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Tissue-resident immune cells in tumor immunity and immunotherapy

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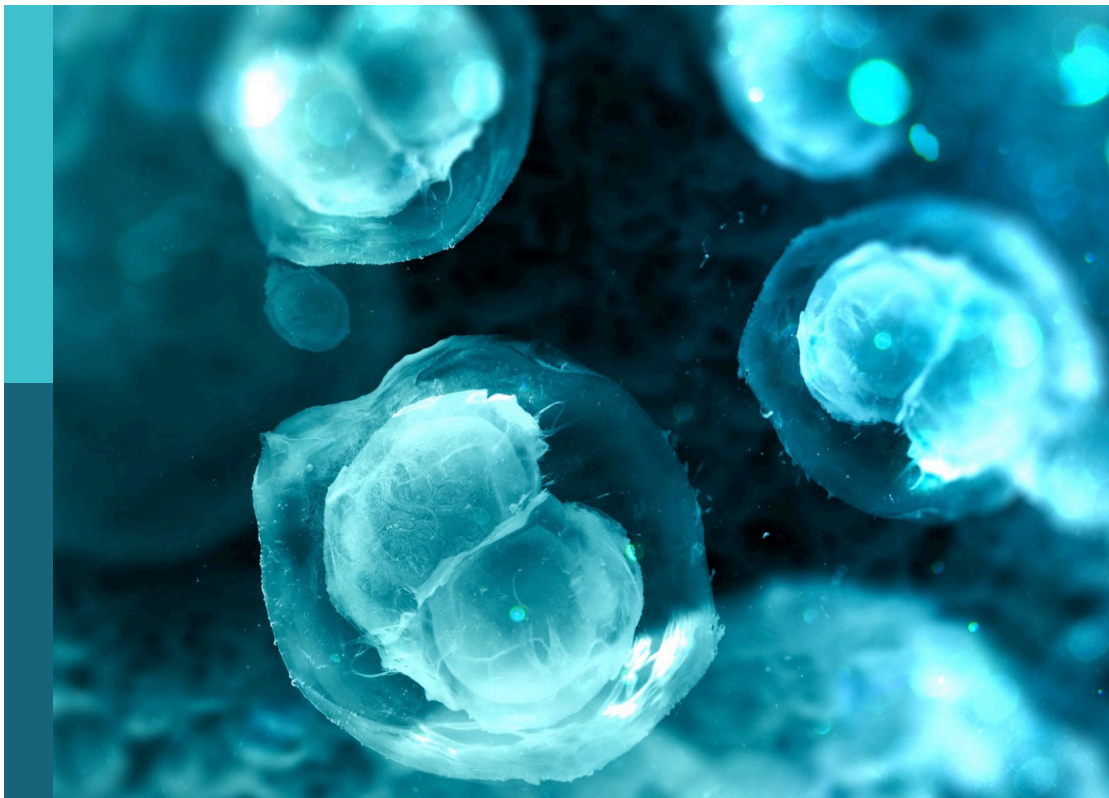

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Tissue-resident immune cells in tumor immunity and immunotherapy

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Editorial: Tissue-resident immune cells in tumor immunity and immunotherapy

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KEYWORDS

tissue-resident immune cell, macrophage, natural killer cells, dendritic cells, tertiary lymphoid structures, tumor immunity

Editorial on the Research Topic
Tissue-resident immune cells in tumor immunity and immunotherapy

Tumor microenvironment (TME) is a complex ecosystem and consists of tumor cells, multiple stromal cells and diverse immune cells (Wu et al., 2021a). From the perspective of immune cells, recent clinical practice reveals that immunotherapies have become remarkable breakthroughs in cancer treatment strategy. Clinical immunotherapies are composed of oncolytic virus, enhanced co-stimulators, vaccines, inhibitory immune checkpoint inhibitors (ICIs) and adoptive cell therapies. As a representative of ICIs, PD1/PD-L1 blockades have been approved for clinical appliance. However, they fail to generate durable responses in most patients. Our understanding of intrinsically and extrinsically immunosuppressive mechanisms for the resistance of ICIs has markedly increased (Wu et al., 2021b). Therefore, the development of strategies to reverse immunosuppressive microenvironment and surmount resistance to immunotherapy have become momentous. Tissue-resident immune cells (TRICs) are immune cells existing in non-immune organs like skin, liver, lung and gastrointestinal tract. They mainly include innate lymphoid cells, macrophages, resident memory T cells, natural killer cells, natural killer T cells, non-classical T cells, and memory B cells (Liu et al.). TRICs are deemed as a bridge that connects innate immunity to adaptive immunity. They enable to remodel the TME and can modulate tumor progression (Liu Z. et al., 2022). However, the potential mechanisms underlying TRICs to host anti-tumor immune response remain unknown. In the present Research Topic, a total of nine articles including two original articles and seven reviews are published to discuss the development, distribution, functionality and therapeutic potential of TRICs in neoplastic disease (Figure 1).

In response to foreign threats or original ontogenesis, these TRICs are capable to resident and adapt to life within diverse tissues. These site-specific immune cell compositions reflect their distinct localization within tissue niches, wherein they form an integral part of the immune sensing network, monitor for local perturbations in

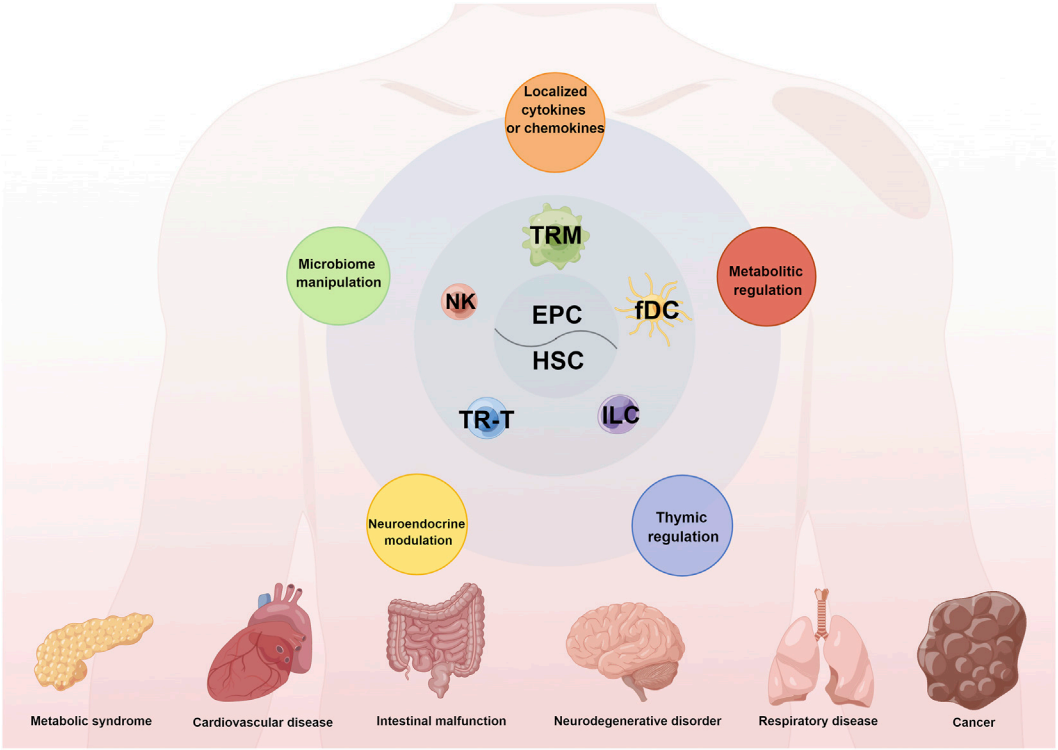

FIGURE 1
The origins, compositions, and functions of TRICs in human diseases. Tissue-resident immune cells (TRICs) mainly originate from embryonic progenitor cells (HSC) or adult haematopoietic stem cell (HSC). And TRICs mainly consist of diverse tissue-resident macrophages (TRM), NK cell, tissue-resident T (TR-T) lymphocytes, innate lymphoid cells (ILC) and follicular dendritic cells (fDC). TRICs have a vital effect on various human diseases like metabolic syndrome, cardiovascular disease and cancer through the integrated mechanisms including metabolic regulation, thymic regulation, neuroendocrine modulation, microbiome manipulation and secretion of cytokines and chemokines.

homeostasis throughout the body and provide defense against malignancies. For example, Liu et al. characterize the neuroendocrine modulation in tissue-specific immune cells, including regulations through autonomic nervous system, sensory nerves and various neuroendocrine factors. Hence, the involvement of neuroendocrine-tissue specific immunity axis in tumor biology requires to be further explored in the future and may represent a potential target for the immunotherapy of tumors.

Given the tissue-specific structure and location of TRICs, tertiary lymphoid structures (TLS) represent one of the most studied examples of TRIC aggregates. TLS are deemed as organized ectopic lymphoid organs developing in non-lymphoid tissues during chronic inflammation and cancer in response to persistent antigen stimulation (Sautès-Fridman et al., 2016). In Rossi et al., several aspects of tumor-associated TLS are described including their composition, the promoting or inhibitory mechanisms of TLS development, and their prognostic value. High level of heterogeneity has been described in TLS cellular composition, which includes innate and adaptive immune cells such as macrophages, dendritic cells, mast cells, and T and B lymphocytes which are recruited and activated by the inflammatory milieu. Upon prolonged inflammatory stimuli including cancer, TLS can present lymphoid aggregates resembling B cell follicles with germinal centers and T cell areas supported by fibroblastic reticular cells, follicular dendritic cells and high endothelial venules (Siliņa et al., 2018). TLS are the sites where continuous tumor-antigens exposures activate local and systemic T and B cell responses, which control tumor growth, as reported for different tumor types (Dieu-Nosjean et al., 2016). A crucial role is played by the non-hematopoietic stromal component, which include fibroblasts, vascular/lymphatic endothelial cells, and epithelial cells, both in maintaining the architecture of TLS and in shaping the local cross-talk with immune cells by the production of proinflammatory cytokines and chemokines, that drive the recruitment of myeloid and lymphoid cells. Among the myeloid compartment, dendritic cells, as antigen presenting cells, are considered sufficient to TLS induction and maintenance, and are associated with better prognosis in cancer (Sautès-Fridman et al., 2019). Also, macrophages and neutrophils are present on TLS, but their role need to be better elucidated. Finally, the detrimental role of Tregs in TLS is described, with the mechanisms underlying their recruitment and expansion, and the suppressive role of follicular T regs, which are considered potential

immunotherapeutic targets for improving the efficacy of ICI therapy. Furthermore, the immunosuppressive landscape induced by Treg was carefully analyzed by Riaz et al. in non-alcoholic fatty liver disease (NAFLD)-associated hepatocellular carcinoma.

Functionally, TRICs can play a dual role since, in the remodeling of the TME, they can foster or suppress tumor response (Liu Z. et al., 2022). For example, the macrophages locating in peritoneum shape a high-energy and chronic inflammatory microenvironment that favors tumor metastasis. Likewise, the peritoneal resident macrophages impair the anti-tumor response of effector T cells and compromise therapeutic efficacy of ICIs (Xia et al.). Similarly, intestinal macrophages contribute to the regeneration of intestinal epithelial cells and the immune homeostasis in intestinal mucosa. These macrophages are capable to polarize into diverse phenotype in response to the endogenous and environmental cues. Ma et al. decipher the origin and mechanisms of maintenance of intestinal macrophages. They also describe the interplays between intestinal macrophages and internal and external stimuli, and highlight the role of intestinal macrophages in the inflammatory bowel disease occurrence and development. In addition, the review by Busà et al. analyze the contribution of innate immune cells, including NK cells, dendritic cells, macrophages, myeloid-derived suppressor cells (MDSCs), and innate lymphoid cells (ILCs), in the modulation of the tumor microenvironment and in shaping the adaptive tumor response.

Immunotherapy has allowed the field of oncology to turn a critical corner. And TRICs maybe major candidates for therapeutic manipulation. As a novel immunometabolic checkpoint, adenosine interacts with its receptors named A1, A2A, A2B, and A3 to promote cancer cell proliferation, neoangiogenesis, immunoescape and metastasis. In TME, adenosine mainly derives from the degradation of ATP, ADP, UTP, and NAD through several ectonucleotidases like CD38, CD39, and CD73. Adenosine not only enable to limit the inflammatory reaction, but also suppress anti-tumor immunosurveillance. Hence, nano-drugs targeting CD39 or CD73 deserve detailed study to increase anti-cancer immune responses (Ferrari et al.). Moreover, Yu et al. generate some novel chimeric antigen receptor (CAR) T cells *via* the construction of tumor-specific cell surface antigen on CAR-T cells. For instance, they create the CAR-T cells overexpressing glucose-regulated protein 78 (GRP78) on cell surface (csGRP78). These engineered CAR-T cells high-efficiently eliminate tumor cells and prolong survival *in vitro* and *in vivo*. In addition, Zhao et al. demonstrate that the use of human or humanized antibody fragments for CAR construction can reduce the immunogenicity of the CAR and improve the therapeutic efficacy. However, these approaches need further clinical investigation. Therefore, developing the therapeutic strategies based on TRICs in the progressive disease is urgently required.

Collectively, this Research Topic describes the origins and biology of TRICs and the function of TRICs in tumorigenesis and malignant progression. It also confirms the importance of TRICs in cancer prevention or treatment and uncovers some unexplored knowledges in the field. In the future, the ontogeny of TRICs especially the key regulators involved in the process should be elaborated. The development of specific druggable targets of TRICs should be further explored and applied in a "personalized medicine" perspective.

Author contributions

ADP and QW contributed to the writing of Editorial.

Conflict of interest

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References

Dieu-Nosjean, M.-C., Giraldo, N. A., Kaplon, H., Germain, C., Fridman, W. H., and Sautès-Fridman, C. (2016). Tertiary lymphoid structures, drivers of the anti-tumor responses in human cancers. *Immunol. Rev.* 271, 260–275. doi:10.1111/imr.12405

Liu, S. Q., Gao, Z. J., Wu, J., Zheng, H. M., Li, B., Sun, S., et al. (2022a). Single-cell and spatially resolved analysis uncovers cell heterogeneity of breast cancer. *J. Hematol. Oncol.* 15, 19. doi:10.1186/s13045-022-01236-0

Liu, Z., Gao, Z., Li, B., Li, J., Ou, Y., Yu, X., et al. (2022b). Lipid-associated macrophages in the tumor-adipose microenvironment facilitate breast cancer progression. *OncoImmunology* 11, 2085432. doi:10.1080/2162402X.2022.2085432

Sautès-Fridman, C., Lawand, M., Giraldo, N. A., Kaplon, H., Germain, C., Fridman, W. H., et al. (2016). Tertiary lymphoid structures in cancers: Prognostic value, regulation, and manipulation for therapeutic intervention. *Front. Immunol.* 7, 407. doi:10.3389/fimmu.2016.00407

Sautès-Fridman, C., Petitprez, F., Calderaro, J., and Fridman, W. H. (2019). Tertiary lymphoid structures in the era of cancer immunotherapy. *Nat. Rev. Cancer* 19, 307–325. doi:10.1038/s41568-019-0144-6

Siliņa, K., Soltermann, A., Attar, F. M., Casanova, R., Uckeley, Z. M., Thut, H., et al. (2018). Germinal centers determine the prognostic relevance of tertiary lymphoid structures and are impaired by corticosteroids in lung squamous cell carcinoma. *Cancer Res.* 78, 1308–1320. doi:10.1158/0008-5472.CAN-17-1987

Wu, Q., Li, B., Li, J., Sun, S., Yuan, J., and Sun, S. (2021a). Cancer-associated adipocytes as immunomodulators in cancer. *Biomark. Res.* 9, 2. doi:10.1186/s40364-020-00257-6

Wu, Q., Yu, X., Li, J., Sun, S., and Tu, Y. (2021b). Metabolic regulation in the immune response to cancer. *Cancer Commun.* 41, 661–694. doi:10.1002/cac2.12182

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Humanized CD30-Targeted Chimeric Antigen Receptor T Cells Exhibit Potent Preclinical Activity Against Hodgkin's Lymphoma Cells

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CD30-directed chimeric antigen receptors (CARs) with single chain antibody fragment (scFv)-binding domains from murine HRS3 show strong cytotoxicity to Hodgkin's Lymphoma cells and have been used in clinical trials. However, murine scFv in CAR might induce specific rejective immune responses in patients, which compromises the therapeutic effects. The use of human or humanized antibody fragments for CAR construction, rather than those derived from mouse antibodies, can reduce the immunogenicity of the CAR. Importantly, this strategy might simultaneously decrease the risk of cytokine-mediated toxicities and improve CAR T cell persistence. Murine HRS3 antibody has been successfully humanized by grafting the complementarity-determining regions (CDRs) from the mouse antibody framework onto human immunoglobulin consensus sequences, followed by an *in vitro* evolutionary strategy to select functional Fab fragments with the same affinity as murine sources. In this study, humanized scFvs were utilized to construct a CD30-directed CAR (hHRS3-CAR), and its effectiveness was compared with that of HRS3-CAR. The hHRS3-CAR-T cells specifically kill CD30-positive tumor cell lines *in vitro* and eliminate lymphoma xenografts in immunodeficient mice with comparable efficiency to HRS3-CAR. The hHRS-CAR-T could be used in clinical trials based on the previously reported advantages of humanized CARs, such as the reduction of immune rejection and better persistence of cells.

Keywords: chimeric antigen receptor, CD30, humanized HRS3 antibody, single-chain variable fragment, Hodgkin's Lymphoma, central memory phenotype

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HIGHLIGHTS

- hHRS3-CAR-T cells were highly efficacious against CD30-positive tumor cells similar to HRS3-CAR-T cells
- hHRS3-CAR-T cells show higher central memory phenotype compared to HRS3-CAR-T cells

Abbreviations: AFM13, HRS3-based bispecific antibody; ALCL, anaplastic large cell lymphoma; CARs, chimeric antigen receptors; CAR-T, chimeric antigen receptor T-cells; CD19, cluster of differentiation 19; hHRS3-CAR, humanized HRS3 scFv-based CAR; CFSE, carboxyfluorescein succinimidyl ester; HL, Hodgkin's lymphoma; HRS3, murine anti-CD30 monoclonal antibody; HRS3-CAR, murine HRS3 scFv-based CAR; PI, Propidium iodide; scFv, single chain antibody fragments; Tcm, central memory cells; Tem, effector memory cells; TNFRSF8, tumor necrosis factor receptor superfamily member 8.

- The CD30-directed CAR-T based on humanized HRS3 scFv might decrease immunogenicity and benefit the clinical therapy

INTRODUCTION

Chimeric antigen receptors (CARs) are artificial proteins whose basic structure is composed of an antigen recognition ectodomain and activation endodomain linked by a spacer and transmembrane domain (Eshhar et al., 1993; Sadelain et al., 2013; Boyiadzis et al., 2016). These are engineered receptors used to specifically direct patient T cells to target tumor cells (Wang et al., 2019). Adoptive cell therapy with the cluster of differentiation 19 (CD19)-directed chimeric antigen receptor T-cells (CAR-T) has induced durable clinical efficacy and has been approved for clinical first-line treatment, which provides a rationale for the development of other CARs (Kochenderfer et al., 2012; Boyiadzis et al., 2016; Turtle et al., 2016b).

Tumor necrosis factor receptor superfamily member 8 (TNFRSF8) is a 120-kDa type I transmembrane glycoprotein, more commonly referred to as CD30 (van der Weyden et al., 2017). In the normal physiological state, cell surface expression of CD30 is limited to activated T, B, and natural killer lymphocytes (Wasik et al., 2013). However, it is also strongly expressed in malignant hematopoietic cells, including Hodgkin's lymphoma (HL), anaplastic large cell lymphoma (ALCL), primary cutaneous ALCL, lymphomatoid papulosis, and certain cases of transformed mycosis fungoides (Schirrmann et al., 2014; van der Weyden et al., 2017). Thus, it was regarded as an ideal therapeutic target and a wide range of agents were developed for CD30-positive malignancies (Schwarting et al., 1989; Younes et al., 2012). Brentuximab vedotin, a CD30 antibody–drug conjugates, has achieved remarkable results against HL and ALCL (Pro et al., 2012; Younes et al., 2012). Recently, it was shown that anti-CD30 CAR-T cells can induce partial or complete remission in HL and ALCL (Wang et al., 2017; Ramos et al., 2020).

Although CD30 remains an excellent target for CAR-T therapy of HL, various obstacles should be overcome to clinically improve their efficacy, such as the persistence of CAR-T cells, trafficking to tumors, and increased tumor cytotoxicity (Ramos et al., 2017; Hong et al., 2018; Grover and Savoldo, 2019; Hombach et al., 2019). The murine anti-CD30 monoclonal antibody (HRS3), and HRS3-based bispecific antibody (AFM13) and recombinant fusion protein combined with cytokines, and CAR-T have been developed and applied to the specific immunotherapy of Hodgkin's lymphoma, including CAR-T therapy (Hombach et al., 1993; Renner et al., 2000; Jahn et al., 2012; Grover and Savoldo, 2019). Currently, most single chain antibody fragments (scFvs) are murine-derived, which, owing to immunogenicity, may weaken the efficacy of the treatment (Zou et al., 2018). One strategy to reduce the immunogenicity of CAR-T cells is to humanize scFv. Humanized CAR-T cells have also received attention because they are less antigenic and have a long survival time *in vivo* (Turtle et al., 2016a; Sommermeyer et al., 2017; Rafiq et al., 2020). Starting from the previously characterized cognate HRS3 mouse monoclonal antibody, the bacterially produced functional Fab fragment was humanized by grafting the CDRs from the mouse antibody framework onto human immunoglobulin consensus sequences, followed by an *in vitro* evolutionary strategy to select functional Fab fragments with the same affinity as murine sources (Schlapschy et al., 2004). The humanized HRS3 antibody is fully functional in recognizing its native receptor antigen. CAR-T based on humanized HRS3-scFv has not been reported.

In this study, murine and humanized HRS3 scFv-based CARs (HRS3-CAR and hHRS3-CAR, respectively) were constructed. We used humanized scFvs to construct hHRS3-CARs, and its effectiveness was compared with that of HRS3-CAR.

MATERIALS AND METHODS

Cell Culture

L428 and L540 Hodgkin's lymphoma-derived cell lines were purchased from Shanghai Fudan-Zhangjiang Bio-Pharmaceutical Co., Ltd. (Shanghai, China). Cells were cultured in RPMI-1640 (Hyclone, United States) supplemented with 20% fetal bovine serum (FBS; Gibco). Raji cells were purchased from ATCC and cultured in RPMI-1640 medium containing 10% FBS. HEK293T cells were cultured in Dulbecco's modified Eagle's medium (Millipore, United States) supplemented with 10% FBS at 37°C and 5% CO_2. The cells were authenticated by via Short Tandem Repeat (STR) profiling and tested negative for *mycoplasma* contamination.

Construction of Plasmids Encoding anti-CD30 CARs

The CD19-specific single-chain antibody CD19-scFv was derived from anti-CD19 mAb as previously described (Yang et al., 2017). The VL and VH regions of murine and humanized HRS3 and HRS3 antibodies were obtained from the published article (Schlapschy et al., 2004). The extracellular domains containing VL and VH regions were synthesized and cloned into a lentiviral backbone containing other parts of CAR: a CD8 hinge spacer and transmembrane region, 4-1BB, and CD3ζ endo-domains under the control of the CMV promoter. The resulting plasmids were named HRS3-CAR and hHRS3-CAR, respectively.

