting transplantation (122).

Transplantation is the last option to rescue end-stage organ failure, which is heavily dependent on the immunosuppressive (IS) medications to protect the graft against alloimmune injury. The IS drugs are non-specific, and therefore cause global immunosuppression and chronic toxicity. It is widely demonstrated that Tregs modulate alloimmune responsiveness and immunosuppress through both TCR-dependent and TCR-independent mechanisms, therefore play a vital role in maintaining immunotolerance. Treg-mediated therapy to be a promising option to taper the magnitude of immunosuppression in transplanted patients for a better long-term graft survival. There is an overwhelming preclinical data in various mouse models of transplantation have demonstrated the efficacy and safety of using Tregs in transplantation settings (11, 38, 123–127). Besides, recent clinical trials using Treg-based therapies in solid organ transplantation also offer the potential of an improved therapeutic efficacy. Although, Tregs are a promising option but the success of Treg based therapy is marred by various limitations. Several cell surface markers have been tested to isolate high purity Tregs from both peripheral/cord blood, selected Tregs should retain their phenotype: of $CD4^+CD127^{low}CD25^+FOXP3^+CD62L^{hi}CCR7^+$ T expressing phenotype for an effective therapeutic candidate. Besides, these phenotype Tregs also display high and sustained FOXP3 and Helios expression and expanded cells should be able to suppress adult peripheral blood T cell proliferation in co-culture assays, retain their purity >95% & viability >90%. In most clinical trials of solid organ transplantation, varying doses of mainly polyclonal Tregs (0.5M-7M) have been tested successfully without any side effects (128). The clinical efficacy and safety of Treg mediated immunotherapy has been successfully tested in liver and kidney transplantation, but not yet in lung transplantation (39, 129). However, e*x vivo* delivery of regulatory T cells for control of alloimmune priming in the donor lung has been tested under pretransplant conditions, which concluded that pre-transplant Treg administration can inhibit alloimmunity within the lung allograft at early time points post-transplant (40).

Future research

The use of Treg-based immunotherapy to promote tolerance in various solid organ transplantations has emerged as a promising approach. The number, metabolism and function of Treg cells are tightly regulated by numerous costimulatory signals and the associated cytokine signaling (130). As a result, it keeps a delicate balance between immunosuppression and excessive immune activation or autoimmunity. Apart from polyclonal Tregs, there are currently numerous new techniques that have been adopted to make produce antigen specific Tregs *in vitro*, which are therapeutically more effective than polyclonal Tregs. Besides, Chimeric Antigen Receptor (CAR)-expressing Tregs and engineered TCRs, and overexpression of FOXP3 platforms have been introduced to produce antigen-specific Tregs, and preclinical results recorded very encouraging results (131–133). Various clinical trials remain compromised by an inability to manufacture a sufficient Treg cell dose; therefore, it is essential to harness the reparative and regulatory potential of Tregs *in-vivo*. In preclinical studies, a variety of therapeutic options have been used to expand Tregs *in vivo*, including costimulatory and coinhibitory signals, such as abatacept/belatacept primary target CD28, CTLA4, PD-1, ICOS, cytokine signaling, and CAR-Tregs (134–137). Blocking CD28/CTLA4-B7 and CD40-CD154 is one of the most extensively studied costimulation pathways using CTLA4-Ig and MR1 (138–141). CTLA4-Ig, alone or in combination with TCR ligation, exhibits therapeutic efficacy by conversing naive T cells into $FOXP3^+$ T cells and by expanding their numbers, thus favoring graft survival (142). Several preclinical studies led to the development of abatacept, which has now been approved for the prevention of Graft-versus-host disease GvHD (143). IL-2 plays a vital role in Treg generation, survival, stability, and function, and signaling via the IL-2 receptor and activation of STAT5 signaling pathways can be utilized to promote Treg expansion *in vivo* (144, 145). A number of mediators have been reported to stimulate Tregs, which include TSG-6, TGF-β, IL-5, IL-9, IL-10, IL-27, IL-35 and IL-33 to facilities immune tolerance and repair process (54, 146–151). In addition to cytokines, some growth factors, especially TSG-6, play crucial role in modulating Treg levels during tissue repair. TSG-6 has been tested extensively in preclinical studies, which demonstrated its positive effects on wound healing and tissue repair (81, 84, 91, 152). Besides, the currently available preclinical data indicates that TSG-6 can function as a potential antifibrotic and angiogenic agent and can regulate pro-inflammatory cytokines and enhance tissue repair in multiple animal models, while suppressing inflammatory reactions induced by ischemia in a variety of disease models (54, 65, 66, 91, 153). In addition to costimulatory and cytokine signaling, CAR-Tregs have been investigated to generate antigen-specific Tregs by expanding Tregs with APCs and specific antigens or engineering them with T-cell receptors. TCR-engineered Tregs are promising, but they are still MHC-restricted,

limiting individual patient application (132, 133). The single-chain variable fragment, extracellular hinge, transmembrane region, and intracellular signaling domains are used in an MHC-independent way to engineer Tregs with chimeric antigen receptor genes. In animal models, CAR-Tregs have shown great potential for treating different diseases, especially allograft rejection and various autoimmune diseases (154, 155), and CAR-Tregs are a potential choice of immunetolerance in clinical transplantation to achieve an effective immunosuppression (154).

Clinical limitations

Several preclinical studies have suggested that Treg infusion after lung transplantation may reduce acute and chronic rejection. In humans, Treg therapy may be substantially limited by several potential pitfalls. A crucial question remains, however, as to whether Treg infusions following lung transplants are safe for humans. Several early phase trials discussed above suggest that there will be no adverse effects associated with Treg infusion after lung transplantation, although no clinical trials have been initiated. Despite these vital regulatory and reparative effects of Tregs in preclinical and clinical transplantation, Treg therapy still faces crucial challenges, which include how to calculate an effective dose, antigen specificity, expansion, and large-scale production for future clinical trials.

Conclusion

We conclude that Tregs are a vital part of the immune response and play a major role in determining the transplant functioning in clinical transplantations, and $FOXP3^+$ Tregs may serve as a relevant biomarker for predicting outcomes of transplantation.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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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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