Lentiviral Preparation and Transduction of T-Cells

Lentiviral DNA vectors were transfected with Lipo6000™ Transfection Reagent (Beyotime, China) according to the manufacturer's protocol. For lentivirus production, 20 μg of core plasmid together with helper plasmids (10 μg pCMVΔ8.9 and 4 μg pMD2. G) were transfected into 293T cells as described previously (Yang et al., 2018). Viral supernatants were collected 48 and 72 h after transfection and filtered through a 0.45 μm filter. After centrifugation at 25,000 rpm for 2.5 h at 4°C, the virus was suspended in 0.1% bovine serum

albumin (BSA) in PBS, dissolved overnight and stored in aliquots at 80°C.

Primary human T cells were isolated from healthy human blood, as previously described (Yang et al., 2019). T cells were cultured in advanced RPMI 1640 medium (Life Technologies, United States) containing 10% FBS (Life Technologies, United States) with 200 U/ml IL-2 (PeproTech, United States). T cells were activated by adding Dynabeads human T-activator CD3/CD28 kit (Life Technologies, United States) according to the manufacturer's instructions. After 48 h, at a 1:1 ratio, and on day 3, T cells were transduced with lentivirus (Multiplicity of infection, MOI = 20) in the presence of Lentiboost (Sirion Biotech), followed by a medium change after 24 h transfer to 24-well plates.

Cytotoxicity Assay

Cytotoxicity assays were conducted using a slightly modified version of a previously described assay, and cytokine concentrations were determined using enzyme-linked immunosorbent assay (ELISA) (Sun et al., 2019). For data analysis, target cells were labeled with carboxyfluorescein succinimidyl ester (CFSE) and dead target cells were identified as double-positive for CFSE and propidium iodide (PI) using a FACS Canto flow cytometer. For each sample, 10000 events of P1 were collected. The percentage of specific target cell death was calculated as follows: 100 × [No. of CFSE and PI double-positive cells] ÷ [(No. for CFSE-positive cells) + (No. of CFSE and PI double-positive cells)].

Flow Cytometry

Flow cytometry was performed using the standard methods (Yang et al., 2019). The following antibodies were used: PE-anti-CD3 (Clone UCHT1, BD Biosciences or Biolegend, 981002), APC-anti-CD4 (BioLegend, 357408), FITC-anti-CD8 (BioLegend, 344704), PE-anti-CD45RO (Biolegend, 304206), APC-anti-CCR7 (BioLegend, 353214), and CD30 APC (Clone BerH8, BD Biosciences). Dead target cells were identified by double staining with CFSE and PI (BD Biosciences). All experiments were analyzed using FlowJo version 10 (Tree Star, Inc.).

Xenograft Mouse Model

The protocol was approved by the Ethics Committee of the West China Hospital of Sichuan University West China Hospital. Six-to eight-week-old female NOD-Prkdcem26ll2rgem26Nju (NCG) mice were purchased from GemPharmatech Co., Ltd (Jiangsu, China) and engrafted with 2×10^5 L428-EGFP-luci cells via tail vein injection. Five days later, CAR- or control T cells that had been expanded *in vitro* for 7 days were injected intravenously. The mice were serially imaged using 2D bioluminescence imaging as previously described to determine tumor progression (Guercio et al., 2021). All captured images were analyzed using Living Image software 4.1 (PerkinElmer, United States). The animals were examined every day for survival and sacrificed when moribund for collection of tissue samples. At the end of the experiment (day 64), the remaining surviving mice were euthanized and animal samples were collected. Each experiment included 6 mice per group and was repeated twice (total $n = 12$ mice per group).

Cytokine Secretion Assays

Effector cells (2.5×10^5 NC, CD19-CAR, HRS3-CAR, or hHRS-CAR-T cells) and target cells (5×10^4) were co-cultured in the absence of IL-2 at a ratio of 5:1 in 96-well plates for 24 h. Supernatants were collected and subjected to ELISA assay (BD Biosciences, United States) measurements according to the manufacturer's instructions.

Statistical Analysis

Graphs were plotted using the GraphPad Prism 8.0 statistical software. Data were analyzed using SPSS 22.0 software (IBM, United States). Statistical differences between two groups were analyzed using the unpaired Student's t-test with Welch correction. Unless otherwise stated, data are presented as mean ± SD, and statistical significance was set at $p < 0.05$.

RESULTS

Humanized HRS3-CAR-T Cells Exert a Similar *In vitro* Anti-Lymphoma Activity Compared to HRS3-BBz T-Cells

We constructed hHRS3-CAR and HRS3-CAR for an initial validation of the humanized CAR, by cloning the scFvs of hHRS3 or HRS3, respectively, into the same CAR scaffold (**Figure 1A**) (Schlapschy et al., 2004). The CAR expressing anti-CD30(HRS3)-scFv was used as the positive control and that expressing anti-CD19-scFv was used as the negative control (CD19-CAR), as previously described (Sun et al., 2019). Comparing the amino acid sequences of VL and VH for HRS3-scFv and hHRS3-scFv, it was found that there were no significant changes in the amino acids in the VL; however, amino acid changes in the VH were apparent (**Figure 1B**).

To verify the function of hHRS-CAR-T, we selected non-HL cells Raji and U937, and the HL cells L428 and L540. We evaluated CD30 protein expression using flow cytometry. CD30 was not detected in Raji or U937 cells, but was detected in the HL cell lines (**Figure 2A**, **Supplementary Figure S1A**). Next, we examined CAR expression and evaluated the ability of CAR-T cells to mediate the lysis of $CD30^+$ HL cells (**Figure 2B**). CAR-T cells lysed $CD30^+$ L428 and L540 cells (**Figure 2C**), but not Raji and U937 cells (**Supplementary Figure S1B**). We assessed the cytotoxicity of CD30-CAR T cells using 24-h cytotoxicity assays (**Figure 2D**). T cells expressing CD19-CAR served as negative controls. CAR-T cells specifically recognized and eliminated HL cells in an effector-to-target ratio-dependent manner (**Figures 2E,F**).

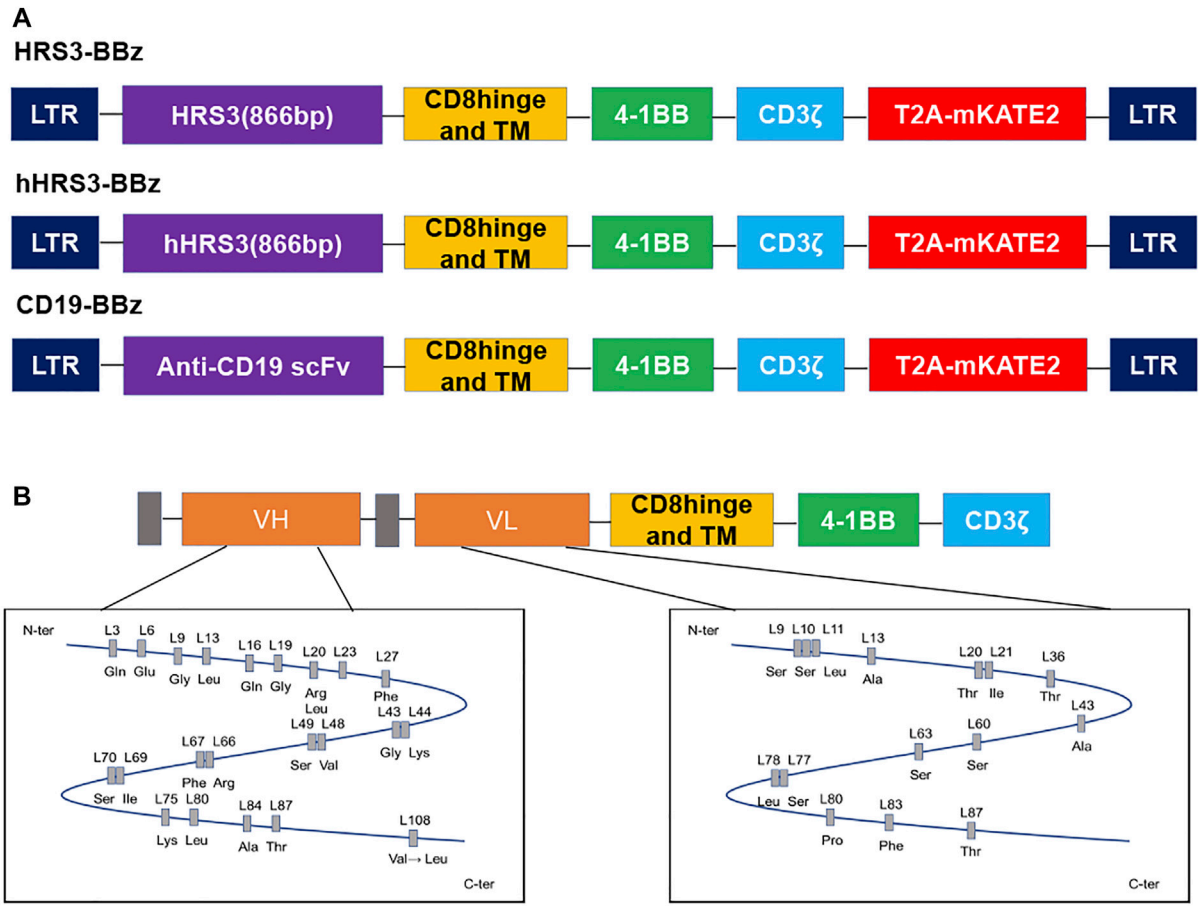

FIGURE 1 | Schematic diagram of the humanized HRS3-chimeric antigen receptor (CAR) construct and its differences from the murine receptor. **(A)** Schematic representation of CD19-CAR, HRS3-CAR, and hHRS3-CAR. All CARs had hinge, transmembrane, and CD8, and a CD3ζ T-cell activation domain. The mKATE2 is far-red fluorescent protein. In this study, the mKATE2 domain was used to trace the expression of CAR by fusing to the N-terminal of CAR via a self-cleaving T2A peptide. **(B)** Humanization of the HRS3 VL domain required 18 amino acid exchanges while retaining one amino acid from the murine framework sequence, whereas for the HRS3 VH domain, 27 amino acids were exchanged and four murine residues were retained (Schlapschy et al., 2004). The figure shows the schematic diagram of amino acids after mutation.

Humanized HRS3-CAR-T Specifically Kills CD30-Positive Cells Similar to HRS3-CAR-T

To test whether T cells expressing hHRS3-CAR were capable of specifically recognizing tumor lines expressing CD30, we co-incubated CAR-T cells with Raji cells expressing CD30 and those that did not (**Figure 2G**). CAR-T cells specifically lysed Raji-CD30 cells, but not Raji cells (**Figure 2H**). The amounts of secreted cytokines, IFN-γ and IL-2 (**Figure 2I**, **Supplementary Figure S2**), were measured. The hHRS3-CAR-T cells recognized all $CD30^+$ tumor lines and secreted high levels of IFN-γ. Very low levels of IFN-γ were observed when hHRS3-CAR-T cells were co-cultured with Raji cells. In contrast, higher levels of IFN-γ were observed when CD19-CAR-T cells were co-cultured with Raji cells.

Expanded hHRS3-CAR-T Cells Express a Higher Central Memory Phenotype

To identify the effect of CAR expression on T cell phenotypes, markers of the T cell subsets were analyzed using FACS. The CD4 to CD8 ratio of CAR^+ T cells was not significantly different between the HRS3-CAR-T and hHRS3-CAR-T cells (**Figures 3A,B**). Notably, participating effector memory (Tem) cells were significantly enriched in CD30-CAR-T cells 4 days after infection, whereas central memory (Tcm) cells were significantly enriched in CAR-T cells on the eighth day after infection (**Figures 3C,D**). The hHRS3-CAR-T cells maintained a higher proportion of $CCR7^+CD45RO^+$ Tcm cells, while HRS3-CAR-T cells had larger populations of $CCR7^-CD45RO^+$ Tem cells compared to their counterparts.

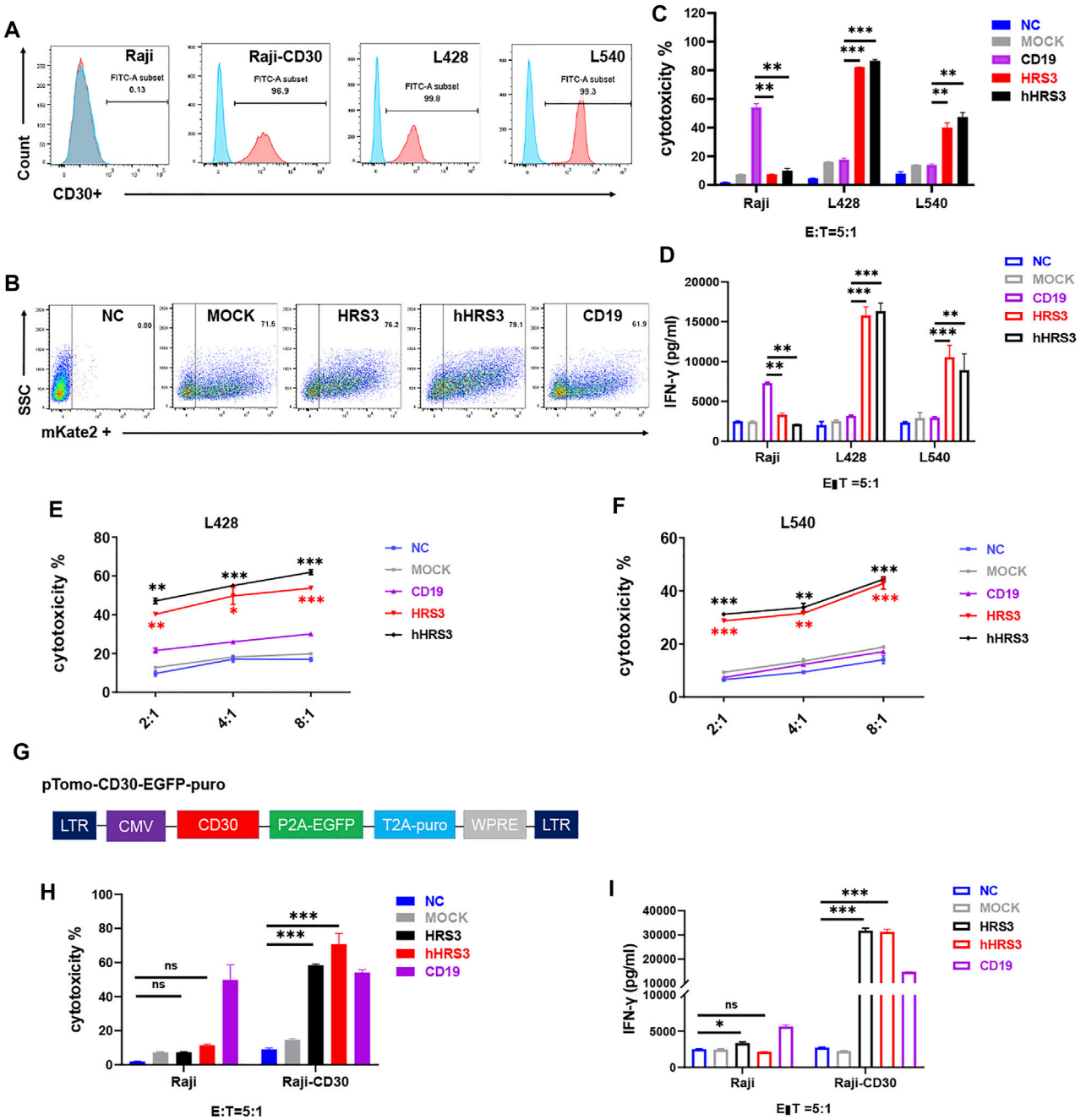

FIGURE 2 | CARs with humanized HRS3-specific scFv recognize CD30$^+$ tumor cells. **(A)** B cell lymphoma and Hodgkin's lymphoma (HL) cell lines were stained with a monoclonal antibody specific for the CD30 antigen and analyzed using flow cytometry. CD30 expression was evident in a majority of the cell lines after staining with an anti-CD30 monoclonal antibody (Pink) when compared to staining with control antibody (Blue). Raji was used as negative control. **(B)** Flow-cytometry analyses in a representative donor showing CAR expression. **(C)** T cells expressing hHRS3-CAR and HRS3-CAR exhibited cytotoxicity against the non-HL and HL cell lines in a 24-h assay. Graphs show mean ± SEM of duplicate wells. One of two representative experiments with different donor cells and similar results is shown. **(D)** IFN-γ concentrations in the supernatants of CD30-CAR-T-cells after stimulation with Raji, L428, and L540 cells for 24 h, analyzed by ELISA. **(E,F)** *In vitro* cytotoxic assay to evaluate cytolytic activity of NC T cells (Blue line), CD19-CAR-T cells (Purple line), HRS3-CAR-T cells (Red line) and hHRS3-CAR-T cells (Black line) on CD30$^+$ lymphoma cell lines, namely L428 and L540 cells. The results are presented as the mean volume ±SD, * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001 vs CD19 (CD19 as negative control). **(G)** Diagram of the full-length overexpressing human CD30 vector. **(H,I)** Detection of Raji and Raji-CD30 cell killing and cytokines (CD19 as positive control). "NC" represents uninfected T cells (negative control). "MOCK" indicates the transgenic plant that contained only the vector construct (negative control). The data were derived from different donor cells and the *in vitro* experiments were repeated independently at least three times. The results are presented as the mean volume ±SD, * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001 vs NC.

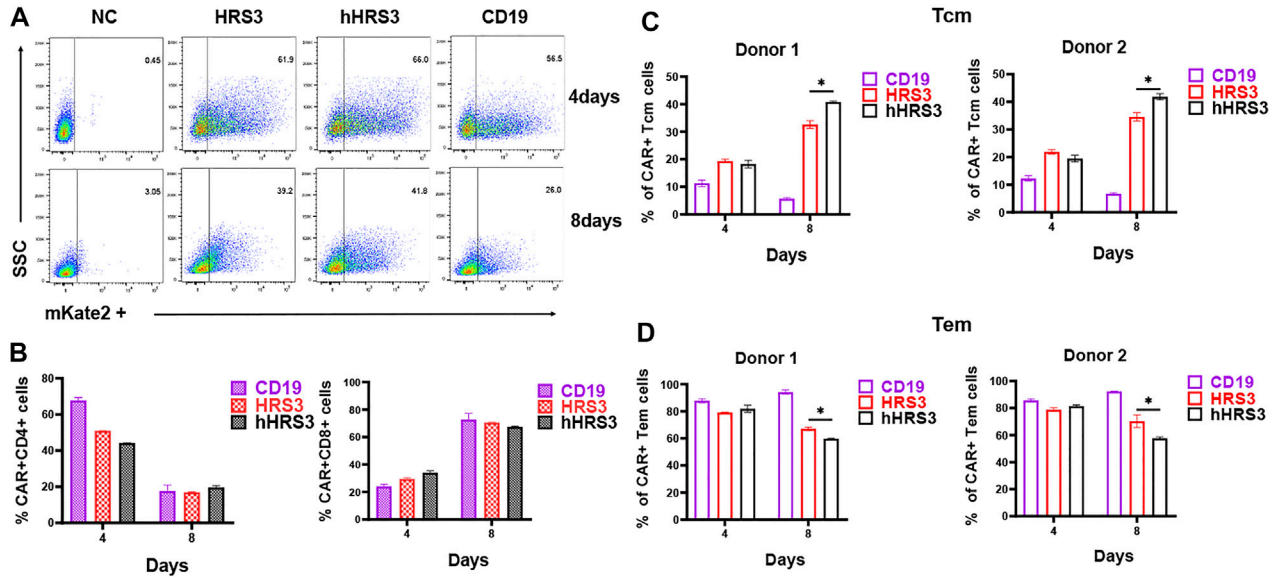

FIGURE 3 | Detection of CAR-T cell phenotypes. **(A)** Three days after culture initiation, the cells were transduced with vectors encoding the indicated CARs, or the cells were left untransduced. Cells were also stained with Protein L to detect CARs. **(B)** Four and 8 days after transduction, the cells were stained with antibodies against CD4, and CD8. There were no significant differences between T cell expression for each CAR on day 4 and 8 post-infection. **(C)** T cell subpopulations were analysed in different CARs by flow cytometry (mean ± SD). T cells are functionally divided into four subsets: central memory ($CCR7^+CD45RO^+$) and effector memory ($CCR7^-CD45RO^+$), according to the cell surface expression of CCR7 and CD45RO. The *in vitro* experiments were independently repeated at least three times using different donor cell sources in figure A, B. The data in Figure **(C,D)** were from two independent experiments with three wells repeated per experiment. The results are presented as the mean volume ±SD, * p-value < 0.05 vs HRS3.

Antitumor Responses by Humanized HRS3 Chimeric Antigen Receptor T Cells *in vivo*

To evaluate the anti-tumor activity of hHRS3-CAR-T and HRS3-CAR-T cells in murine models, NCG mice (six per group) were injected intravenously with L428 tumor cells (2×10^6 cells per mouse). After 5 days (day 0), the tumor-bearing mice were imaged using a bioluminescence imaging system for Luc expression, and the mice were randomly divided into four groups for different treatments (**Figures 4A,B**). NC T cells, CD19-CAR-T cells, HRS3-CAR-T cells, and hHRS3-CAR-T cells (1×10^7 cells per mouse) were infused intravenously as a single dose. hHRS3-CAR-T cells showed excellent efficacy comparable to HRS3-CAR cells. In contrast, CD19-CAR-T and NC T cells exhibited progressive tumor growth (**Figure 4C**). When tumor sizes were assessed 21 days after CAR-T cell infusion, we found that T cells expressing hHRS3-CAR were more effective at reducing tumor sizes than T cells expressing HRS3-CAR (**Figure 4D**); however, survival and weight change were not statistically different when treatment with hHRS3-CAR-T and HRS3-CAR-T cells was compared. Mice in the control group died after 40 days, whereas mice in the HRS3-CAR-T and hHRS3-CAR-T groups died after 63 days. Similar relative survival curves were obtained in repeated experiments, and the survival curve data from both experiments were combined for analysis (**Figure 5A**). Owing to significant liver ascites and death in the control group, body weight changes were only recorded for 35 days (**Supplementary Figure S3**). Previous studies have reported a class of HL tumor models that preferentially caused accumulation of tumors in the liver abdomen (Guercio et al., 2021). Macroscopic analysis of the sacrificed mice showed large tumor masses located preferentially in the liver (**Figure 5B**). Therefore, the anti-tumor activity of hHRS3-CAR-T cells was comparable to that of HRS3-CAR-T cells in this model.

Considering that hHRS3-CAR Tcm cells were significantly enriched *in vitro*, we evaluated its proportion *in vivo*. The results showed that the difference in Tcm cell ratio was not statistically significant in the blood, spleen, and bone marrow of mice treated with hHRS3-CAR-T cells versus HRS3-CAR (**Figures 5C–E**).

DISCUSSION

To date, several clinical trials have been conducted in adults with relapsed/refractory $CD30^+$ lymphoma using different CD30-CAR-T cells (Di Stasi et al., 2009; Wang et al., 2017). Compared with the great success achieved by CD19-redirected CAR, CD30-target CAR still has room for improvement. Previous studies have demonstrated that the transfer of autologous human T-cells expressing foreign proteins, including CARs derived from murine scFvs, can elicit cellular and humoral immune responses

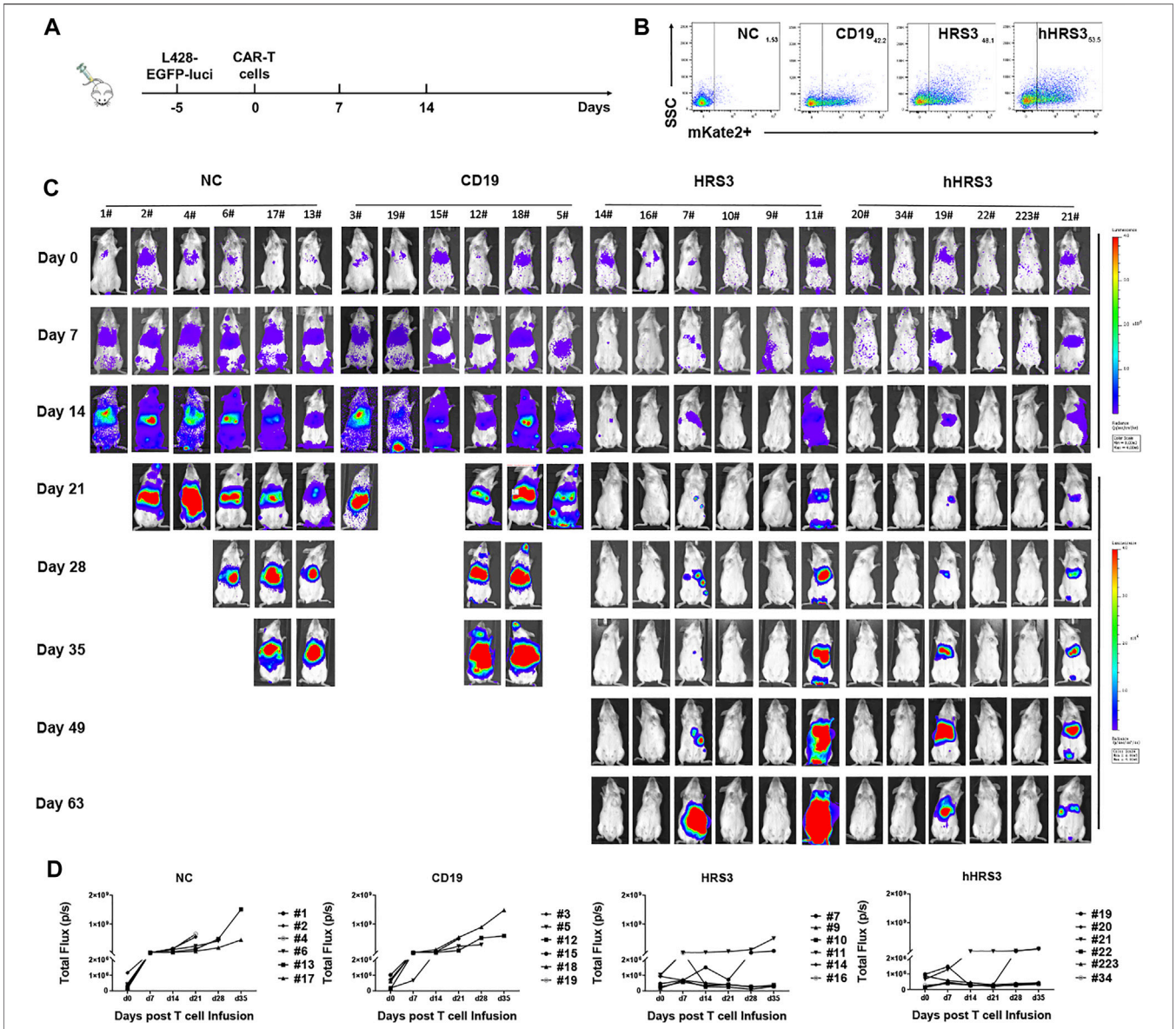

FIGURE 4 | Humanized HRS3-CAR-T cells eradicate tumors *in vivo*. **(A)** The panel shows the *in vivo* xenograft immunodeficient mouse model in which HL L428-EGFP-luci cells (2×10^6 cells per mouse) were systemically infused into NCG mice ($n = 6$ for both experimental and control groups, day -5). Effector cells or un-transduced T cells (1×10^7 cells per mouse) were infused intravenously at the time of tumor establishment (day 0), which was assessed by IVIS Imaging. **(B)** Expression of CAR-T in each group inoculated. **(C)** IVIS Imaging of tumor growth from day 0 to day 63 (end-of-imaging). **(D)** Bioluminescence of each mouse xenograft treated with NC T cells, CD19-CAR-T cells, HRS3-CAR-T cells, and hHRS3-CAR-T cells. Each experiment included 6 mice per group and was repeated twice (total $n = 12$ mice per group). Data are representative of two independent experiments.

that may compromise CAR-T effects (Lamers et al., 2006; Wang et al., 2020; Wagner et al., 2021). The use of human or humanized antibody fragments for CAR construction, rather than those derived from mouse antibodies, such as the production of modified CD19 and Her-2 CAR-T, combination of humanized or fully human fragments, and modification of the extracellular hinge region and/or transmembrane domain, can reduce the immunogenicity of the CAR (Hombach et al., 2010; Jonnalagadda et al., 2015). Importantly, this strategy might simultaneously decrease the risk of cytokine-mediated toxicity and improve CAR-T cell persistence (Sommermeyer et al., 2017; Rafiq et al., 2020).

HRS3 is derived from a murine antibody (da Costa et al., 1992; Hombach et al., 1998); humanized HRS3 scFv was derived from HRS3 mAb, which may represent another class of anti-CD30 antibodies (Schlapschy et al., 2004). Notably, previous studies

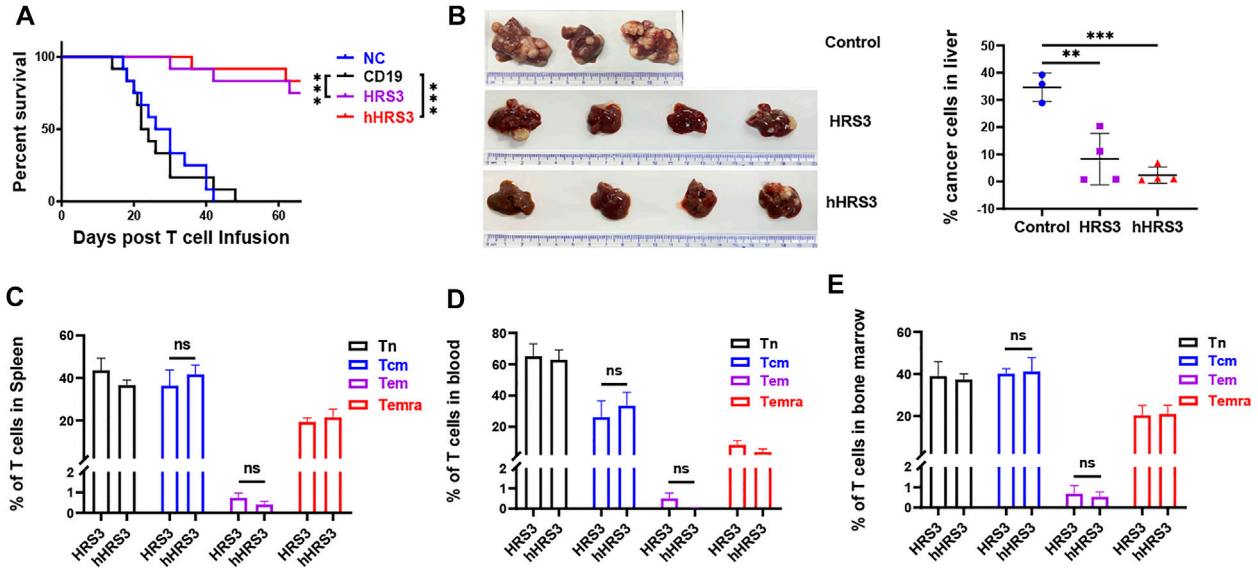

FIGURE 5 | Central memory cells (Tcm) expressing hHRS3-CAR were enriched in mice and conferred long-lasting immunity to HL. **(A)** Survival of mice is shown as a Kaplan-Meier curve (data shown combined from two independent experiments, n = 12/group). Survival was not statistically different for NC mice versus CD19; p = 0.8037. $p < 0.0001$ for the comparison of HRS3 group versus CD19 mice. $p < 0.0001$ for the comparison of hHRS3 group versus CD19 mice. NC, non-transduced T cells. **(B)** Flow cytometry detection of tumor cell involvement in the liver of mice in each group. The control group was CD19 group (n = 3), and the experimental group was HRS3 and hHRS3 groups (n = 4). **(C)** Spleen, **(D)** blood, and **(E)** bone marrow from surviving CD30-CAR-treated mice (n = 3) were analysed for the number of CAR-T cell subsets using flow cytometry (mean ± SD). T cells are functionally divided into four subsets: naïve ($CCR7^+CD45RO^-$); central memory ($CCR7^+CD45RO^+$); effector memory ($CCR7^-CD45RO^+$); and terminal effector ($CCR7^-CD45RO^-$), according to the cell surface expression of CCR7 and CD45RO. Each experiment included 6 mice per group and was repeated twice (total n = 12 mice per group). **(B)** data shown are representative or **(C–E)** combined from two independent experiments (n = 12). * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001 vs control (CD19).

have demonstrated that the humanized Fab fragment was fully functional with respect to CD30 binding; however, whether the CAR possessing this molecule as an extracellular recognition domain can safely and effectively eliminate target cells remains requires further investigation. In this study, we found that hHRS3-CAR-T cells were highly efficacious against antigen-specific tumor cells in both *in vitro* and *in vivo* with responses that were comparable to those of HRS3-CAR-T cells. Besides scFv, other elements of CAR may also produce rejection. In this study, the sequence of the hinge spacer and transmembrane domain in the CAR were all derived from human CD8α to minimize immunogenicity. Furthermore, the linker of (GGGGS)3 that connects the heavy and light chains in the scFv has been widely used in antibodies and CARs. However, these strategies do not completely eliminate immunogenicity. Even a fully human antibody fragment might also lead to immune reactions in the human; therefore, to conclusively determine whether they are nonimmunogenic requires their application in human clinical trials.

Retrospective analysis of published CAR-T cell clinical studies revealed that an elevated proportion of Tcm or less differentiated CAR-T cells provided superior antitumor efficacy, and Tcm-derived CAR-T cells are functionally superior to those made from bulk $CD8^+$ T cells (Chang et al., 2015; Tao et al., 2020). Tcm provides effective long-term memory responses because they have the capacity to persist long term in the circulation, have a high proliferative capacity, and can replenish other memory T-cell subsets, including Tem (Gehad et al., 2018; Mauriello et al., 2020). Our results showed that hHRS3-CAR could produce the higher proportion of Tcm compared to parental CAR. Similar results were also observed in previous reports (Zhao et al., 2019). However, the mechanism underlying this effect remain to be elucidated up to now. We speculate that it is due to the continuous stimulation caused by antigen, such as murine scFv, accelerating the differentiation of Tcm to Tem. Although the advantages of humanized CAR-T have been proven in clinical trials, the improvement was not significant in our experiment, which may be due to the huge differences in the immune environment in immunodeficient mice and human. For example, the humanized anti-CD19 CAR did not show a difference in the ability to kill target cells *in vitro* and in mice compared to parental CAR, but it exhibited significantly lower side effects in the patients with B-cell lymphoma (Heard et al., 2021).

In conclusion, we focused on developing a CD30-specific CAR using humanized HRS3-scFv. hHRS3-CAR-T cells specifically killed $CD30^+$ tumor cells *in vitro* with efficiency similar to that of murine HRS3-CAR-T, and these T cells significantly inhibited the established $CD30^+$ human lymphoma tumor cells in immunodeficient mice. These findings indicate that hHRS3-CAR-T cells are a promising cellular therapeutic agent and warrants further clinical exploration.

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.

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Ethics Committee of West China Hospital of Sichuan University.

AUTHOR CONTRIBUTIONS

JG, DY, and XZ: conception and design. JG and DY: methodology, validation, data collection, analysis, writing-original draft and editing. SH and WY: methodology, validation, data collection, analysis. YZ and XZ: administrative, writing, or material support.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcell.2021.775599/full#supplementary-material

REFERENCES

Boyiadzis, M., Bishop, M. R., Abonour, R., Anderson, K. C., Ansell, S. M., Avigan, D., et al. (2016). The Society for Immunotherapy of Cancer Consensus Statement on Immunotherapy for the Treatment of Hematologic Malignancies: Multiple Myeloma, Lymphoma, and Acute Leukemia. *J. Immunotherapy Cancer* 4, 90. doi:10.1186/s40425-016-0188-z

Chang, Z. L., Silver, P. A., and Chen, Y. Y. (2015). Identification and Selective Expansion of Functionally superior T Cells Expressing Chimeric Antigen Receptors. *J. Transl Med.* 13, 161. doi:10.1186/s12967-015-0519-8

da Costa, L., Carde, P., Lumbroso, J. D., Ricard, M., Pfreundschuh, M., Bosq, J., et al. (1992). Immunoscintigraphy in Hodgkin's Disease and Anaphastic Large Cell Lymphomas: Results in 18 Patients Using the Iodine Radiolabeled Monoclonal Antibody HRS-3. *Ann. Oncol.* 3 (Suppl. 4), S53–S57. doi:10.1093/annonc/3.suppl_4.s53

Di Stasi, A., De Angelis, B., Rooney, C. M., Zhang, L., Mahendravada, A., Foster, A. E., et al. (2009). T Lymphocytes Coexpressing CCR4 and a Chimeric Antigen Receptor Targeting CD30 Have Improved Homing and Antitumor Activity in a Hodgkin Tumor Model. *Blood* 113 (25), 6392–6402. doi:10.1182/blood-2009-03-209650

Eshhar, Z., Waks, T., Gross, G., and Schindler, D. G. (1993). Specific Activation and Targeting of Cytotoxic Lymphocytes through Chimeric Single Chains Consisting of Antibody-Binding Domains and the Gamma or Zeta Subunits of the Immunoglobulin and T-Cell Receptors. *Proc. Natl. Acad. Sci.* 90 (2), 720–724. doi:10.1073/pnas.90.2.720

Gehad, A., Teague, J. E., Matos, T. R., Huang, V., Yang, C., Watanabe, R., et al. (2018). A Primary Role for Human central Memory Cells in Tissue Immunosurveillance. *Blood Adv.* 2 (3), 292–298. doi:10.1182/bloodadvances.2017011346

Grover, N. S., and Savoldo, B. (2019). Challenges of Driving CD30-Directed CAR-T Cells to the Clinic. *BMC Cancer* 19 (1), 203. doi:10.1186/s12885-019-5415-9

Heard, A., Chang, J., Warrington, J. M., and Singh, N. (2021). Advances in CAR Design. *Best Pract. Res. Clin. Haematol.* 34 (3), 101304. doi:10.1016/j.beha.2021.101304

Hombach, A., Heuser, C., Sircar, R., Tillmann, T., Diehl, V., Pohl, C., et al. (1998). An Anti-CD30 Chimeric Receptor that Mediates CD3-zeta-independent T-Cell Activation against Hodgkin's Lymphoma Cells in the Presence of Soluble CD30. *Cancer Res.* 58 (6), 1116–1119.

Hombach, A. A., Rappl, G., and Abken, H. (2019). Blocking CD30 on T Cells by a Dual Specific CAR for CD30 and Colon Cancer Antigens Improves the CAR T Cell Response against CD30– Tumors. *Mol. Ther.* 27 (10), 1825–1835. doi:10.1016/j.ymthe.2019.06.007

Hombach, A., Hombach, A. A., and Abken, H. (2010). Adoptive Immunotherapy with Genetically Engineered T Cells: Modification of the IgG1 Fc 'spacer' Domain in the Extracellular Moiety of Chimeric Antigen Receptors Avoids 'off-Target' Activation and Unintended Initiation of an Innate Immune Response. *Gene Ther.* 17 (10), 1206–1213. doi:10.1038/gt.2010.91

Hombach, A., Jung, W., Pohl, C., Renner, C., Sahin, U., Schmits, R., et al. (1993). A CD16/CD30 Bispecific Monoclonal Antibody Induces Lysis of Hodgkin's Cells by Unstimulated Natural Killer cellsIn ANDIn Vivo. *Int. J. Cancer* 55 (5), 830–836. doi:10.1002/ijc.2910550523

Hong, L. K., Chen, Y., Smith, C. C., Montgomery, S. A., Vincent, B. G., Dotti, G., et al. (2018). CD30-Redirected Chimeric Antigen Receptor T Cells Target CD30+ and CD30− Embryonal Carcinoma via Antigen-dependent and Fas/FasL Interactions. *Cancer Immunol. Res.* 6 (10), 1274–1287. doi:10.1158/2326-6066.CIR-18-0065

Jahn, T., Zuther, M., Friedrichs, B., Heuser, C., Guhlke, S., Abken, H., et al. (2012). An IL12-IL2-antibody Fusion Protein Targeting Hodgkin's Lymphoma Cells Potentiates Activation of NK and T Cells for an Anti-tumor Attack. *PLoS One* 7 (9), e44482. doi:10.1371/journal.pone.0044482

Jonnalagadda, M., Mardiros, A., Urak, R., Wang, X., Hoffman, L. J., Bernanke, A., et al. (2015). Chimeric Antigen Receptors with Mutated IgG4 Fc Spacer Avoid Fc Receptor Binding and Improve T Cell Persistence and Antitumor Efficacy. *Mol. Ther.* 23 (4), 757–768. doi:10.1038/mt.2014.208

Kochenderfer, J. N., Dudley, M. E., Feldman, S. A., Wilson, W. H., Spaner, D. E., Maric, I., et al. (2012). B-cell Depletion and Remissions of Malignancy along with Cytokine-Associated Toxicity in a Clinical Trial of Anti-CD19 Chimeric-Antigen-Receptor-Transduced T Cells. *Blood* 119 (12), 2709–2720. doi:10.1182/blood-2011-10-384388

Lamers, C. H. J., Sleijfer, S., Vulto, A. G., Kruit, W. H. J., Kliffen, M., Debets, R., et al. (2006). Treatment of Metastatic Renal Cell Carcinoma with Autologous T-Lymphocytes Genetically Retargeted against Carbonic Anhydrase IX: First Clinical Experience. *Jco* 24 (13), e20–e22. doi:10.1200/JCO.2006.05.9964

Marika Guercio, M., Domenico Orlando, D., Stefano Di Cecca, S., Matilde Sinibaldi, M., Iolanda Boffa, I., Simona Caruso, S., et al. (2021). CD28.OX40 Co-stimulatory Combination Is Associated with Long *In Vivo* Persistence and High Activity of CAR.CD30 T-Cells. *haematol* 106 (4), 987–999. doi:10.3324/haematol.2019.231183

Mauriello, A., Manolio, C., Cavalluzzo, B., Avallone, A., Borrelli, M., Morabito, A., et al. (2020). Immunological Effects of Adjuvants in Subsets of Antigen Presenting Cells of Cancer Patients Undergoing Chemotherapy. *J. Transl Med.* 18 (1), 34. doi:10.1186/s12967-020-02218-x

Pro, B., Advani, R., Brice, P., Bartlett, N. L., Rosenblatt, J. D., Illidge, T., et al. (2012). Brentuximab Vedotin (SGN-35) in Patients with Relapsed or Refractory Systemic Anaplastic Large-Cell Lymphoma: Results of a Phase II Study. *Jco* 30 (18), 2190–2196. doi:10.1200/JCO.2011.38.0402

Rafiq, S., Hackett, C. S., and Brentjens, R. J. (2020). Engineering Strategies to Overcome the Current Roadblocks in CAR T Cell Therapy. *Nat. Rev. Clin. Oncol.* 17 (3), 147–167. doi:10.1038/s41571-019-0297-y

Ramos, C. A., Ballard, B., Zhang, H., Dakhova, O., Gee, A. P., Mei, Z., et al. (2017). Clinical and Immunological Responses after CD30-specific Chimeric Antigen Receptor-Redirected Lymphocytes. *J. Clin. Invest.* 127 (9), 3462–3471. doi:10.1172/JCI94306

Ramos, C. A., Grover, N. S., Beaven, A. W., Lulla, P. D., Wu, M.-F., Ivanova, A., et al. (2020). Anti-CD30 CAR-T Cell Therapy in Relapsed and Refractory Hodgkin Lymphoma. *Jco* 38 (32), 3794–3804. doi:10.1200/JCO.20.01342

Renner, C., Hartmann, F., Jung, W., Deisting, C., Juwana, M., and Pfreundschuh, M. (2000). Initiation of Humoral and Cellular Immune Responses in Patients with Refractory Hodgkin's Disease by Treatment with an Anti-cd16/cd30 Bispecific Antibody. *Cancer Immunol. Immunother.* 49 (3), 173–180. doi:10.1007/s002620050617

Sadelain, M., Brentjens, R., and Rivière, I. (2013). The Basic Principles of Chimeric Antigen Receptor Design. *Cancer Discov.* 3 (4), 388–398. doi:10.1158/2159-8290.CD-12-0548

Schirrmann, T., Steinwand, M., Wezler, X., Ten Haaf, A., Tur, M. K., and Barth, S. (2014). CD30 as a Therapeutic Target for Lymphoma. *BioDrugs* 28 (2), 181–209. doi:10.1007/s40259-013-0068-8

Schlapschy, M., Gruber, H., Gresch, O., Schafer, C., Renner, C., Pfreundschuh, M., et al. (2004). Functional Humanization of an Anti-CD30 Fab Fragment for the Immunotherapy of Hodgkin's Lymphoma Using an *In Vitro* Evolution Approach. *Protein Eng. Des. Selection* 17 (12), 847–860. doi:10.1093/protein/gzh098

Schwarting, R., Gerdes, J., Durkop, H., Falini, B., Pileri, S., and Stein, H. (1989). BER-H2: a New Anti-ki-1 (CD30) Monoclonal Antibody Directed at a Formol-Resistant Epitope. *Blood* 74 (5), 1678–1689. doi:10.1182/blood.v74.5.1678.1678

Sommermeyer, D., Hill, T., Shamah, S. M., Salter, A. I., Chen, Y., Mohler, K. M., et al. (2017). Fully Human CD19-specific Chimeric Antigen Receptors for T-Cell Therapy. *Leukemia* 31 (10), 2191–2199. doi:10.1038/leu.2017.57

Sun, B., Yang, D., Dai, H., Liu, X., Jia, R., Cui, X., et al. (2019). Eradication of Hepatocellular Carcinoma by NKG2D-Based CAR-T Cells. *Cancer Immunol. Res.* 7 (11), 1813–1823. doi:10.1158/2326-6066.CIR-19-0026

Tao, L., Farooq, M. A., Gao, Y., Zhang, L., Niu, C., Ajmal, I., et al. (2020). CD19-CAR-T Cells Bearing a KIR/PD-1-Based Inhibitory CAR Eradicate CD19+HLA-C1− Malignant B Cells while Sparing CD19+HLA-C1+ Healthy B Cells. *Cancers* 12 (9), 2612. doi:10.3390/cancers12092612

Turtle, C. J., Hanafi, L.-A., Berger, C., Gooley, T. A., Cherian, S., Hudecek, M., et al. (2016a). CD19 CAR-T Cells of Defined CD4+:CD8+ Composition in Adult B Cell ALL Patients. *J. Clin. Invest.* 126 (6), 2123–2138. doi:10.1172/JCI85309

Turtle, C. J., Hanafi, L.-A., Berger, C., Hudecek, M., Pender, B., Robinson, E., et al. (2016b). Immunotherapy of Non-hodgkin's Lymphoma with a Defined Ratio of CD8 + and CD4 + CD19-specific Chimeric Antigen Receptor-Modified T Cells. *Sci. Transl. Med.* 8 (355), 355ra116. doi:10.1126/scitranslmed.aaf8621

van der Weyden, C. A., Pileri, S. A., Feldman, A. L., Whisstock, J., and Prince, H. M. (2017). Understanding CD30 Biology and Therapeutic Targeting: a Historical Perspective Providing Insight into Future Directions. *Blood Cancer J.* 7 (9), e603. doi:10.1038/bcj.2017.85

Wagner, D. L., Fritsche, E., Pulsipher, M. A., Ahmed, N., Hamieh, M., Hegde, M., et al. (2021). Immunogenicity of CAR T Cells in Cancer Therapy. *Nat. Rev. Clin. Oncol.* 18 (6), 379–393. doi:10.1038/s41571-021-00476-2

Wang, C.-M., Wu, Z.-Q., Wang, Y., Guo, Y.-L., Dai, H.-R., Wang, X.-H., et al. (2017). Autologous T Cells Expressing CD30 Chimeric Antigen Receptors for Relapsed or Refractory Hodgkin Lymphoma: An Open-Label Phase I Trial. *Clin. Cancer Res.* 23 (5), 1156–1166. doi:10.1158/1078-0432.CCR-16-1365

Wang, D., Zeng, C., Xu, B., Xu, J.-H., Wang, J., Jiang, L.-J., et al. (2020). Anti-CD30 Chimeric Antigen Receptor T Cell Therapy for Relapsed/refractory CD30+ Lymphoma Patients. *Blood Cancer J.* 10 (1), 8. doi:10.1038/s41408-020-0274-9

Wang, X., Scarfò, I., Schmidts, A., Toner, M., Maus, M. V., and Irimia, D. (2019). Dynamic Profiling of Antitumor Activity of CAR T Cells Using Micropatterned Tumor Arrays. *Adv. Sci.* 6 (23), 1901829. doi:10.1002/advs.201901829

Wasik, M. A., Jimenez, G. S., and Weisenburger, D. D. (2013). Targeting CD30 in Malignant Tissues: Challenges in Detection and Clinical Applications. *Pathobiology* 80 (5), 252–258. doi:10.1159/000347192

Yang, D., Cheng, D., Tu, Q., Yang, H., Sun, B., Yan, L., et al. (2018). HUWE1 Controls the Development of Non-small Cell Lung Cancer through Down-Regulation of P53. *Theranostics* 8 (13), 3517–3529. doi:10.7150/thno.24401

Yang, D., Sun, B., Dai, H., Li, W., Shi, L., Zhang, P., et al. (2019). T Cells Expressing NKG2D Chimeric Antigen Receptors Efficiently Eliminate Glioblastoma and Cancer Stem Cells. *J. Immunotherapy Cancer* 7 (1), 171. doi:10.1186/s40425-019-0642-9

Yang, D., Sun, B., Zhang, X., Cheng, D., Yu, X., Yan, L., et al. (2017). Huwe1 Sustains Normal Ovarian Epithelial Cell Transformation and Tumor Growth through the Histone H1.3-H19 Cascade. *Cancer Res.* 77 (18), 4773–4784. doi:10.1158/0008-5472.CAN-16-2597

Younes, A., Gopal, A. K., Smith, S. E., Ansell, S. M., Rosenblatt, J. D., Savage, K. J., et al. (2012). Results of a Pivotal Phase II Study of Brentuximab Vedotin for Patients with Relapsed or Refractory Hodgkin's Lymphoma. *Jco* 30 (18), 2183–2189. doi:10.1200/JCO.2011.38.0410

Zhao, Y., Liu, Z., Wang, X., Wu, H., Zhang, J., Yang, J., et al. (2019). Treatment with Humanized Selective CD19CAR-T Cells Shows Efficacy in Highly Treated B-ALL Patients Who Have Relapsed after Receiving Murine-Based CD19CAR-T Therapies. *Clin. Cancer Res.* 25 (18), 5595–5607. doi:10.1158/1078-0432.CCR-19-0916

Zou, Y., Xu, W., and Li, J. (2018). Chimeric Antigen Receptor-Modified T Cell Therapy in Chronic Lymphocytic Leukemia. *J. Hematol. Oncol.* 11 (1), 130. doi:10.1186/s13045-018-0676-3

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OPINION
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Potentiating Cancer Immune Therapy *via* Nanomaterials and Purinergic Signaling

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INTRODUCTION

Adenosine, an autacoid nucleoside interacting with P1 receptors, activates four G protein-coupled receptors named A1, A_{2A}, A_{2B}, and A_3, crucially regulating several human pathologies (Borea et al., 2018). It affects both neoplastic and immune cells, promoting cancer cell proliferation, neo-angiogenesis, immunoescape, and metastasis (Arab and Hadjati, 2019). Extracellular nucleotides such as ATP, ADP, and UTP also function as cell-to-cell communication signals by binding and activating P2 receptors belonging to the P2X and P2Y subfamilies (Kennedy, 2021). These receptors are further subdivided into different subtypes (Khakh et al., 2021). The differential expressions of P1 and P2 receptors both in immune and tumor cells generate a complex picture. Cancers are able to convert extracellular ATP into immunosuppressive adenosine, through the activation of CD39 ectonucleotidase that hydrolyzes ATP to AMP, and a subsequent CD73 enzyme that transforms AMP into adenosine, with the stimulation of adenosine receptors on immune cells activating numerous immunosuppressive effects (Borea et al., 2018; Boison and Yegutkin 2019). The shift from P2 to P1 activation is important for limiting the inflammatory response, thus preventing tissue damage, but may also deleteriously inhibit immunosurveillance (Antonioli et al., 2013; Allard et al., 2019). Targeting CD39 and CD73 has, therefore, become a new way to fight cancer (Perrot et al., 2019; Moesta et al., 2020; Li et al., 2019). This review conjugates the current knowledge of purinergic signaling in cancer biology with techniques involving nanomaterials to increase anticancer immune responses.

P1 Receptors and Cancer

Two hallmarks connecting adenosine to cancer include 1) solid tumors develop hypoxia and increase adenosine from nanomolar to micromolar concentrations and 2) the A_{2A} receptor is an essential brake of immune cells (Sitkovsky M. V., 2020; Hatfild and Sitkovsky, 2020). The hypoxic activation of the master oxygen-sensitive transcriptional regulator HIF-1α upregulates ecto-5′-nucleotidase (CD73), generating adenosine accumulation associated with poor prognosis in many neoplasms (Borea et al., 2017). Adenosine activates cAMP-elevating A_{2A} receptors to inhibit $CD8^+$, $CD4^+$ lymphocytes, and natural killer (NK) cells but stimulates B and T regulatory lymphocytes (Treg), tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells (MDSCs), thus establishing a typically immunosuppressive tumor microenvironment (TME) (Vijayan et al., 2017). This encouraged immunologists to recognize adenosine as a new "immune checkpoint regulator" that stimulated the classic anti-cytotoxic T-like antigen 4 (CTLA4) and anti–programmed death-ligand 1 (PD-L1) to increase immunoescape (Sitkovsky M. V., 2020). Indeed, CTLA4 and PD-L1 inhibitors have been well-tolerated in cancer patients, improving overall morbidity and survival

versus standard chemotherapy. However, efficacy may be limited to relatively few patients in some tumor types, reflecting the presence of alternative immunosuppressive factors in TME. Notably, anti-PD1 therapy increased immunosuppressant A_{2A} receptors on $CD8^+$ T cells; moreover, patients resistant to immunotherapy showed CD73 upregulation, suggesting that adenosine machinery counteracted the effects of immune checkpoint inhibitor drugs (Zarek et al., 2008). One improvement strategy has been implemented to inhibit (Kotulová et al., 2021) the hypoxia-HIF-1α-A_{2A} receptor-mediated pathway in the TME through A_{2A} receptor antagonists (Hatfield and Sitkovsky, 2020; Willingham et al., 2020). Accordingly, genetic silencing of the A_{2A} receptor strongly increased inflammation and tumor rejection in mice (Ohta and Sitkovsky, 2001; Ohta et al., 2006; Sitkovsky M. V., 2020). A series of phase I/II clinical trials, evaluating the safety and efficacy of A_{2A} receptor blockers/CD73 inhibitors including oleclumab, CPI-006, BMS-986179, and NZV-930 and A_{2A} receptor antagonists such as ciforadenant, inupadenant, taminadenant, AZD4635, and preladenant alone or coadministered with immune checkpoint inhibitors such as anti-PD1 or anti-PDL1, are under evaluation (Arab and Hadjati, 2019; Arab et al., 2021; Franco et al., 2021; Thompson and Powell, 2021).

Beyond targeting the A_{2A} receptor, anticancer immunotherapy can also be potentiated by inhibiting the A2B receptor, a subtype also capable of stimulating cAMP in T cells. Phase I clinical trials of A2B blockers in patients with advanced cancer are underway (Franco et al., 2021). Arguably, this pharmacological approach might only succeed in patients bearing hypoxic tumors with a sufficient number of tumor-reactive T cells, yet this consideration remains to be resolved (Sitkovsky M. V., 2020; Fong et al., 2020).

P2 Receptors and Cancer

The TME is rich in ATP and its metabolites modulating tumor and immune cell biology and responses (Di Virgilio et al., 2018). The contribution of P2 receptors to cancer biology has been intensively investigated (Chiarella et al., 2021). The ATP-activated P2X7 receptor has emerged as a pivotal membrane molecule in tumors as it is expressed by cancer cells and by macrophages, dendritic cells, and lymphocytes infiltrating the tumor mass (De Marchi et al., 2019).

Tumor cell cytotoxicity (apoptosis or necrosis) due to prolonged P2X7 receptor activation and pore formation was a desirable anti-tumor response of this membrane molecule (Feng et al., 2006; Fu et al., 2009; Bian et al., 2013; Avanzato et al., 2016). However, subsequent identification of P2X7 receptor variants, with more precise characterization of the responses and measurement of cancer cell expression levels, indicated this subtype was upregulated in many tumor types (McLarnon, 2017; Di Virgilio et al., 2018, Zhang et al., 2019a; 2019b). More significantly, P2X7 receptor stimulation by low extracellular ATP concentrations was pro-tumorigenic, favoring cancer cell survival, proliferation, motility, and chemoresistance (Adinolfi et al., 2012; Schneider et al., 2015; Arnaud-Sampaio et al., 2020). In addition to the P2X7 receptor subtype, the P2X4, P2X5, $P2Y_6$, and $P2Y_{12}$ receptors also have involvement in tumor biology (Roger et al., 2015). P2X4 and P2X7 receptor subtype expressions concurred with tumor cell proliferation (He et al., 2020). In contrast, P2X5 receptor mediated an anti-proliferative (Zhang et al., 2020) effect by inducing tumor cell differentiation. Cumulative reports have indicated pro-neoplastic $P2Y_2$ receptor-mediated responses conferring resistance to cell apoptosis, stimulation of tumor replication, and dissemination (Limami et al., 2012; Choi et al., 2013; Schumacher et al., 2013). The lack of expression of the P2X7 receptor in P2X7 KO mice induced a decrease in $CD8^+$ lymphocytes while the number of Treg cells increased (De Marchi et al., 2019).

From a pharmacological and therapeutic perspective, P2 receptors have high potential to complement radiation therapy against resistant, highly malignant cancers. The stimulation of P2X7, $P2Y_6$, and $P2Y_{12}$ receptors was significant in the DNA damage response induced by γ-irradiation of adenocarcinoma A549 cells (Ide et al., 2014). B16 melanoma cells both *in vitro* and *in vivo* responded similarly to P2X7 receptor antagonists (Tanamachi et al., 2017). The use of single P2 receptor subtype inhibitors was often sufficient to block tumor cell growth and dissemination (Drill et al., 2021). The growth of human high-grade gliomas was inhibited by P2X7 subtype antagonists (Kan et al., 2020); receptor inhibitors, such as emodin, and the *Uncaria tomentosa* extract effectively counteracted the P2X7 receptor-mediated breast cancer spread (Zhu et al., 2021). P2X7 receptor antagonization could also usefully reduce pain in cancer patients with metastases. In particular, the P2X7 receptor antagonists AFC5261 and A-740003 were promising in animal models (Li et al., 2018; De Marchi et al., 2019; Falk et al., 2019). Further identification and characterization of new P2X7 receptor modulators and inhibitors were recommended (Hempel et al., 2013). Also for consideration, the expression of P2X7 and other P2 receptors by immune cells participated in immunosurveillance (Jelassi et al., 2013; Grassi and Conti, 2021). The awareness of the importance of P2-mediated signaling in cancer pathogenesis and progression (**Figure 1A**) has prompted therapeutic strategies targeting extracellular nucleotides. In this light, nanomaterials may improve anticancer outcomes by modulating the immune and tumor cell purinome.

Nanomaterials and Immunosurveillance

Although TME immunosurveillance may be markedly heterogeneous, most anticancer agents rely on the reactivation of homeostatic immune defense mechanisms (Joyce and Fearon, 2015; Terry et al., 2017; Ni et al., 2021). Initially, innovative nanomaterials improved upon conventional treatments yet soon drew criticism when nanoparticles elicited toxic effects from immunological alterations (Lenders et al., 2020). Nonetheless, rationally tailored nanomaterials have renewed interest in penetrant TME modulators (Zhang et al., 2021) that address tumor immune evasion (Guevara et al., 2021) by immunotherapy enhancement to promote immunogenic tumor cell death (Aikins et al., 2020) (Nogrady, 2021). The multiple cell types comprising the TME provide alternative nanomaterial targets, and their

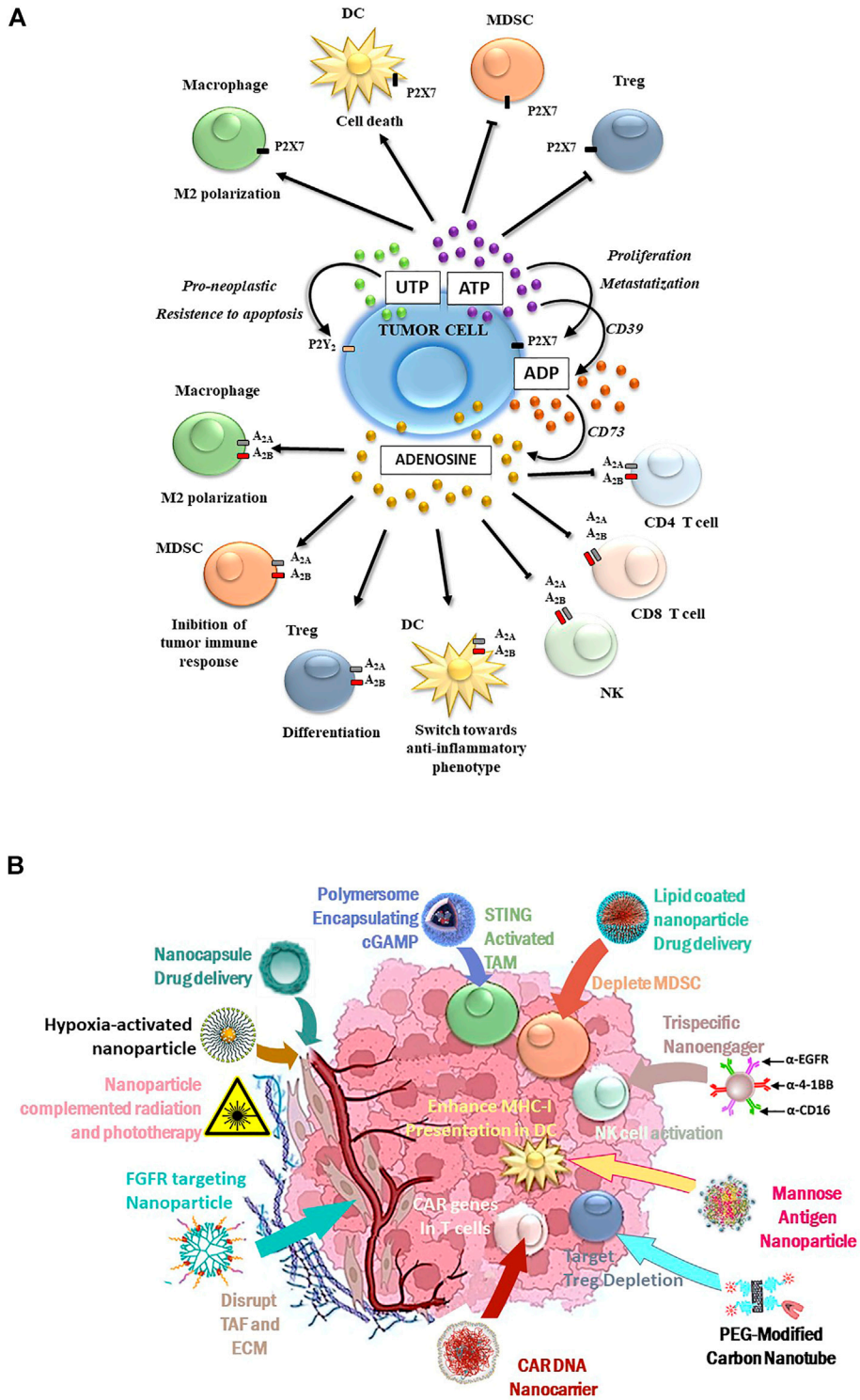

FIGURE 1 | (A) Purinergic-mediated responses occurring in the TME: P2 receptor-induced activities are summarized in the upper part of the figure, while P1 receptor-mediated responses are depicted in the lower figure part. **(B)** Schematic diagram for tailored nanoparticles targeting the TME and its immunological components to potentiate cancer immune therapy.

involvement in intervention design can be reciprocal (Song et al., 2017). For example, to counteract tumor adenosine accumulation, lipid nanoparticles mediating the knockdown of the corresponding A_{2A} receptor in memory T cells could rescue $CD8^+$ T-cell chemotaxis for infiltration into the TME of head and neck squamous cell carcinomas (Newton et al., 2021). Nanoparticle-based delivery approaches also include cell membrane–camouflaged nanocarriers (Grimaudo, 2021) such as tumor-associated macrophage membrane-coated nanoparticles (Chen et al., 2021). Cell membrane-bioinspired nanoparticles can provide superior immune regulation, nanocapsule drug delivery (Zhang et al., 2019c; Irvine and Dane, 2020), tumor targeting, and biocompatibility (Mu et al., 2021).

Yet diversity among tumorigenic cells and between individuals may still Yet, thwart nano-based delivery systems. The improved knowledge of various chronological stages of TME development remains necessary for more effective nanoplatform implementation (Yang et al., 2021) to target the more persistent subpopulation of cancer stem cells (Duan et al., 2021). The highest immunotherapeutic efficacy occurs when nanoparticles achieve precise and timely delivery, specifically targeting neoplastic cells with minimal harm to healthy cells (Muluh et al., 2021). Addressing TME traits, hypoxia-activated nanoparticles have theranostic applications (Wang et al., 2019). Since TME hypoxia blocked antitumor immunity (Singleton et al., 2021), tumor hypoxia-activated polymeric micelles were used to both activate strong cytotoxicity and stimulate a systemic antitumor immunity that effectively eradicated breast cancer in preclinical murine models (Liu et al., 2021). Hypoxia-modifier nanoparticles (Yuan et al., 2021) targeting the blood–brain barrier, enhanced immunotherapy of glioblastoma (Meng et al., 2021), a particularly aggressive form of cancer involving intracellular purine alterations (Debom et al., 2021; Giuliani et al., 2021). Cancer metastasis treatment remains a highlight of nanomedicine-based immunotherapy (Zhang et al., 2019). Excellent efficacy was observed for TME-activated nanoparticle chemodynamic immunotherapy of melanoma-derived lung metastasis (Zhai et al., 2021).

How Nanomaterials Can Be Used to Modulate TME Purinergic Signaling

Compared to the relatively heterogenous tumor-cell population, non-tumorigenic supportive cells within the TME such as tumor-associated fibroblasts (TAFs) may present a more consistent target for nanoparticle intervention (Li et al., 2021), yet some limitations persist. nanomaterial-based TME modulation impinging upon purinergic signaling pathways can serve to additionally recruit the immune system to provide more integrative therapy (Laplane et al., 2019; Shi and Lammers, 2019). Nanomaterials can be adapted to modulate purinergic signaling in a number of ways since nanoparticles can be size-tailored to have diameters that match pore sizes present in leaky TME vasculature, thus establishing size-related penetration and accumulation (Yu et al., 2020). Moreover, nanoparticles can assist with improved delivery of drugs such as A_{2A} antagonists that counteracted immunosuppression (Arruga et al., 2021). It is notable that the purinergic signaling network is subjected to modulation by microRNA (miRNA) (Ferrari et al., 2016), and over 30 miRNAs directly or indirectly modulate P1 and P2 receptors and ectoenzymes, with miR-187 capable of modulating both P2X7 and CD73 (Guo et al., 2022). Notably, miRNA that bind the 3′ untranslated region of the P2X7 receptor can affect the development of breast cancer by influencing the P2X7 receptor expression (Zhu et al., 2021). Nanoparticles are well-suited for precision medicine strategies to deliver purinergic signaling-specific miRNA and silencing RNA (siRNA) therapeutics (Kara et al., 2022). It has already been demonstrated that the nanoparticle delivery of siRNA-CD73 to the central nervous system blocked the CD73 expression in the glioblastoma immune microenvironment, inducing apoptosis to delay tumor growth (Azambuja et al., 2020). Smart nanomaterials can be engineered to exploit TME-specific purinergic pathway anomalies. A hydrogel of alginate conjugated with an ATP-specific aptamer hybridized with immunoadjuvant CpG oligonucleotides enabled the release of immune adjuvants in synchrony with low-dose repeated chemo/radiotherapies. This achieved a remarkable synergistic response; in addition to eliminating tumors, the evoked immune memory rejected re-challenged tumors and inhibited distant tumor metastases when combined with immune checkpoint blockade (Sun et al., 2021).

Nanoparticles Modulating TME Purinergic Pathways Potentiate Immune Therapy

Innate immune interactions include macrophage responsiveness to damage-associated molecular patterns (DAMPs) originating from the cancer cells. M2-like tumor-associated macrophages (TAMs) can efficiently engulf neighboring apoptotic cells abundant in solid tumors, an early immunosuppressive mechanism preventing a DAMP-mediated immune response. The MER proto-oncogene tyrosine kinase (MerTK) can promote an “eat me” signal on dying cells to enhance efferocytosis (Ou et al., 2021). Consequently, apoptotic cells are eliminated before releasing intracellular ATP and cyclic GMP that would otherwise activate the ATP-gated P2X7 channels of TAMs and also cytosolic nucleic acid sensor pathways, including cyclic GMP-AMP synthase (cGAS) producing cyclic guanosine monophosphate–adenosine monophosphate (cGAMP), a second messenger binding and activating the adapter protein, stimulating interferon gene (STING), expressed in TAMs and other cells of the TME. The production of stress-responsive cytokines would ultimately cause M2 macrophages to be polarized toward an immune-activated M1 phenotype (Zhao et al., 2021). Appropriately, macrophages have become key targets for nanoparticle intervention (Medrano-Bosch et al., 2021). A nanoparticle-incorporating STING activator cGAMP enhanced the antitumor immunity in PD-L1-insensitive models of triple-negative breast cancer (Cheng et al., 2018) and improved the clinical outcome of immunotherapy for melanoma (Shae et al., 2019). Cationic silica nanoparticles induced necrotic cell death and activation of the STING in the TME to enhance antitumor immunity (An

TABLE 1 | Examples of immunomodulatory nanoparticle types, tumor microenvironment (TME) interactions and co-involved purinergic pathways.

Nanomaterial	Size (nm)	TME target	Co-involved purinergic ecto-enzyme receptor subtype	Reference
CAR DNA Nanocarrier	155 ± 40	murine $CD8^+$ T cell	P2X7	Smith et al. (2017)
FGFR targeting nanoparticle	10-200	Tumor Associated Fibrobasts (TAF)	CD73	Li et al. (2021)
Hypoxia-activated nanoparticle	254 ± 27	Hypoxia	CD39, CD73, A_{2A}, $P2Y_2$, P2X7, $P2Y_{11}$	Wang et al. (2019)
Lipid coated nanoparticle drug delivery	≈30	Myeloid-Derived Supressor Cells (MDSC)	P2X7, A_{2B}	Zhang et al. (2019)
Mannose antigen nanoparticle	210	Dendritic Cell (DC	P2X7	Pei et al. (2021)
Nanocapsule drug delivery	100-200	Tumor Extracellular Matrix	P2X4, P2X7, $P2Y_{12}$	Irvine and Dane, (2020)
PEG-modified carbon nanotube	101 ± 41	Regulatory T cells (T_{reg})	A_{2A}, A_{2B}	Sacchetti et al. (2013)
Polymersome encapsulating cGAMP	20-100	Tumor Associated Macrophage (TAM)	P2X7	Shae et al. (2019)
Trispecific nanoengager	112 ± 7	Natural Killer (NK) cell	CD39, A_{2A}, A_{2B}, A_3, P2X7	Au et al. (2020)

et al., 2018). Inhalable nanoparticulate agonists of STING synergized with radiotherapy to provide the long-term control of lung metastases (Liu et al., 2019). Combining nanoparticles with compatible forms of therapy such as radiation therapy (Huang et al., 2021) or photodynamic therapy (Jin et al., 2021) improved antitumor efficacy by promoting immunogenic cell death.

Nanomaterials are also capable of enhancing the trained acquired immune response (Magadán et al., 2021), and they have been rationally designed to enhance T-cell expansion, navigate physical barriers, and modulate the TME to overcome barriers to T-cell-based immunotherapies (Gong et al., 2021). Engineered immunomodulating nano-adapter particle rafts such as trispecific natural killer cell nanoengagers (Au et al., 2020) carry more than one monoclonal antibody (mAb) to bridge effector and tumor cells. More effective responses than simply mixing the parental mAbs with T cells, NK cells, natural killer (NK) cells, or macrophages were observed (Jiang et al., 2021). Nanogels selectively released an interleukin-15 cargo upon T-cell receptor activation and expanded T cells in tumors 16-fold relative to the systemic administration of free cytokines. The higher doses of cytokines could be administered, without toxic side effects, to potentiate human chimeric antigen receptor (CAR)-T cell therapy (Tang et al., 2018). Nanoparticle versatility, exemplified in **Table 1** and **Figure 1B**, has meant that numerous clinical nanomaterials and drugs potentiating immunotherapy are currently under development (Li et al., 2020; Hu and Huang, 2022).

DISCUSSION

The TME, heavily conditioned by nucleotide/nucleoside release and hydrolysis, makes purinergic signaling an extremely attractive target for strategic modulation of both cancer and immune cells, but responses to antagonists or agonists are highly context-dependent (Hreich et al., 2021). The inhibitors of specific purinome components have successfully blocked tumor progression and metastasis in animal models and preclinical studies, yet improved specific therapeutic strategies are needed. The recent implementation of nanomaterials has shown that they can be very effective agents, acting on their own, delivering mRNA or improving mAb presentation to disrupt the TME refractoriness to immune therapy.

AUTHOR CONTRIBUTIONS

DF, SG, SM, MN, AT, and JB wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

REFERENCES

Adinolfi, E., Raffaghello, L., Giuliani, A. L., Cavazzini, L., Capece, M., Chiozzi, P., et al. (2012). Expression of P2X7 Receptor IncreasesIn VivoTumor Growth. *Cancer Res.* 72, 2957–2969. doi:10.1158/0008-5472.can-11-1947

Aikins, M. E., Xu, C., and Moon, J. J. (2020). Engineered Nanoparticles for Cancer Vaccination and Immunotherapy. *Acc. Chem. Res.* 53, 2094–2105. doi:10.1021/acs.accounts.0c00456

Allard, D., Chrobak, P., Allard, B., Messaoudi, N., and Stagg, J. (2019). Targeting the CD73-Adenosine axis in Immuno-Oncology. *Immunol. Lett.* 205, 31–39. doi:10.1016/j.imlet.2018.05.001

An, M., Yu, C., Xi, J., Reyes, J., Mao, G., Wei, W.-Z., et al. (2018). Induction of Necrotic Cell Death and Activation of STING in the Tumor Microenvironment via Cationic Silica Nanoparticles Leading to Enhanced Antitumor Immunity. *Nanoscale* 10, 9311–9319. doi:10.1039/c8nr01376d

Antonioli, L., Pacher, P., Vizi, E. S., and Haskó, G. (2013). CD39 and CD73 in Immunity and Inflammation. *Trends Mol. Med.* 19, 355–367. doi:10.1016/j.molmed.2013.03.005

Arab, S., and Hadjati, J. (2019). Adenosine Blockage in Tumor Microenvironment and Improvement of Cancer Immunotherapy. *Immune Netw.* 2719, e23. doi:10.4110/in.2019.19.e23

Arab, S., Alizadeh, A., and Asgharzade, S. (2021). Tumor-resident Adenosine-Producing Mesenchymal Stem Cells as a Potential Target for Cancer Treatment. *Clin. Exp. Med.* 21, 205–213. doi:10.1007/s10238-020-00674-9

Arnaud-Sampaio, V. F., Rabelo, I. L. A., Ulrich, H., and Lameu, C. (2020). The P2X7 Receptor in the Maintenance of Cancer Stem Cells, Chemoresistance and Metastasis. *Stem Cel Rev Rep* 16, 288–300. doi:10.1007/s12015-019-09936-w

Arruga, F., Serra, S., Vitale, N., Guerra, G., Papait, A., Baffour Gyau, B., et al. (2021). Targeting of the A2A Adenosine Receptor Counteracts Immunosuppression *In Vivo* in a Mouse Model of Chronic Lymphocytic Leukemia. *Haematologica* 106, 1343–1353. doi:10.3324/haematol.2019.242016

Au, K. M., Park, S. I., and Wang, A. Z. (2020). Trispecific Natural Killer Cell Nanoengagers for Targeted Chemoimmunotherapy. *Sci. Adv.* 6, eaba8564. doi:10.1126/sciadv.aba8564

Avanzato, D., Genova, T., Fiorio Pla, A., Bernardini, M., Bianco, S., Bussolati, B., et al. (2016). Activation of P2X7 and P2Y11 Purinergic Receptors Inhibits Migration and Normalizes Tumor-Derived Endothelial Cells via cAMP Signaling. *Sci. Rep.* 6, 32602. doi:10.1038/srep32602

Azambuja, J. H., Schuh, R. S., Michels, L. R., Iser, I. C., Beckenkamp, L. R., Roliano, G. G., et al. (2020). Blockade of CD73 Delays Glioblastoma Growth by Modulating the Immune Environment. *Cancer Immunol. Immunother.* 69, 1801–1812. doi:10.1007/s00262-020-02569-w

Bian, S., Sun, X., Bai, A., Zhang, C., Li, L., Enjyoji, K., et al. (2013). P2X7 Integrates PI3K/AKT and AMPK-PRAS40-mTOR Signaling Pathways to Mediate Tumor Cell Death. *PLoS One* 8, e60184. doi:10.1371/journal.pone.0060184

Boison, D., and Yegutkin, G. G. (2019). Adenosine Metabolism: Emerging Concepts for Cancer Therapy. *Cancer Cell* 36, 582–596. doi:10.1016/j.ccell.2019.10.007

Borea, P. A., Gessi, S., Merighi, S., Vincenzi, F., and Varani, K. (2017). Pathological Overproduction: the Bad Side of Adenosine. *Br. J. Pharmacol.* 174, 1945–1960. doi:10.1111/bph.13763

Borea, P. A., Gessi, S., Merighi, S., Vincenzi, F., and Varani, K. (2018). Pharmacology of Adenosine Receptors: The State of the Art. *Physiol. Rev.* 98, 1591–1625. doi:10.1152/physrev.00049.2017

Chen, C., Song, M., Du, Y., Yu, Y., Li, C., Han, Y., et al. (2021). Tumor-Associated-Macrophage-Membrane-Coated Nanoparticles for Improved Photodynamic Immunotherapy. *Nano Lett.* 21, 5522–5531. doi:10.1021/acs.nanolett.1c00818

Cheng, N., Watkins-Schulz, R., Junkins, R. D., David, C. N., Johnson, B. M., Montgomery, S. A., et al. (2018). A Nanoparticle-Incorporated STING Activator Enhances Antitumor Immunity in PD-L1-Insensitive Models of Triple-Negative Breast Cancer. *JCI Insight* 3, 120638. doi:10.1172/jci.insight.120638

Chiarella, A. M., Ryu, Y. K., Manji, G. A., and Rustgi, A. K. (2021). Extracellular ATP and Adenosine in Cancer Pathogenesis and Treatment. *Trends Cancer* 7, 731–750. doi:10.1016/j.trecan.2021.04.008

Choi, J. H., Ji, Y. G., and Lee, D. H. (2013). Uridine Triphosphate Increases Proliferation of Human Cancerous Pancreatic Duct Epithelial Cells by Activating P2Y2 Receptor. *Pancreas* 42, 680–686. doi:10.1097/mpa.0b013e318271bb4b

De Marchi, E., Orioli, E., Pegoraro, A., Sangaletti, S., Portararo, P., Curti, A., et al. (2019). The P2X7 Receptor Modulates Immune Cells Infiltration, Ectonucleotidases Expression and Extracellular ATP Levels in the Tumor Microenvironment. *Oncogene* 38, 3636–3650. doi:10.1038/s41388-019-0684-y

Debom, G. N., Rubenich, D. S., and Braganhol, E. (2021). Adenosinergic Signaling as a Key Modulator of the Glioma Microenvironment and Reactive Astrocytes. *Front. Neurosci.* 15, 648476. doi:10.3389/fnins.2021.648476

Di Virgilio, F., Sarti, A. C., Falzoni, S., De Marchi, E., and Adinolfi, E. (2018). Extracellular ATP and P2 Purinergic Signalling in the Tumour Microenvironment. *Nat. Rev. Cancer* 18, 601–618. doi:10.1038/s41568-018-0037-0

Drill, M., Jones, N. C., Hunn, M., O'Brien, T. J., and Monif, M. (2021). Antagonism of the ATP-Gated P2X7 Receptor: a Potential Therapeutic Strategy for Cancer. *Purinergic Signal.* 17, 215–227. doi:10.1007/s11302-021-09776-9

Duan, H., Liu, Y., Gao, Z., and Huang, W. (2021). Recent Advances in Drug Delivery Systems for Targeting Cancer Stem Cells. *Acta Pharmaceutica Sinica B* 11, 55–70. doi:10.1016/j.apsb.2020.09.016

Falk, S., Appel, C. K., Bennedbæk, H. B., Al-Dihaissy, T., Unger, A., Dinkel, K., et al. (2019). Chronic High Dose P2X7 Receptor Inhibition Exacerbates Cancer-Induced Bone Pain. *Eur. J. Pharmacol.* 845, 48–55. doi:10.1016/j.ejphar.2018.12.032

Feng, Y. H., Li, X., Zeng, R., and Gorodeski, G. I. (2006). Endogenously Expressed Truncated P2X7 Receptor Lacking the C-Terminus Is Preferentially Upregulated in Epithelial Cancer Cells and Fails to Mediate Ligand-Induced Pore Formation and Apoptosis. *Nucleosides Nucleotides Nucleic Acids* 25, 1271–1276. doi:10.1080/15257770600890921

Ferrari, D., Bianchi, N., Eltzschig, H. K., and Gambari, R. (2016). MicroRNAs Modulate the Purinergic Signaling Network. *Trends Mol. Med.* 22, 905–918. doi:10.1016/j.molmed.2016.08.006

Fong, L., Hotson, A., Powderly, J. D., Sznol, M., Heist, R. S., Choueiri, T. K., et al. (2020). Adenosine 2A Receptor Blockade as an Immunotherapy for Treatment-Refractory Renal Cell Cancer. *Cancer Discov.* 10, 40–53. doi:10.1158/2159-8290.cd-19-0980

Franco, R., Rivas-Santisteban, R., Navarro, G., and Reyes-Resina, I. (2021). Adenosine Receptor Antagonists to Combat Cancer and to Boost Anti-cancer Chemotherapy and Immunotherapy. *Cells* 11, 2831. doi:10.3390/cells10112831

Fu, W., McCormick, T., Qi, X., Luo, L., Zhou, L., Li, X., et al. (2009). Activation of P2X(7)-Mediated Apoptosis Inhibits DMBA/TPA-induced Formation of Skin Papillomas and Cancer in Mice. *BMC Cancer* 9, 114. doi:10.1186/1471-2407-9-114

Giuliani, P., Carluccio, M., and Ciccarelli, R. (2021). Role of Purinome, A Complex Signaling System, in Glioblastoma Aggressiveness. *Front. Pharmacol.* 12, 632622. doi:10.3389/fphar.2021.632622

Gong, N., Sheppard, N. C., Billingsley, M. M., June, C. H., and Mitchell, M. J. (2021). Nanomaterials for T-Cell Cancer Immunotherapy. *Nat. Nanotechnol* 16, 25–36. doi:10.1038/s41565-020-00822-y

Grassi, F., and Conti, B. D. P. (2021). The P2X7 Receptor in Tumor Immunity. *Front Cel Dev Biol* 9, 694831. doi:10.3389/fcell.2021.694831

Grimaudo, M. A. (2021). Nanotechnology for the Development of Nanovaccines in Cancer Immunotherapy. *Adv. Exp. Med. Biol.* 1295, 303–315. doi:10.1007/978-3-030-58174-9_13

Guevara, M. L., Persano, F., and Persano, S. (2021). Nano-immunotherapy: Overcoming Tumour Immune Evasion. *Semin. Cancer Biol.* 69, 238–248. doi:10.1016/j.semcancer.2019.11.010

Guo, J., Yang, P., Li, Y. F., Tang, J. F., He, Z. X., Yu, S. G., et al. (2022). MicroRNA: Crucial Modulator in Purinergic Signalling Involved Diseases. *Purinergic Signal.* doi:10.1007/s11302-022-09840-y

Hatfield, S. M., and Sitkovsky, M. V. (2020). Antihypoxic Oxygenation Agents with Respiratory Hyperoxia to Improve Cancer Immunotherapy. *J. Clin. Invest.* 130, 5629–5637. doi:10.1172/jci137554

He, J., Zhou, Y., Arredondo Carrera, H. M., Sprules, A., Neagu, R., Zarkesh, S. A., et al. (2020). Inhibiting the P2X4 Receptor Suppresses Prostate Cancer Growth *In Vitro* and *In Vivo*, Suggesting a Potential Clinical Target. *Cells* 9, 2511. doi:10.3390/cells9112511

Hempel, C., Nörenberg, W., Sobottka, H., Urban, N., Nicke, A., Fischer, W., et al. (2013). The Phenothiazine-Class Antipsychotic Drugs Prochlorperazine and Trifluoperazine Are Potent Allosteric Modulators of the Human P2X7 Receptor. *Neuropharmacology* 75, 365–379. doi:10.1016/j.neuropharm.2013.07.027

Hreich, S. J. D., Benzaquen, J., Hofman, P., and Vouret-Craviari, V. (2021). To Inhibit or to Boost the ATP/P2RX7 Pathway to Fight Cancer-That Is the Question. *Purinergic Signal.* 17, 619–631. doi:10.1007/s11302-021-09811-9

Hu, M., and Huang, L. (2022). Strategies Targeting Tumor Immune and Stromal Microenvironment and Their Clinical Relevance. *Adv. Drug Deliv. Rev.* 183, 114137.

Huang, Z., Wang, Y., Yao, D., Wu, J., Hu, Y., and Yuan, A. (2021). Nanoscale Coordination Polymers Induce Immunogenic Cell Death by Amplifying Radiation Therapy Mediated Oxidative Stress. *Nat. Commun.* 12, 145. doi:10.1038/s41467-020-20243-8

Ide, S., Nishimaki, N., Tsukimoto, M., and Kojima, S. (2014). Purine Receptor P2Y6 Mediates Cellular Response to γ-ray-induced DNA Damage. *J. Toxicol. Sci.* 39, 15–23. doi:10.2131/jts.39.15

Irvine, D. J., and Dane, E. L. (2020). Enhancing Cancer Immunotherapy with Nanomedicine. *Nat. Rev. Immunol.* 20, 321–334. doi:10.1038/s41577-019-0269-6

Jelassi, B., Anchelin, M., Chamouton, J., Cayuela, M. L., Clarysse, L., Li, J., et al. (2013). Anthraquinone Emodin Inhibits Human Cancer Cell Invasiveness by Antagonizing P2X7 Receptors. *Carcinogenesis* 34, 1487–1496. doi:10.1093/carcin/bgt099

Jiang, C. T., Chen, K. G., Liu, A., Huang, H., Fan, Y. N., Zhao, D. K., et al. (2021). Immunomodulating Nano-Adaptors Potentiate Antibody-Based Cancer Immunotherapy. *Nat. Commun.* 12, 1359. doi:10.1038/s41467-021-21497-6

Jin, F., Liu, D., Xu, X., Ji, J., and Du, Y. (2021). Nanomaterials-Based Photodynamic Therapy with Combined Treatment Improves Antitumor Efficacy through Boosting Immunogenic Cell Death. *Int. J. Nanomedicine* 16, 4693–4712. doi:10.2147/ijn.s314506

Joyce, J. A., and Fearon, D. T. (2015). T Cell Exclusion, Immune Privilege, and the Tumor Microenvironment. *Science* 348, 74–80. doi:10.1126/science.aaa6204

Kan, L. K., Seneviratne, S., Drummond, K. J., Williams, D. A., O'Brien, T. J., and Monif, M. (2020). P2X7 Receptor Antagonism Inhibits Tumour Growth in Human High-Grade Gliomas. *Purinergic Signal.* 16, 327–336. doi:10.1007/s11302-020-09705-2

Kara, G., Calin, G. A., and Ozpolat, B. (2022). RNAi-based Therapeutics and Tumor Targeted Delivery in Cancer. *Adv. Drug Deliv. Rev.* 182, 114113. doi:10.1016/j.addr.2022.114113

Kennedy, C. (2021). The P2Y/P2X divide: How it Began. *Biochem. Pharmacol. May* 187, 114408. Epub 2021 Jan 11. PMID: 33444568. doi:10.1016/j.bcp.2021.114408

Khakh, B. S., Burnstock, G., Kennedy, C., King, B. F., North, R. A., Séguéla, P., et al. (2001). International union of Pharmacology. XXIV. Current Status of the Nomenclature and Properties of P2X Receptors and Their Subunits. *Pharmacol. Rev.* 53, 107–118.

Kotulová, J., Hajdúch, M., and Džubák, P. (2021). Current Adenosinergic Therapies: What Do Cancer Cells Stand to Gain and Lose? *Int. J. Mol. Sci.* 22, 12569.

Laplane, L., Duluc, D., Bikfalvi, A., Larmonier, N., and Pradeu, T. (2019). Beyond the Tumour Microenvironment. *Int. J. Cancer* 145, 2611–2618. doi:10.1002/ijc.32343

Lenders, V., Koutsoumpou, X., Sargsian, A., and Manshian, B. B. (2020). Biomedical Nanomaterials for Immunological Applications: Ongoing Research and Clinical Trials. *Nanoscale Adv.* 2, 5046–5089. doi:10.1039/d0na00478b

Li, P., Zhang, Q., Xiao, Z., Yu, S., Yan, Y., and Qin, Y. (2018). Activation of the P2X7 Receptor in Midbrain Periaqueductal gray Participates in the Analgesic Effect of Tramadol in Bone Cancer Pain Rats. *Mol. Pain* 14, 1744806918803039. doi:10.1177/1744806918803039

Li, W., Little, N., Park, J., Foster, C. A., Chen, J., and Lu, J. (2021). Tumor-Associated Fibroblast-Targeting Nanoparticles for Enhancing Solid Tumor Therapy: Progress and Challenges. *Mol. Pharm.* 18, 2889–2905. doi:10.1021/acs.molpharmaceut.1c00455

Li, W., Peng, A., Wu, H., Quan, Y., Li, Y., Lu, L., et al. (2020). Anti-Cancer Nanomedicines: A Revolution of Tumor Immunotherapy. *Front. Immunol.* 11, 601497. doi:10.3389/fimmu.2020.601497

Li, X. Y., Moesta, A. K., Xiao, C., Nakamura, K., Casey, M., Zhang, H., et al. (2019). Targeting CD39 in Cancer Reveals an Extracellular ATP- and Inflammasome-Driven Tumor Immunity. *Cancer Discov.* 9, 1754–1773. doi:10.1158/2159-8290.cd-19-0541

Limami, Y., Pinon, A., Leger, D. Y., Pinault, E., Delage, C., Beneytout, J. L., et al. (2012). The P2Y2/Src/p38/COX-2 Pathway Is Involved in the Resistance to Ursolic Acid-Induced Apoptosis in Colorectal and Prostate Cancer Cells. *Biochimie* 94, 1754–1763. doi:10.1016/j.biochi.2012.04.006

Liu, J., Ai, X., Cabral, H., Liu, J., Huang, Y., and Mi, P. (2021). Tumor Hypoxia-Activated Combinatorial Nanomedicine Triggers Systemic Antitumor Immunity to Effectively Eradicate Advanced Breast Cancer. *Biomaterials* 273, 120847. doi:10.1016/j.biomaterials.2021.120847

Liu, Y., Crowe, W. N., Wang, L., Lu, Y., Petty, W. J., Habib, A. A., et al. (2019). An Inhalable Nanoparticulate STING Agonist Synergizes with Radiotherapy to Confer Long-Term Control of Lung Metastases. *Nat. Commun.* 10, 5108. doi:10.1038/s41467-019-13094-5

Magadán, S., Mikelez-Alonso, I., Borrego, F., and González-Fernández, Á. (2021). Nanoparticles and Trained Immunity: Glimpse into the Future. *Adv. Drug Deliv. Rev.* 175, 113821. doi:10.1016/j.addr.2021.05.031

McLarnon, J. G. (2017). Roles of Purinergic P2X7 Receptor in Glioma and Microglia in Brain Tumors. *Cancer Lett.* 28 (402), 93–99. doi:10.1016/j.canlet.2017.05.004

Medrano-Bosch, M., Moreno-Lanceta, A., and Melgar-Lesmes, P. (2021). Nanoparticles to Target and Treat Macrophages: The Ockham's Concept. *Pharmaceutics* 13, 1340. doi:10.3390/pharmaceutics13091340

Meng, L., Wang, C., Lu, Y., Sheng, G., Yang, L., Wu, Z., et al. (2021). Targeted Regulation of Blood-Brain Barrier for Enhanced Therapeutic Efficiency of Hypoxia-Modifier Nanoparticles and Immune Checkpoint Blockade Antibodies for Glioblastoma. *ACS Appl. Mater. Inter.* 13, 11657–11671. doi:10.1021/acsami.1c00347

Moesta, A. K., Li, X. Y., and Smyth, M. J. (2020). Targeting CD39 in Cancer. *Nat. Rev. Immunol.* 20, 739–755.

Mu, D., He, P., Shi, Y., Jiang, L., and Liu, G. (2021). Bioinspired Membrane-Coated Nanoplatform for Targeted Tumor Immunotherapy. *Front. Oncol.* 11, 819817. doi:10.3389/fonc.2021.819817

Muluh, T. A., Chen, Z., Li, Y., Xiong, K., Jin, J., Fu, S., et al. (2021). Enhancing Cancer Immunotherapy Treatment Goals by Using Nanoparticle Delivery System. *Int. J. Nanomedicine* 16, 2389–2404. doi:10.2147/ijn.s295300

Newton, H. S., Chimote, A. A., Arnold, M. J., Wise-Draper, T. M., and Conforti, L. (2021). Targeted Knockdown of the Adenosine A_{2A} Receptor by Lipid NPs Rescues the Chemotaxis of Head and Neck Cancer Memory T Cells. *Mol. Ther. Methods Clin. Dev.* 21, 133–143. doi:10.1016/j.omtm.2021.03.001

Ni, Y., Zhou, X., Yang, J., Shi, H., Li, H., Zhao, X., et al. (2021). The Role of Tumor-Stroma Interactions in Drug Resistance within Tumor Microenvironment. *Front. Cel Dev Biol* 9, 637675. doi:10.3389/fcell.2021.637675

Nogrady, B. (2021). How Nanotechnology Can Flick the Immunity Switch. *Nature* 595, 18–19. doi:10.1038/d41586-021-01790-6

Ohta, A., Gorelik, E., Prasad, S. J., Ronchese, F., Lukashev, D., Wong, M. K., et al. (2006). A2A Adenosine Receptor Protects Tumors from Antitumor T Cells. *Proc. Natl. Acad. Sci. USA* 103, 13132–13137. doi:10.1073/pnas.0605251103

Ohta, A., and Sitkovsky, M. (2001). Role of G-Protein-Coupled Adenosine Receptors in Downregulation of Inflammation and protection from Tissue Damage. *Nature* 414, 916–920. doi:10.1038/414916a

Ou, L., Zhang, A., Cheng, Y., and Chen, Y. (2021). The cGAS-STING Pathway: A Promising Immunotherapy Target. *Front. Immunol.* 12, 795048. doi:10.3389/fimmu.2021.795048

Pei, M., Xu, R., Zhang, C., Wang, X., Li, C., and Hu, Y. (2021). Mannose-functionalized Antigen Nanoparticles for Targeted Dendritic Cells, Accelerated Endosomal Escape and Enhanced MHC-I Antigen Presentation. *Colloids Surf. B Biointerfaces* 197, 111378. doi:10.1016/j.colsurfb.2020.111378

Perrot, I., Michaud, H. A., Giraudon-Paoli, M., Augier, S., Docquier, A., Gros, L., et al. (2019). Blocking Antibodies Targeting the CD39/CD73 Immunosuppressive Pathway Unleash Immune Responses in Combination Cancer Therapies. *Cell Rep* 27, 2411–2425. e9. doi:10.1016/j.celrep.2019.04.091

Roger, S., Jelassi, B., Couillin, I., Pelegrin, P., Besson, P., and Jiang, L. H. (2015). Understanding the Roles of the P2X7 Receptor in Solid Tumour Progression and Therapeutic Perspectives. *Biochim. Biophys. Acta* 10 (Pt B), 2584–2602. doi:10.1016/j.bbamem.2014.10.029

Sacchetti, C., Rapini, N., Magrini, A., Cirelli, E., Bellucci, S., Mattei, M., et al. (2013). *In Vivo* targeting of Intratumor Regulatory T Cells Using PEG-Modified Single-Walled Carbon Nanotubes. *Bioconjug. Chem.* 24, 852–858. doi:10.1021/bc400070q

Schneider, G., Glaser, T., Lameu, C., Abdelbaset-Ismail, A., Sellers, Z. P., Moniuszko, M., et al. (2015). Extracellular Nucleotides as Novel, Underappreciated Pro-metastatic Factors that Stimulate Purinergic Signaling in Human Lung Cancer Cells. *Mol. Cancer* 14, 201. doi:10.1186/s12943-015-0469-z

Schumacher, D., Strilic, B., Sivaraj, K. K., Wettschureck, N., and Offermanns, S. (2013). Platelet-Derived Nucleotides Promote Tumor-Cell Transendothelial Migration and Metastasis via P2Y2 Receptor. *Cancer Cell* 24, 130–137. doi:10.1016/j.ccr.2013.05.008

Shae, D., Becker, K. W., Christov, P., Yun, D. S., Lytton-Jean, A. K. R., Sevimli, S., et al. (2019). Endosomolytic Polymersomes Increase the Activity of Cyclic Dinucleotide STING Agonists to Enhance Cancer Immunotherapy. *Nat. Nanotechnol* 14, 269–278. doi:10.1038/s41565-018-0342-5

Shi, Y., and Lammers, T. (2019). Combining Nanomedicine and Immunotherapy. *Acc. Chem. Res.* 52, 1543–1554. doi:10.1021/acs.accounts.9b00148

Singleton, D. C., Macann, A., and Wilson, W. R. (2021). Therapeutic Targeting of the Hypoxic Tumour Microenvironment. *Nat. Rev. Clin. Oncol.* 18, 751–772. doi:10.1038/s41571-021-00539-4

Sitkovsky, M. V. (2020b). Lessons from the A2A Adenosine Receptor Antagonist-Enabled Tumor Regression and Survival in Patients with Treatment-Refractory Renal Cell Cancer. *Cancer Discov.* 10, 16–19. doi:10.1158/2159-8290.cd-19-1280

Sitkovsky, M. V. (2020a). Sufficient Numbers of Anti-tumor T Cells Is a Condition of Maximal Efficacy of Anti- Hypoxia-A2-Adenosinergic Drugs during Cancer Immunotherapy. *Curr. Opin. Pharmacol* 53, 98–100. doi:10.1016/j.coph.2020.07.011

Smith, T. T., Stephan, S. B., Moffett, H. F., McKnight, L. E., Ji, W., Reiman, D., et al. (2017). *In Situ* programming of Leukaemia-specific T Cells Using Synthetic DNA Nanocarriers. *Nat. Nanotechnol* 12, 813–820. doi:10.1038/nnano.2017.57

Song, W., Musetti, S. N., and Huang, L. (2017). Nanomaterials for Cancer Immunotherapy. *Biomaterials* 148, 16–30. doi:10.1016/j.biomaterials.2017.09.017

Sun, L., Shen, F., Tian, L., Tao, H., Xiong, Z., Xu, J., et al. (2021). ATP-responsive Smart Hydrogel Releasing Immune Adjuvant Synchronized with Repeated Chemotherapy or Radiotherapy to Boost Antitumor Immunity. *Adv. Mater.* 33, e2007910. doi:10.1002/adma.202007910

Tanamachi, K., Nishino, K., Mori, N., Suzuki, T., Tanuma, S. I., Abe, R., et al. (2017). Radiosensitizing Effect of P2X7 Receptor Antagonist on Melanoma *In Vitro* and *In Vivo*. *Biol. Pharm. Bull.* 40 (6), 878–887. doi:10.1248/bpb.b17-00083

Tang, L., Zheng, Y., Melo, M. B., Mabardi, L., Castaño, A. P., Xie, Y. Q., et al. (2018). Enhancing T Cell Therapy through TCR-Signaling- Responsive Nanoparticle Drug Delivery. *Nat. Biotechnol.* 36, 707–716. doi:10.1038/nbt.4181

Terry, S., Savagner, P., Ortiz-Cuaran, S., Mahjoubi, L., Saintigny, P., Thiery, J. P., et al. (2017). New Insights into the Role of EMT in Tumor Immune Escape. *Mol. Oncol.* 11, 824–846. doi:10.1002/1878-0261.12093

Thompson, E. A., and Powell, J. D. (2021). Inhibition of the Adenosine Pathway to Potentiate Cancer Immunotherapy: Potential for Combinatorial Approaches. *Annu. Rev. Med.* 72, 331–348. doi:10.1146/annurev-med-060619-023155

Vijayan, D., Young, A., Teng, M. W. L., and Smyth, M. J. (2017). Targeting Immunosuppressive Adenosine in Cancer. *Nat. Rev. Cancer* 17, 709–724. doi:10.1038/nrc.2017.86

Wang, Y., Shang, W., Niu, M., Tian, J., and Xu, K. (2019). Hypoxia-active Nanoparticles Used in Tumor Theranostic. *Int. J. Nanomedicine* 14, 3705–3722. doi:10.2147/ijn.s196959

Willingham, S. B., Hotson, A. N., and Miller, R. A. (2020). Targeting the A2AR in Cancer; Early Lessons from the Clinic. *Curr. Opin. Pharmacol.* 53, 126–133. doi:10.1016/j.coph.2020.08.003

Yang, M., Li, J., Gu, P., and Fan, X. (2021). The Application of Nanoparticles in Cancer Immunotherapy: Targeting Tumor Microenvironment. *Bioact Mater.* 6, 1973–1987. doi:10.1016/j.bioactmat.2020.12.010

Yu, W., Liu, R., Zhou, Y., and Gao, H. (2020). Size-Tunable Strategies for a Tumor Targeted Drug Delivery System. *ACS Cent. Sci.* 6, 100–116. doi:10.1021/acscentsci.9b01139

Yuan, C. S., Deng, Z. W., Qin, D., Mu, Y. Z., Chen, X. G., and Liu, Y. (2021). Hypoxia-modulatory Nanomaterials to Relieve Tumor Hypoxic Microenvironment and Enhance Immunotherapy: Where Do We Stand. *Acta Biomater.* 125, 1–28. doi:10.1016/j.actbio.2021.02.030

Zarek, P. E., Huang, C. T., Lutz, E. R., Kowalski, J., Horton, M. R., and Linden, J. (2008). A2A Receptor Signaling Promotes Peripheral Tolerance by Inducing T-Cell Anergy and the Generation of Adaptive Regulatory T Cells. *Blood* 111, 251–259. doi:10.1182/blood-2007-03-081646

Zhai, T., Zhong, W., Gao, Y., Zhou, H., Zhou, Z., Liu, X., et al. (2021). Tumor Microenvironment-Activated Nanoparticles Loaded with an Iron-Carbonyl Complex for Chemodynamic Immunotherapy of Lung Metastasis of Melanoma *In Vivo*. *ACS Appl. Mater. Inter.* 13, 39100–39111. doi:10.1021/acsami.1c11485

Zhang, P., Zhai, Y., Cai, Y., Zhao, Y., and Li, Y. (2019). Nanomedicine-Based Immunotherapy for the Treatment of Cancer Metastasis. *Adv. Mater.* 31, e1904156. doi:10.1002/adma.201904156

Zhang, W. J., Hu, C. G., Zhu, Z. M., and Luo, H. L. (2020). Effect of P2X7 Receptor on Tumorigenesis and its Pharmacological Properties. *Biomed. Pharmacother.* 125, 109844. doi:10.1016/j.biopha.2020.109844

Zhang, Y., Bush, X., Yan, B., and Chen, J. A. (2019c). Gemcitabine Nanoparticles Promote Antitumor Immunity against Melanoma. *Biomaterials* 189, 48–59. doi:10.1016/j.biomaterials.2018.10.022

Zhang, Y., Cheng, H., Li, W., Wu, H., and Yang, Y. (2019a). Highly-expressed P2X7 Receptor Promotes Growth and Metastasis of Human HOS/MNNG Osteosarcoma Cells via PI3K/Akt/GSK3beta/beta-Catenin and mTOR/HIF1alpha/VEGF Signaling. *Int. J. Cancer* 145, 1068–1082. doi:10.1002/ijc.32207

Zhang, Y., Ding, J., and Wang, L. (2019b). The Role of P2X7 Receptor in Prognosis and Metastasis of Colorectal Cancer. *Adv. Med. Sci.* 64, 388–394. doi:10.1016/j.advms.2019.05.002

Zhang, Y., Han, X., and Nie, G. (2021). Responsive and Activable Nanomedicines for Remodeling the Tumor Microenvironment. *Nat. Protoc.* 16, 405–430. doi:10.1038/s41596-020-00421-0

Zhao, R., Cao, J., Yang, X., Zhang, Q., Iqbal, M. Z., Lu, J., et al. (2021). Inorganic Material Based Macrophage Regulation for Cancer Therapy: Basic Concepts and Recent Advances. *Biomater. Sci.* 9, 4568–4590. doi:10.1039/d1bm00508a

Zhu, X., Li, Q., Song, W., Peng, X., and Zhao, R. (2021). P2X7 Receptor: a Critical Regulator and Potential Target for Breast Cancer. *J. Mol. Med. (Berl)* 99, 349–358. doi:10.1007/s00109-021-02041-x

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REVIEW
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The Role of Tissue-Resident Macrophages in the Development and Treatment of Inflammatory Bowel Disease

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Inflammatory bowel disease (IBD), comprising Crohn's disease and ulcerative colitis, is a refractory disease with many immune abnormalities and pathologies in the gastrointestinal tract. Because macrophages can distinguish innocuous antigens from potential pathogens to maintain mucosa barrier functions, they are essential cells in the intestinal immune system. With numerous numbers in the intestinal tract, tissue-resident macrophages have a significant effect on the constant regeneration of intestinal epithelial cells and maintaining the immune homeostasis of the intestinal mucosa. They also have a significant influence on IBD through regulating pro-(M1) or anti-inflammatory (M2) phenotype polarization according to different environmental cues. The disequilibrium of the phenotypes and functions of macrophages, disturbed by intracellular or extracellular stimuli, influences the progression of disease. Further investigation of macrophages' role in the progression of IBD will facilitate deciphering the pathogenesis of disease and exploring novel targets to develop novel medications. In this review, we shed light on the origin and maintenance of intestinal macrophages, as well as the role of macrophages in the occurrence and development of IBD. In addition, we summarize the interaction between gut microbiota and intestinal macrophages, and the role of the macrophage-derived exosome. Furthermore, we discuss the molecular and cellular mechanisms participating in the polarization and functions of gut macrophages, the potential targeted strategies, and current clinical trials for IBD.

Keywords: inflammatory bowel disease, ulcerative colitis, Crohn's disease, tissue-resident macrophage, macrophage polarization

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1 INTRODUCTION

In recent years, the morbidity of inflammatory bowel disease (IBD) has increased globally, especially in Africa and Asia, and is spreading worldwide with accelerated speed (Windsor and Kaplan, 2019). IBD is an idiopathic, refractory and chronic disease, with clinical symptoms including abdominal pain, rectal bleeding, weight loss, diarrhea, and anemia, that requires life-long medication (Roda et al., 2020; Seyed Tabib et al., 2020). Conventionally, IBD is mainly divided into Crohn's disease (CD) and ulcerative colitis (UC), along with different locations and inflammatory types, respectively (Torres et al., 2017; Ungaro et al., 2017). CD affects almost every part of the gastrointestinal tract and tissue layers, and the most frequent locations are the terminal ileum and colon (Torres et al., 2017; Roda et al., 2020). However, UC mainly involves the superficial intestinal mucosa of the colon,

including the proximal end of the rectum (Ungaro et al., 2017; Kobayashi et al., 2020). Approximately 5 million people worldwide are affected with IBD, yet the etiology of IBD is not known, and there are no cures so far (Kaplan, 2015). The continuation of this trend is likely to increase the worldwide population of patients affected by IBD to tens of millions or more in the next few decades (Ananthakrishnan, 2015). More importantly, recent discoveries revealed that patients with IBD are more susceptible to colorectal cancer (Nadeem et al., 2020). Thus, the prevention and treatment of IBD are extremely necessary and important.

The etiology of IBD arises from intricate interactions between the immune system, genetic predisposition, environmental cues, and the gut microbiome (Kobayashi et al., 2020; Roda et al., 2020). Under ideal conditions, intestinal inflammation is a self-limiting process that combats harmful pathogens and rapidly returns to homeostasis (Schett and Neurath, 2018). However, a disequilibrium between the mucosal immune system and the commensal ecosystem occurs in the exacerbation of IBD, because of the imbalance of the immunological mechanisms underlying the resolution of inflammation (Hunter, 2012; Schett and Neurath, 2018; Graham and Xavier, 2020). Intestinal macrophages are an integral part of the normal intestinal tissues that can engulf microorganisms and present antigens to activate T cells, as well as prevent excessive inflammation through specific molecular and cellular mechanisms (Smythies et al., 2005; Garrett et al., 2010; Mowat, 2018). According to the microenvironmental cues, macrophages could polarize into two different phenotypes: the classically activated (M1) or alternatively activated (M2) macrophages (Funes et al., 2018; Locati et al., 2020). When the intestinal barrier function is impaired, the efferocytosis effect leads to macrophages skewing toward M2 subtypes functionally, producing cytokines, chemokines and lipid mediators that participate in healing the intestinal mucosal barrier and maintaining homeostasis (Elliott et al., 2017; Na et al., 2019). Various cytokines and other soluble factors, including prostaglandin E2 (PGE2), bone morphogenetic protein 2 (BMP2), and WNT ligands, can be produced by gut macrophages that stimulate the expansion of epithelial cells and participate in the enteric nervous system or intestinal mucosal barrier to help maintain tissue homeostasis (Bain and Mowat, 2014). Meanwhile, intestinal macrophages maintain tolerance *via* promoting the proliferation of antigen-specific $CD4^+CD25^+$ regulatory T (T-reg) cells primarily by producing interleukin (IL)-10 in order to inhibit unrestrained inflammation as a response to innocuous commensal microbes (O'Garra et al., 2004; Hedrich and Bream, 2010).

In addition, the lack of response in patients with IBD to anti-tumor necrosis factor (TNF) therapy was found to be related to a defect in the IL-10 signaling pathway, which is crucial for biologics-induced macrophages skewing toward M2 subsets (Vos et al., 2012; Roda et al., 2016; Koelink et al., 2020). Therefore, intestinal macrophages play major roles in the establishment and maintenance of intestinal homeostasis. As a consequence, disequilibrium of intestinal macrophages could lead to a loss of tolerance to harmless microbes and food antigens, which is considered to underlie the excessive inflammation in IBD (de Souza and Fiocchi, 2016). In view of these findings, it is important to analyze the potential contribution of macrophages to intestinal chronic inflammation, which may offer us novel insights into the pathogenesis and therapeutic strategies in IBD.

In this review, we outline the characteristics of the origin and maintenance of macrophages, as well as their interaction with other immune cells. Then, we delineate the cellular and molecular mechanisms that underlie the differentiation and function of intestinal macrophages. Furthermore, we describe the interaction between gut microbiota and intestinal macrophages, and the role of the macrophage-derived exosome. Finally, potential treatment targets, applicable drugs and current clinical trials for IBD are discussed.

2 ORIGIN AND MAINTENANCE OF INTESTINAL MACROPHAGES

The gastrointestinal tract harbors copious amounts of tissue macrophages, orchestrating innate and adaptive immune systems, helping to maintain homeostasis *via* tolerance to commensal microbes, as well as resisting potentially harmful pathogens (Yip et al., 2021). In the past, the origin of tissue macrophages was considered to be the blood monocytes, deriving from precursors in the bone marrow (van Furth et al., 1972; Bain and Mowat, 2014). With the development of novel techniques, there is mounting evidence showing that monocytes are not the exclusive origin of tissue-resident macrophages (Wu and Hirschi, 2020). Macrophages arise in two different ways: they can be differentiated from blood-derived monocytes and embryo-derived precursors, deriving from the yolk sac and/or fetal liver, established in the tissue before birth (Shaw et al., 2018; Wu and Hirschi, 2020). Intestinal tissue-resident macrophages, derived from embryonic precursors, maintain themselves *via* self-renewal *in situ* throughout adulthood but also require constant replenishment by circulating monocytes in the blood (Shaw et al., 2018). This is in contrast to macrophages in other tissues, such as microglia in the central nervous system and Langerhans cells in the liver, that maintain themselves independently and self-renew *in situ* throughout adult life (Yona et al., 2013; Ginhoux and Guilliams, 2016).

With progress in recent research, traditional understanding has been challenged, and embryo-derived macrophages were found to sustain themselves in the adult intestine (Shaw et al., 2018). In adult mice, parts of intestinal macrophages, characterized by high expression levels of CD4 and apoptotic cell-uptake receptor Tim-4 ($Tim\text{-}4^+CD4^+$ macrophages), were demonstrated to be long-lived and locally self-renewed independent of monocytes, while $Tim\text{-}4^-CD4^+$ macrophages were found to have a slow turnover from blood monocytes (Shaw et al., 2018). In contrast, $Tim\text{-}4^-CD4^-$ macrophages were short-lived and relied on continuous replenishment of blood monocytes (Shaw et al., 2018). Similar results have been found in duodenal transplant patients' mucosa, along with short-lived monocyte-derived macrophage subsets, different and dominant subsets with a slower replenishment rate were confirmed (Bujko et al., 2018). Similarly, another study in

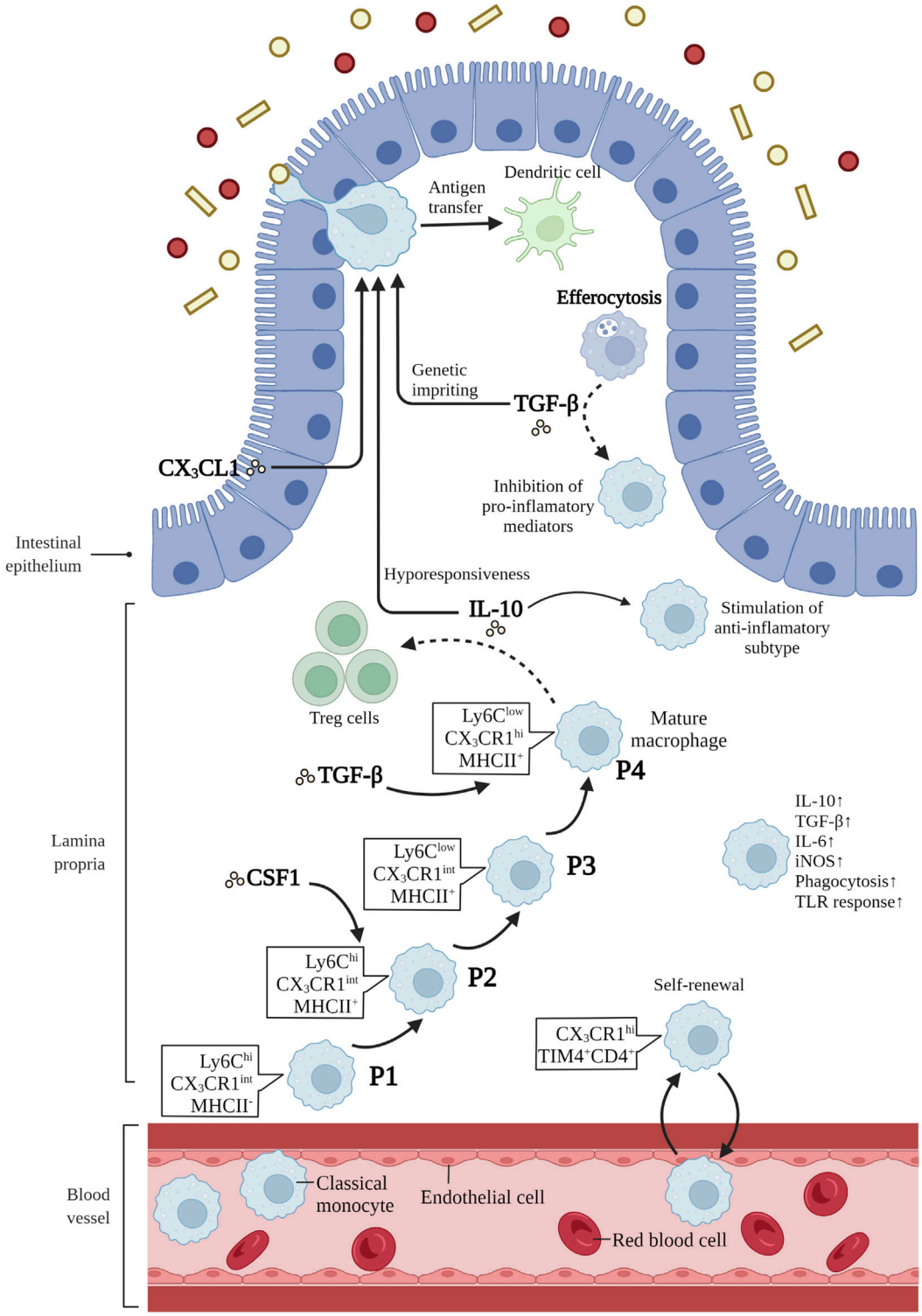

FIGURE 1 | Differentiation and function of intestinal macrophages. Intestinal macrophages are replenished by blood-derived monocytes through a series of differential intermediates in a way of "monocyte waterfall". lymphocyte antigen 6C-high ($Ly6C^{hi}$) CX3C-chemokine receptor 1-intermediate (CX_3CR1^{int}) major histocompatibility complex II ($MHCII^-$) (P1) monocytes enter the lamina propria, acquiring the expression of MHCII (P2), and then downregulating Ly6C expression (P3). Finally, these cells upregulate the expression of CX_3CR1, CD64, and F4/80, giving rise to mature macrophages (P4). T cell immunoglobulin mucin receptor 4-positive ($Tim\text{-}4^+$) $CD4^+$ macrophages have been demonstrated to be long-lived and locally self-renewed independent of monocytes. Macrophage colony-stimulating factor 1 (CSF1) is a critical cytokine in the survival, proliferation and differentiation of intestinal macrophages. IL-10 is crucial in inducing subtype of anti-inflammatory

(*Continued*)

FIGURE 1 | macrophages, maintaining the hyporesponsiveness to toll-like receptors (TLRs) stimulation, and promoting antigen-specific regulatory T (Treg) cells. CX3C-chemokine ligand 1 (CX_3CL1), secreted by intestinal epithelial cells, plays a critical role in the differentiation and function of mouse intestinal macrophages. In addition, CX_3CR1^+ macrophages form transepithelial dendrites (TEDs) with the ability to capture potential pathogens and transfer antigens to dendritic cells. The transforming growth factor-β (TGF-β) is essential for the terminal differentiation of macrophages in intestinal mucosa, and genetic imprinting of intestinal macrophages in mice. Besides, efferocytosis produce the secretion of TGF-β by macrophages, restraining the production of pro-inflammatory mediators. Mature intestinal macrophages are accompanied with the upregulation of IL-10, TGF-β, IL-6, iNOS, and the ability of phagocytosis and TLR responsiveness. Created with BioRender.com.

mice found that self-renewal embryo-derived macrophages and bone marrow-derived monocytes concomitantly reside in the lamina propria and muscularis externa layer of the intestine, persisting throughout adulthood (De Schepper et al., 2018). Depletion of self-maintaining macrophages could lead to a series of problems, such as injury of the submucosal vascular network, damage of enteric neurons, impaired neuro-evoked gastrointestinal section, and weakened intestinal motility (De Schepper et al., 2018). As noted above, parabiosis experiments (Guilliams et al., 2013; Hashimoto et al., 2013; Jakubzick et al., 2013; Epelman et al., 2014) and genetic fate-mapping experiments (Ginhoux et al., 2010; Hoeffel et al., 2012; Schulz et al., 2012; Yona et al., 2013) have proved that macrophages in the adult gut originating from yolk-sac precursors maintain self-renewal throughout life, thus existing independently of bone-marrow precursors for long-lasting maintenance. Given that there are different intestinal macrophage subsets with strong heterogeneity and distinct functional characteristics, future studies on macrophages in IBD should be focusing on altering the inflammatory phenotype, in parallel with keeping the protective tissue-resident subpopulations intact.

It has been proposed that macrophage precursors might compete for a limited number of niches, and the balance between embryo-derived macrophages and bone-marrow derived monocytes is delicately orchestrated by niche accessibility, availability and precursor plasticity (Guilliams and Scott, 2017; Guilliams and Svedberg, 2021). The orchestration of the niche assures that monocytes can differentiate efficiently into macrophages based on tissue-specific microenvironments only when the niche is available (Guilliams and Scott, 2017). Niche imprints play a strategic role in dictating macrophage identity and capacity for self-maintenance rather than only their origin, as was described previously (Mass and Gentek, 2021; Viola and Boeckxstaens, 2021). When the body is in a steady state, embryonic macrophages continuously renew and maintain themselves in adult tissues (Louwe et al., 2021). In the case of infection and tissue damage causing large consumption of tissue-resident macrophages, blood circulating monocytes can be used for long-term reconstruction of tissue-resident macrophages (Louwe et al., 2021). Intestinal macrophages are replenished by blood-derived monocytes through a series of differential intermediates in a step-wise continuum way, which is a process taking around 5–6 days, and has become known as the monocyte waterfall (Bain et al., 2013; Bain et al., 2014). (**Figure 1**). Lymphocyte antigen 6C-high ($Ly6C^{hi}$) CX3C-chemokine receptor 1- intermediate ($CX3CR1^{int}$) major histocompatibility complex II ($MHCII^-$) (P1) monocytes seem similar to their counterparts in blood in aspects of both phenotype and morphology (Schridde et al., 2017). As these cells enter the lamina propria, they first acquire the expression of MHCII (P2), and then downregulate Ly6C expression (P3), together with the other proteins of extravasation, and finally, upregulating the expression of CX_3CR1, CD64, and F4/80, giving rise to mature macrophages (P4) (Bain et al., 2013; Bain et al., 2014) (**Figure 1**). Notably, more and more evidences suggest that there is an analogous waterfall in the human intestinal mucosa, with classical $CD14^{hi}CCR2^+CD11c^{hi}$ monocytes gradually differentiating into $CD14^{low}CCR2^-CD11c^{low}$ macrophages (Bain et al., 2013; Bernardo et al., 2018; Bujko et al., 2018). However, there are still many questions to be answered about this process. How do macrophages differentiate into specific compartments in the distinct micro-environment of the duodenum with powerful absorptive ability or the cecum with myriad microbes? Is there a role for tissue-resident macrophages in memorizing past inflammatory injury within the intestinal tissue? Based on the aforementioned findings and potential challenges, facilitating the restoration of inflammation-imprinted resident macrophages and their niche in homeostasis may bring about a promising therapeutic avenue to prevent secondary complications in these patients.

3 FUNCTION OF INTESTINAL MACROPHAGES IN IBD

IBD is characterized by a destructive and relapsing inflammation of the gastrointestinal tract that harbors many macrophages that accumulate in the mucosa and are involved in the pathogenetic process. In IBD, the inflamed colonic mucosa can recruit a large number of macrophages, vigorously secreting IL-1, IL-6, TNF-α, IL-12, and IL-23, as well as producing reactive oxygen species (ROS), reactive nitrogen intermediates (RNIs), and proteases that could degrade the extracellular matrix. However, the characteristics of intestinal-resident macrophages in CD and UC are distinct with respect to distribution, ability to clear pathogens, and involvement in fibrosis. Macrophages mainly spread within the intestinal mucosa in UC, however, in CD, these cells also infiltrate in the muscular layer and mesenteric fat tissue (Mahida et al., 1989; Kredel et al., 2013). In the past, there was a simplified statement that CD has even been regarded as a primary immunodeficiency of macrophage (Casanova and Abel, 2009). Therefore, impaired cytokine secretion by macrophages was thought to be the reason for defective pathogenic clearance in CD (Smith et al., 2009). It is common to observe that intestinal macrophages contain *E. coli* in CD, but not in UC (Elliott et al., 2015). In contrary, bacterial pathogens were responded actively by macrophages in patients with UC (Rahman et al., 2010). As a

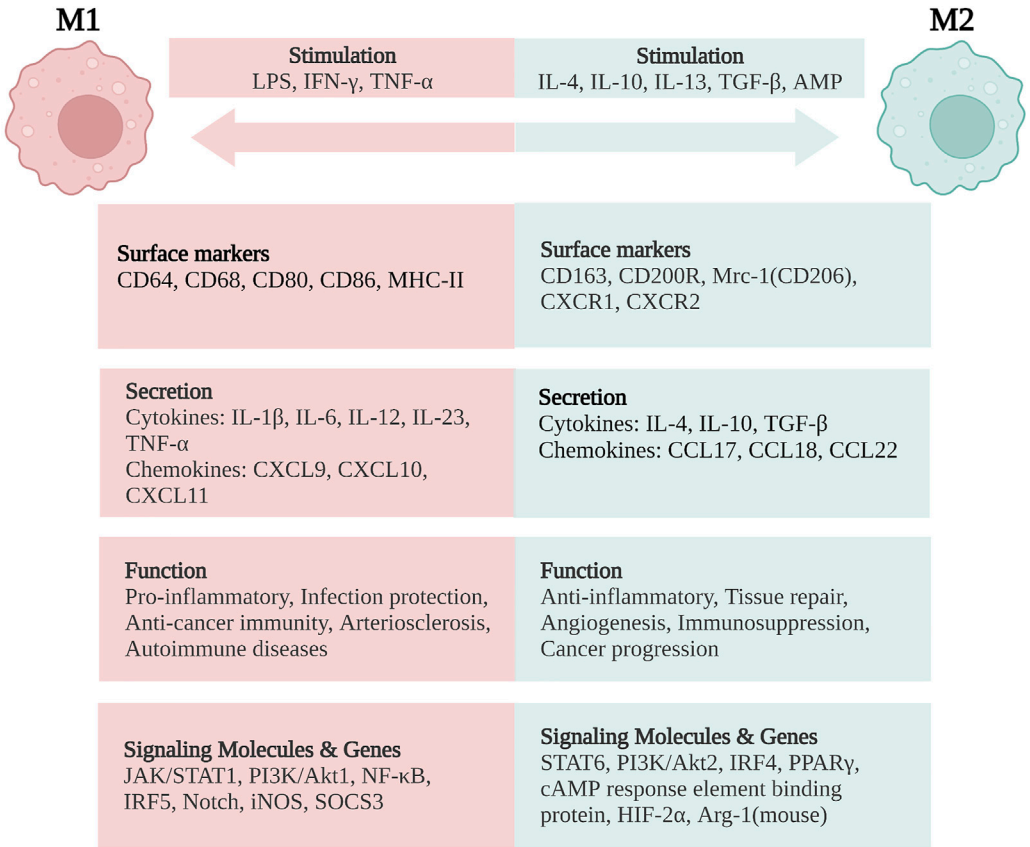

FIGURE 2 | Macrophage polarization and phenotypes. M1 macrophages are stimulated by LPS, IFN-γ, and TNF-α, while M2 macrophages are stimulated by IL-4, IL-10, IL-13, TGF-β, and AMP. M1 macrophages are characterized by the functions of pro-inflammatory, infection protection, anti-cancer immunity, arteriosclerosis, and autoimmune diseases. M2 macrophages exert the functions of anti-inflammatory, tissue repair, angiogenesis, and immunosuppression, but are related with cancer progression. LPS, lipopolysaccharide; IFN, interferon; TNF, tumor necrosis factor; IL, interleukin; TGF, transforming growth factor; AMP, adenosine monophosphate; CD, cluster of differentiation; MHC, major histocompatibility complex; Mrc-1, mannose receptor C-type 1 (also known as CD206); CXCR, CXC chemokine receptor; CXCL, CXC chemokine ligand; CCL, CC chemokine ligand; JAK/STAT, Janus kinase/signal transducer and activator of transcription; PI3K/Akt, phosphatidylinositol-3-kinase/Akt; NF-κB, nuclear factor-κB; IRF, interferon regulatory factor; iNOS, inducible nitric oxide synthase; SOCS3, suppressor of cytokine signaling-3; PPARγ, peroxisome proliferator activated receptor-γ; HIF-2α, hypoxia-inducible factor-2α; Arg-1, arginase-1. Created with BioRender.com.

consequence, the formation of granulomas is generally found in CD patients but not the UC patients, because of the failure to eradicate invading pathogens effectively (Cook and Dixon, 1973). Notably, the degree of fibrosis is more pronounced in CD than in UC (Yamagata et al., 2011). Excessive wound-healing processes involve various steps with macrophages, resulting in the development of fibrosis and fibrotic strictures subsequently. In fibrotic CD, several factors produced by macrophages promote the formation of fibrosis, such as MMP-2, IL-13, TNF-α, and transforming growth factor-β (TGF-β) (Fichtner-Feigl et al., 2006; Bailey et al., 2012).

3.1 Macrophage Subsets in IBD

Mature macrophage phenotypes and functions are highly plastic and heterogeneous in response to environmental cues (Mosser and Edwards, 2008). Conventionally, a dichotomic nomenclature describes activated macrophages as having two opposite states: classically activated (M1) and alternatively activated (M2) macrophages (Gordon, 2003; Aggarwal et al., 2014). The imbalance of M1/M2 phenotypes has been demonstrated to be involved in colitis exacerbation in the murine model of IBD (Zhu et al., 2014). Here, we summarize the stimulators of M1 and M2 macrophages, molecular marker expression, signaling pathways involved, major cytokines and chemokines secreted, as well as their functions in IBD.

In general, classically activated (M1) macrophages are characterized by secreting cytokines such as IL-12, IL-23, IL-1β, TNF-α, ROS, and RNIs, inducing inflammation and clearing pathogens. In lamina propria of inflamed gut, pro-inflammatory M1 macrophages break down the tight junction proteins, damage the epithelial barrier, and induce epithelial cell apoptosis, leading to excessive inflammation (Cosín-Roger et al., 2013; Steinbach and Plevy, 2014; Lissner et al., 2015). In addition, the M1 subsets are also involved in the induction of T helper 1 cells (Th1) and Th17 responses. The transformation of macrophages into the M1 phenotype involves many key transcription factors, such as Janus kinase/signal transducer and transcription activator 1 (JAK/STAT1) (Hu et al., 2002; Gordon and Martinez, 2010),

TABLE 1 | Different subclasses of M2 macrophage.

Subclass	Stimuli	Cell markers	Chemokines	Cytokines and mediators	Functions
M2a	IL-4, IL-13	Mouse: Arg1, Fizz1, Chi3l3, CD206 Human: CD206, CD163^{low}, IL-1R, CD14^{low}	CCL13, CCL17, CCL18, CCL22, CCL24	IL-10, Arg1, TGF-β, IGF, Fibronectin	Anti-inflammatory, wound healing, elimination of parasites
M2b	LPS, IC, IL-1β	Mouse: CD80, CD86, IL-10^{high}, IL- 12^{low}, HLA-DR^{low}, MHC II Human: CD14^{high}, CD80^{high}, CD200R^{low}	CCL1	IL-1, IL-6, IL-10, TNF-α	Th2 activation, immunoregulation, promoting infection, tumor progression
M2c	IL-10, Glucocorticoids , TGF-β	Mouse: Arg1, CD206 Human: CD86^{low}, CD163^{high}, CD206, TLR1, TLR8	CCL16, CCL18, CXCL13	IL-10, TGF-β, Arg1, MerTK	Anti-inflammatory, tissue remodeling, phagocytosis, immunosuppression
M2d	A2R ligands, LPS, IL-6	Mouse: IL-10^{high}, IL-12^{low}, TNF-α^{low}, VEGF Human: VEGF, IL-10, TGF-β	CCL5, CXCL10, CXCL16	IL-10, VEGF, TGF-β	Anti-inflammatory, angiogenesis, tumor progression

A2R, A2 adenosine receptor; Arg-1, arginase-1; CCL, chemokine (C-C motif) ligand; CD, cluster of differentiation; CXCL, chemokine (CX-C motif) ligand; Chi3l3, chitinase 3-like 3 (also known as Ym1); FIZZ1, found in inflammatory zone 1; IC, immune complex; IGF, insulin-like growth factor; LPS, lipopolysaccharide; MerTK, Mer receptor tyrosine kinase; TLR, Toll-like receptor; VEGF, vascular endothelial growth factor.

phosphoinositide 3-kinase (PI3K)/Akt1 (Lu et al., 2017; Vergadi et al., 2017), nuclear factor (NF)-κB (Oeckinghaus et al., 2011; Zhang et al., 2018), interferon regulatory factor 5 (IRF5) (Chistiakov et al., 2018), and Notch (Xu et al., 2012; Siebel and Lendahl, 2017). On the other hand, sustained M1 phenotype expression has been shown to aggravate the inflammatory response and ultimately cause tissue damage (**Figure 2**).

On the contrary, alternatively activated (M2) macrophages are usually characterized by upregulated factors such as IL-4, IL-10, mannose receptor C-type 1 (Mrc-1, also known as CD206), CD163, arginase-I, IL-1 receptor antagonist, and stabilin-1, which could prevent excessive inflammation, and promote tissue healing. M2 polarization is mainly induced by transcription factors including STAT6 (Ohmori and Hamilton, 1997; Martinez et al., 2009), PI3K/Akt2 (Lu et al., 2017; Vergadi et al., 2017), IRF4 (Satoh et al., 2010; Chistiakov et al., 2018), peroxisome proliferator-activated receptor (PPAR)γ (Bouhlel et al., 2007; Odegaard et al., 2007), and cyclic adenosine monophosphate (cAMP) response element binding protein (Ruffell et al., 2009; Luan et al., 2015). M2 macrophages promote resolving inflammation and remodeling tissue by producing several factors, especially IL-10. Some research has shown that the inhibition of M2 polarization or the synthesis of its cytokines may result in the exacerbation of colitis (Hunter et al., 2010; Zhu et al., 2014). M2 macrophages activate TGF-β by promoting Th2 responses to further promote fibrosis that is intimately involved in tissue remodeling (Murray and Wynn, 2011; Locati et al., 2013; Wynn and Vannella, 2016). In addition, M2 macrophages promote matrix metalloproteinases (MMPs) that control extracellular matrix (ECM) turnover to remove debris, apoptotic cells, and various ECM components, which could help to prevent tissue-damaging M1 macrophage responses (Murray and Wynn, 2011). Moreover, the expression of Arg1, Fizz1, and Ym1 has been shown to decrease inflammatory responses and promote tissue remodeling (Lawrence and Natoli, 2011; Kühl et al., 2015). According to different stimuli and transcriptional changes, the M2 macrophages could be subdivided into several subsets (M2a, M2b, M2c, and M2d subsets) with distinct pathophysiological characteristics (The details are summarized in **Table 1**) (Rőszer, 2015; Wang et al., 2019; Du et al., 2021). Based on these findings, the importance of macrophage polarization in the progression and prognosis of inflammation is becoming increasingly appreciated (**Figure 2**).

3.2 Intestinal Macrophages and Gut Microbiota in IBD

Gut commensal microbiota has continuous interaction with epithelial cells, which is essential in shaping the function of intestinal barrier structure (Hayes et al., 2018). The dysbiosis of gut microbiota has been considered as the key of IBD etiopathology (Gilbert et al., 2018). Pathogen/microbial-associated molecular patterns (PAMPs/MAMPs) are microbial signature molecules, which are recognized and attached by the innate intracellular receptors of macrophages. The macrophages present antigens to activate T cells, leading to the production of cytokines, chemokines, and antimicrobial peptides (AMPs) to maintain the integrity of intestinal barrier (Wells et al., 2011). Besides, the recruitment and differentiation of intestinal macrophage also need the crucial mediator of the microbiota, aside from the induction of local cytokines, growth factors, and chemokines (Fiocchi, 2008). A few of studies have shown in germ-free mice or in mice with depletion of microbiota with broad-spectrum antibiotics, could decrease the number of monocyte-derived or tissue-resident macrophages (Bain et al., 2014; Muller et al., 2014; Shaw et al., 2018). The metagenomic sequencing of microbial RNA has revealed that there are significant alterations in microbial composition, location and biodiversity between IBD patients and healthy individuals (Wallace et al., 2014).

For instance, the adherent invasive *E. coli* (AIEC) showed significant increased amount in IBD patients, and it is involved in

IBD pathogenesis through evading the immune system of the host, linking to intestinal epithelial cells, and promoting excessive inflammation (Lloyd-Price et al., 2019). Besides, AIEC is associated with the ability to transverse the intestinal wall into the lamina propria, even survive and replicate within macrophages by escaping autophagy (Palmela et al., 2018). *Campylobacter* concisus (C. concisus) is a kind of adherent and invasive proteobacterium, participating in the pathogenesis of IBD (Underwood et al., 2016). Some C. concisus strains acquired zonula occludens toxin (zot) gene from a virus (prophage), damaging epithelial tight junctions, inducing macrophage production of undue pro-inflammatory cytokines, such as TNF-α, and ultimately causing the chronic relapse of IBD (Zhang et al., 2014). Akkermansia muciniphila (A. muciniphila) is a Gram-negative anaerobic bacterium, that revealed protective potential with reduced abundance both in CD and UC (Png et al., 2010). A. muciniphila or a specific outer membrane protein Amuc_1100 could improve colitis, with the decreased number of infiltrating macrophages and $CD8^+$ cytotoxic T lymphocytes in the gut (Wang et al., 2020). In a word, it is recognized as a crucial challenge to reconstitute the relationship between the host and the microbiome to an effective symbiotic state.

3.3 Macrophage-Derived Exosome

Macrophage-derived exosome has been shown to play an important role in the pathogenesis of inflammatory exacerbation and resolution by interacting with intestinal epithelial cells (IECs) and other cells. Exosome-derived miR-21a-5p from M1 macrophages could be absorbed by IECs, correlated with downregulation of E-cadherin and activation of group 2 innate lymphoid cells, aggravating dextran sulfate sodium (DSS)-induced colitis in mice (Lu et al., 2021a). In addition, the M2 macrophage-derived exosome miR-590–3p targets large tumor suppressor kinase 1 and then activates Yes-associated protein/β-catenin transcription in IECs, hence reducing colonic inflammation, strengthening mucosal healing, and alleviating DSS-induced colitis in mice (Deng et al., 2021).

4 REGULATION MECHANISM OF MACROPHAGES IN IBD

Generally speaking, as specialized phagocytic cells of the innate immune system, macrophages exhibit multiple functions such as the maintenance of systematic homeostasis, protective defense of the host, and regulation of inflammatory response, as well as re-establishing tissue barriers upon mucosal disruption (You et al., 2016; Dionne et al., 2017). In addition, intestinal macrophages have also been found to secrete various cytokines to maintain tissue homeostasis (Grainger et al., 2017). Here, we summarize the critical regulation mechanisms of macrophages in IBD.

CSF-1. Macrophage colony-stimulating factor 1 (CSF1) is the primary cytokine, which shows a critical role in the survival, proliferation and differentiation of intestinal macrophages. It has been demonstrated that the number of intestinal macrophages appears significant decrease in Csf1-null mice and these with the administration of anti-CSFR1 antibody (MacDonald et al., 2010). Besides, following anti-CSF1R treatment, the depletion of macrophage leads to restraining Paneth cell differentiation, reducing $Lrg5^+$ stem cells, and ultimately impairing the development of IECs (Sehgal et al., 2018).

IL-10/IL-10R Axis. The IL-10/IL-10-receptor (IL-10R) axis is crucial in regulating phenotype of intestinal macrophages in both mice and humans. Lack of IL-10 signaling results in macrophage hyper-responsiveness to toll-like receptors (TLRs) stimulation, causing the development of spontaneous intestinal inflammation (Zigmond et al., 2014). Disrupted IL-10/IL-10R axis can affect several regulatory pathways involved in macrophage activation, such as STAT1, STAT3, nuclear factor-κB (NF-κB), and TREM-1, as well as increase chromatin accessibility to pro-inflammatory genes (Hirotani et al., 2005; Schenk et al., 2005; Simon et al., 2016). Besides, macrophage-derived IL-10 has been demonstrated that it is crucial for maintaining and promoting antigen-specific Treg cells in the intestinal mucosa in mice (Hadis et al., 2011).

CX_3CL1-CX_3CR1. The CX_3CL1-CX_3CR1 axis plays a critical role in the differentiation and function of mouse intestinal macrophages, considering that the high expression of CX_3CR1 and their special localization near by the CX_3CL1-positive intestinal epithelial cells. Lack of CX_3CR1 expression of intestinal macrophages leads to the decrease of IL-10 and defect in promoting Treg cells in the lamina propria, which may exacerbate the DSS-induced colitis in mice (Hadis et al., 2011). In patients with CD, a missense mutation in the CX_3CR1 gene was identified, which was related with an impaired antifungal ability and may lead to progression of extra-intestinal inflammatory diseases (Leonardi et al., 2018). In addition, CX_3CR1^+ macrophages can form transepithelial dendrites (TEDs) with the ability to capture soluble antigens or potential pathogens, and even migrate into the colonic lumen to execute the mission (Rescigno et al., 2001; Vallon-Eberhard et al., 2006).

TGF-β. The TGF-β/TGF-β-receptor (TGF-βR) axis is essential for the terminal differentiation of macrophages in intestinal mucosa, and genetic imprinting of intestinal macrophages in mice, including CX3CR1, IL-10, and αvβ5 integrin genes (Schridde et al., 2017). In line with this, the expression of the Runt-related transcription factor 3 (RUNX3), which is regulated by TGF-β, is a unique feature of intestinal macrophages (Lavin et al., 2014). In addition, macrophage turnover is regulated by the TGF-β/TGF-βR axis by controlling the expression of CCL8 (Schridde et al., 2017). Besides, efferocytosis produce the secretion of TGF-β by macrophages, restraining the production of pro-inflammatory mediators by a mechanism of autocrine/paracrine way involving TGF-β (Fadok et al., 1998).

5 TARGETING MACROPHAGES FOR IBD

There is a huge driving force for exploring innovative treatment options in IBD, because existing therapeutic strategies remain ineffective in many patients (Papamichael et al., 2015). It has been

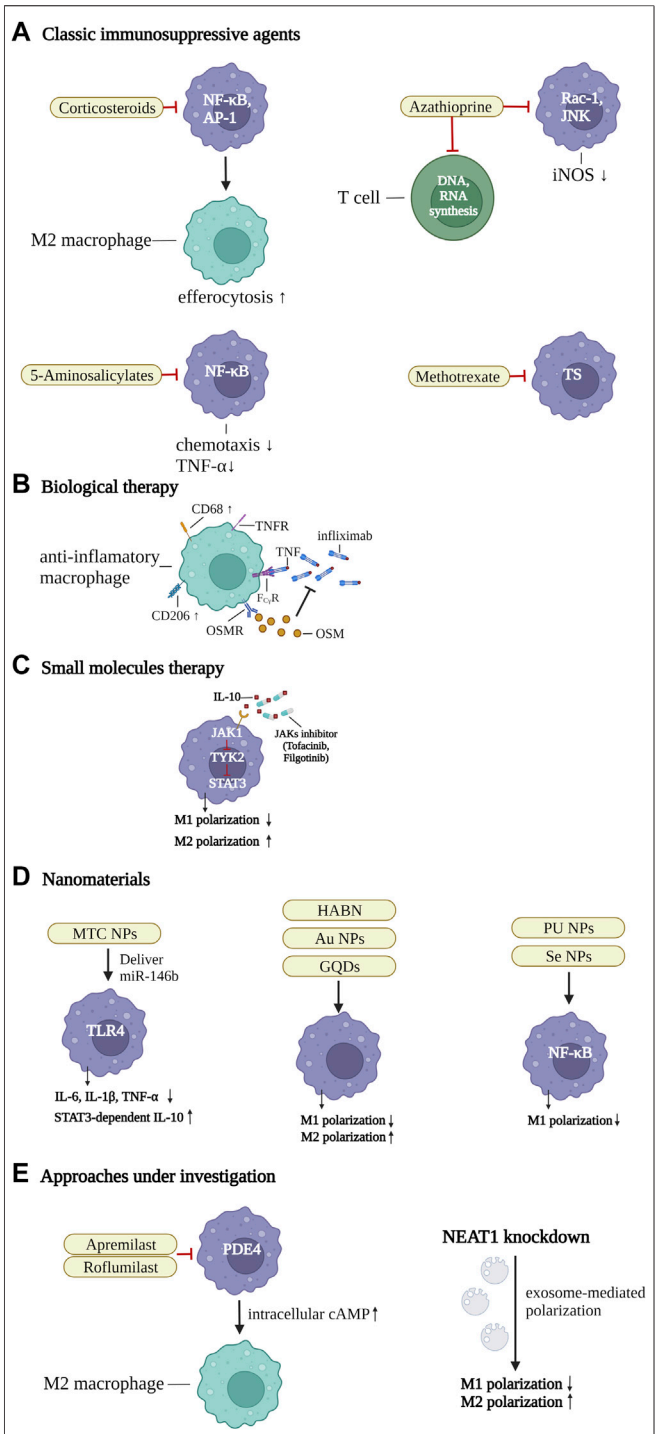

FIGURE 3 | Therapeutic strategies of IBD by targeting the macrophages. **(A)** classic immunosuppressive agents. Corticosteroids inhibit nuclear factor-κB (NF-κB) and activator protein 1 (AP-1), thereby enhancing efferocytosis and inducing the differentiation of M2 macrophages. 5-Aminosalicylate suppresses NF-κB to downregulate TNF-α effects and control the chemotaxis of macrophages. Azathioprine not only restricts T-cell activation by abolishing DNA or RNA synthesis, but also suppresses Ras-related C3 botulinum toxin substrate 1 (Rac1) activity and the phosphorylation of JUN N-terminal kinase, and the expression of iNOS. Methotrexate regulates pro-inflammatory gene expression of macrophages, by controlling thymidylate synthase (TS), **(B)** (*Continued*)

FIGURE 3 | biological therapy. Infliximab blocks TNF and TNF receptor (TNFR)-mediated inflammatory response in intestinal macrophages, by binding with Fcγ receptor (FcγR), and inducing $CD68^{+}CD206^{+}$ regulatory macrophages. The numbers of oncostatin M (OSM) and the receptor (OSMR-β) are associated with unresponsiveness to TNF-α blockers. **(C)** small molecules. JAK inhibitors inhibit the IL-10-JAK1-tyrosine kinase 2-STAT3 signaling pathway, which is crucial for maintaining mature macrophages in intestinal homeostasis. In addition, they can downregulate M1 and promote the differentiation of M2 macrophages. **(D)** nanomaterials. A series of macrophage targeting nanoparticles (NPs) could be synthesized to deliver specific pathway inhibitors or agonists to control inflammatory-related pathways and promote the polarization of anti-inflammatory macrophages, such as mannose-modified trimethyl chitosan (MTC) NPs, Hyaluronic acid-bilirubin nanomedicine (HABN), gold (Au) NPs, graphene quantum dots (GQDs), polyurethane (PU) NPs, and selenium (Se) NPs. **(E)** approaches under investigation. Apremilast and roflumilast are two new phosphodiesterase 4 (PDE4) blockers. The intracellular cAMP level was increased by inhibition of PDE4, thereby blocking inflammation-related factors and promoting pro-resolving macrophages. Inhibition of the Nuclear Enriched Abundant Transcript 1 (NEAT1), a new nuclear long non-coding RNA, can regulate the intestinal mucosal barrier and induce exosome-mediated differentiation of macrophages. Created with BioRender.com.

shown that modulating the polarization of M2 macrophages or suppressing inflammation-related cytokines could be an innovative therapeutic strategy to regulate intestinal inflammation and restore tissue homeostasis (Na et al., 2019; Du et al., 2021). Modulating the phenotypes of macrophages by inhibiting the pro-inflammatory M1 subset and/or inducing the anti-inflammatory M2 subset, may ameliorate IBD effectively (Ma et al., 2019). It has also been shown that macrophage functions can be affected and modulated by classic IBD agents (such as, infliximab or mesalazine) in many ways, for instance, by downregulating inflammatory signaling pathways and/or skewing to the M2 subpopulation (Bantel et al., 2000; Vos et al., 2012). In the following paragraphs, the current treatment strategies involving macrophages and potential new options to enhance the accumulation of the pro-resolving subpopulation in IBD are summed up in detail (**Figure 3**).

5.1 Classic Immunosuppressive Agents

Conventional treatment strategies usually rely on modulating the systemic immune responses by mitigating progression of mucosal inflammation. These conventional strategies are mainly based on immunomodulatory medications such as corticosteroids, aminosalicylates, antibiotics, and thiopurines, as well as folic acid antagonists (methotrexate) (Sales-Campos et al., 2015; Chen et al., 2021).

As the central medication for acute exacerbations of IBD patients, both UC and CD, corticosteroids remain the cornerstone of therapeutic options at present (Mowat et al., 2011). Corticosteroids comprise a series of drugs that exerts anti-inflammatory effects by binding to particular cytosolic receptors and suppressing transcription factors, for instance, NF-κB and activator protein 1 (Oakley and Cidlowski, 2013). Moreover, the activation of immune cells and the production of adhesion molecules are suppressed by corticosteroids in inflamed sites (Sales-Campos et al., 2015; Chen et al., 2021). In addition, corticosteroids have several effects on macrophages, ranging from

enhancing efferocytosis to inducing the polarization of the M2 subpopulation (Giles et al., 2001; Ehrchen et al., 2007). Nevertheless, if corticosteroids are administrated chronically, the occurrence of side effects (e.g., osteoporosis, metabolic disease, cardiovascular syndrome), along with the non-responsive subpopulation, will appear and remain a primary obstruction to sustained remission (Ford et al., 2011; Sales-Campos et al., 2015).

5-Aminosalicylates could control the chemotaxis of macrophages or promote the proliferation of intestinal mucosa cells by downregulating TNF-α effects as well as suppressing NF-κB signaling pathways directly, which play an integral role in pro-inflammatory macrophages in UC patients (Kaiser et al., 1999; Bantel et al., 2000). In addition, 5-aminosalicylates are often combined with corticosteroids in UC patients to reinforce the anti-inflammatory effects (Allgayer, 2003).

Azathioprine and methotrexate are the other two classic immunosuppressive drugs for IBD (Feagan et al., 2000). They not only restrict T-cell activation by abolishing DNA or RNA synthesis but suppress RAC1 activity *via* binding to 6-thioguanine triphosphates, resulting in anti-inflammatory functions in the mouse model by inhibiting the phosphorylation of JUN N-terminal kinase and the expression of iNOS. (Elion, 1989; Marinković et al., 2014). By controlling thymidylate synthase, methotrexate regulates pro-inflammatory gene expression of macrophages (Municio et al., 2016). Based on the aforementioned evidence, macrophage-modulating strategies play an important role in treating IBD patients.

5.2 Biological Therapy

TNF-α is a pro-inflammatory mediator that can be produced by macrophages and other cells including B cells, T cells, natural killer (NK) cells, dendritic cells (DCs), neutrophils, and epithelial cells (Kany et al., 2019). Macrophages are the major producers of TNF-α and interestingly are also highly responsive to TNF-α (Parameswaran and Patial, 2010).There is increasing evidence reinforcing that TNF-α plays a crucial role in the pathogenesis of IBD (Targan et al., 1997; D'Haens et al., 1999; Rutgeerts et al., 1999). The anti-TNF treatment remains a crucial strategy for IBD patients, given that it has the ability to promote the differentiation of regulatory macrophages (Vos et al., 2012).

TNF-α blockers have been used to prevent TNF-α-mediated intestinal mucosal damage and reduce or terminate the biological effects of TNF-α. Since the anti-TNF agent infliximab was approved by U.S. Food and Drug Administration in 1997, biologics have become the primary medications in the treatment of IBD patients (Stack et al., 1997; Targan et al., 1997). Thalidomide is a TNF-α inhibitor, which has been demonstrated to be effective for inducing remission in pediatric CD and adults with refractory CD (Sabate et al., 2002; Lazzerini et al., 2013). By blocking the production of interferon-regulatory factor-5 (IRF-5), thalidomide significantly inhibits the differentiation of M1 macrophage, with downregulated surfaced markers including CD86 and CCR7, as well as decreased pro-inflammatory cytokines including IL-12 and IFN-γ, that attenuates intestinal inflammation and facilitates mucosal healing (Lu et al., 2021b). Several new anti-TNFα agents, such as adalimumab, certolizumab pegol, and golimumab, have been applied in clinical research (Danese et al., 2015; Billmeier et al., 2016; Holleran et al., 2017; Chudy-Onwugaje et al., 2019; Na et al., 2019). The ENVISION I study is a phase III double-blind randomized controlled clinical trial (RCT) investigating the clinical efficacy of adalimumab in pediatric patients with moderate-to-severe UC (Croft et al., 2021). After 8 weeks of treatment, the adalimumab group had significantly more frequent clinical remission than the placebo group (adalimumab group vs. placebo group; 60 vs. 19.8%, p = 0.0001) (Croft et al., 2021). Therefore, moderate-to-severe UC in children could be efficaciously and safely treated by adalimumab (Croft et al., 2021).

TNF-α inhibitors can specifically bind to receptors to form immune complexes (ICs) to neutralize TNF-α. Once bound to the antibody, TNF-α receptor activation is blocked, leading to decreased permeability of cytomembranes and paracellular tight junctions, as well as blocking the accumulation of inflammatory cells in the local intestinal mucosa (Baert et al., 1999; D'Haens et al., 1999; Rutgeerts et al., 1999; Koch and Nusrat, 2012; Vos et al., 2012; Olesen et al., 2016). In addition, by hindering lipid rafts, infliximab could rebuild equilibrium between the intestinal mucosal barrier and adherent invasive *E. coli* in CD (Yakymenko et al., 2018). In addition, anti-TNF medications could not only reduce the secretion of inflammatory cytokines and infiltration of T cells but also skew monocytes to differentiation into regulatory macrophages ($CD68^+CD206^+$), concurrently (Vos et al., 2012; Olesen et al., 2016). Compared to anti-TNF-α monotherapy, TNF-α inhibitors combined with thiopurines have been demonstrated to promote the differentiation of regulatory macrophages and enhance immunosuppressive efficacy (Vos et al., 2012). However, a majority of IBD patients are intolerant to the therapeutic regimen of anti-TNFα combined with thiopurine, which makes the strategy difficult to carry out (Jharap et al., 2010). Hence, ongoing studies should focus on finding novel alternatives for anti-TNFα combined treatment.

In addition, anti-TNF agents can induce alternative macrophage polarization *via* Fcγ receptor ligation (Lucas et al., 2005; Colombel et al., 2010; Pander et al., 2011; Vos et al., 2011; Vos et al., 2012) and increase IL-10 production of macrophages (Pander et al., 2011; Vos et al., 2011; Levin et al., 2016; Bloemendaal et al., 2017; Koelink et al., 2020). After TNF-α blocker treatment, there was obvious differentiation of the M2 subpopulation in patients with mucosal healing, whereas those without mucosal healing had no such induction (Vos et al., 2012). However, the anti-inflammatory subpopulation cannot be induced by biologics without the Fc fragment, for example, certolizumab pegol, indicating that the research on novel antibody-based strategies may be a broad avenue for IBD therapy (Boyer et al., 2016).

Moreover, the expression of oncostatin M (OSM) is elevated in IBD patients and is associated with unresponsiveness to TNF-α blockers (West et al., 2017). As a member of the IL-6 cytokine family, OSM comprises IL-11, IL-31, and leukemia inhibitory factor (Rose and Bruce, 1991). When the OSM gene was deleted or blocked, experimental colitis was significantly alleviated in

animal models with resistance to TNF-α blockers (West et al., 2017). It's worth noting that OSM aggravates the inflammatory response and damages the intestinal mucosal barrier *via* initiating the expression of cytokines, chemokines, and adhesion factors, which produce large numbers of the receptor (OSMR-β) (Pothoven and Schleimer, 2017; West et al., 2017). Thus, OSM emerges as a novel therapeutic target for IBD patients, especially for those who are non-responsive to TNF-α inhibitors, and it may also promote remodeling of the intestinal tissue.

Even if some protocols of IBD therapy use biologics in an early top-down way in high-risk CD patients, the ineffectiveness of the therapy is still an intractable problem that drives patients to increase dosage of medications or try other therapeutic regimens (Berg et al., 2019; Maronek et al., 2020; Roda et al., 2020). As mentioned above, TNF-α blockers can exert anti-inflammatory effects and remodel the intestinal mucosal barrier by inducing the polarization of M2 macrophages, but further research is needed, especially in unresponsive patients.

5.3 Small Molecules

Janus kinases (JAKs) are non-receptor tyrosine kinases that comprise JAK1, JAK2, JAK3, and tyrosine kinase 2 (TYK2) (Darnell et al., 1994). Once the JAK cytokines bond to the cell-membrane receptors, the signaling transducers with activators of transcription (STATs) are activated, leading to STAT homo-dimerization and activate downstream transcription (Boland and Vermeire, 2017). Cytokines essential for intestinal homeostasis and those that are well-described mediators of pathological responses in IBD are all dependent on JAK/STAT-mediated signaling (Villarino et al., 2017; Paramsothy et al., 2018; Salas et al., 2020). Inhibition of the JAK/STAT pathway results in a decrease in several pro-inflammatory factors, for instance, IL-6, IL-12, and IL-23 (Schwartz et al., 2017). Therefore, JAK inhibitors may represent an emerging option for treating IBD (Na et al., 2019; Salas et al., 2020).

Tofacitinib, an oral pan-JAK blocker, has recently been approved for oral treatment of moderate-to-severe active UC (Kremer et al., 2012; Machado et al., 2018; Tripathi and Feuerstein, 2019; Rogler, 2020). It inhibits JAK1 and JAK3 preferentially, parts of the tyrosine kinase family, modulates cytokine secretion, and controls immunomodulation of IBD (Shivaji et al., 2019; Sandborn et al., 2021). Three phase III trials, with 1,732 patients in total, showed that tofacitinib was more effective efficacy than placebo in inducing and maintaining remission in patients with moderately to severely active UC (Sandborn et al., 2017). However, another two phase II double-blind RCT in CD patients, tofacitinib did not show significant results compared with placebo (Sandborn et al., 2014; Panés et al., 2017). Patients on high steroid dosages, some distinct disease characteristics, and details in research design (e.g., no endoscopic confirmation in center) may explain why the lack of efficacy was observed in CD, as opposed to UC.

Filgotinib, a selective JAK1 inhibitor, has emerged as a potential reliable therapy strategy in IBD patients. A phase II double-blind RCT, the FITZROY study, included patients with moderate-to-severe CD (Vermeire et al., 2017). After 10 weeks of treatment, patients who were given filgotinib (200 mg, once daily) showed significantly more frequent clinical remission than patients taking placebo (filgotinib group vs. placebo group; 47 vs. 23%, $p = 0.0077$) (Vermeire et al., 2017). Another phase IIb/III double-blind RCT in patients with moderate-to-severe UC, the SELECTION study, showed that filgotinib (200 mg, once daily) was well tolerated and effective in induction and maintenance of clinical remission compared with placebo (Feagan et al., 2021). A number of studies focusing on the efficacy of this JAK inhibitor in CD patients are under way. The Divergence 2 study (NCT03077412), focusing on analyzing the efficacy of filgotinib in the treatment of perianal fistula in CD, has been completed but no results have yet been reported. Another phase III double-blind RCT, The DIVERSITY1 (NCT02914561), will evaluate the effectiveness and safety of filgotinib in induction and maintenance therapy in patients with moderate-to-severe CD groups, who are biologic-naive and biologic-experienced. In addition, the DIVERSITYLTE trial (NCT02914600), aimed at evaluating the long-term safety in CD patients, is the extension of the DIVERSITY1 study.

JAK blockers could modulate functions of T cells, but also inhibit the IL-10-JAK1-tyrosine kinase 2-STAT3 signaling pathway, which is crucial for maintaining mature macrophages in intestinal homeostasis (Pattison et al., 2012). Interestingly, recent studies have shown that tofacitinib may affect the phenotype of macrophages, which was not investigated in previous related studies (Shiratori et al., 2018; De Vries et al., 2019). Studies have found that tofacitinib can downregulate M1 and promote M2 polarization (CD206, CD163, and IL-10) in murine bone marrow-derived or blood-derived macrophages (Shiratori et al., 2018; De Vries et al., 2019). In UC patients, tofacitinib significantly inhibited the M1 pro-inflammatory pathway, resulting in reduced expression of inflammatory factors; the M2 anti-inflammatory pathway was not affected, such as IL-10 secretion and expression of CD39 and CD206, even though the M2 markers were not upregulated (Cordes et al., 2020).

Targeting multiple pro-inflammatory cytokines, especially the JAK-STAT signaling pathway, may play an important role in novel IBD strategies, especially when patients are unresponsive to current regimens. The therapeutic mechanism of macrophage involvement in JAK blockers, especially in the process of resolving intestinal inflammation, needs to be confirmed in further research. In addition, side effects of JAK blockers are still of concern, such as the threat of thromboembolic events, opportunistic infections, or unknown long-term effects. The potential value of JAK blockers in the treatment of IBD needs to be further evaluated in high-quality and long-term RCTs, based on the risk-benefit ratio criterion.

5.4 Nanomaterials

Traditional medical regimens mainly rely on immunosuppressive agents, which could result in off-target systemic adverse effects and toxic reactions. As an emerging and prospective strategy, nanotechnology could be used for targeted delivery of medications to the inflamed lesions, enhancing local

concentration as well as minimizing systematic adverse effects (Hur et al., 2012; Beloqui et al., 2016; Sohail et al., 2019). It has been shown that macrophage-targeted drug delivery systems can improve drug-delivery efficiency and decrease side effects by making full use of the phagocytic function of macrophages. This strategy has been widely explored in rheumatoid arthritis (Barrera et al., 2000), adiposis (Bu et al., 2013), and malignant tumors such as hepatocellular carcinoma and melanoma (Piaggio et al., 2016).

Intriguingly, macrophage-targeting nanoparticles have been synthesized to deliver inhibitors or agonists of inflammation-related pathways to modulate transition of macrophage phenotype (Singh et al., 2017). Nanomaterials-based therapeutics have shown great promise in the therapy of IBD, because of their useful properties in food and medicine (Cui et al., 2020). For example, miR-146b can be specifically delivered to intestinal macrophages by mannose-modified trimethyl chitosan (MTC)-conjugated nanoparticles (NPs), resulting in remarkable restoration of mucosal barrier function in the mice models (Deng et al., 2019). MTC-NPs can dramatically inhibit the activation of the M1 subset and induce the M2 phenotype by suppressing the TLR4 signaling pathway, thereby inhibiting the secretion of IL-6, IL-1β, and TNF-α (Deng et al., 2019). Moreover, by producing STAT3-dependent IL-10, the proliferation of co-cultured colonic epithelial cells was significantly increased (Deng et al., 2019). In addition, surface-functionalized polyurethane (PU) NPs showed remarkable immunosuppression of THP-1 macrophages, suppressing the secretion of inflammation-related cytokines in phenotypic M1, controlling the NF-κB signaling pathways, and ultimately modulating the differentiation of macrophages (Huang et al., 2018; Nakkala et al., 2021). Additionally, oral nanomedicine delivery systems for treating IBD have great promise, because of their expandability, biocompatibility, and potential targeted efficacy. Hyaluronic acid-bilirubin nanomedicine (HABN) is responsive to ROS, accumulating in inflammatory mucosa and remodeling the colonic tissue in experimental colitis. Importantly, HABN showed significant treatment efficacy *via* differentiating pro-inflammatory M1-like subtypes into the M2-like subtypes and elevating the expression of anti-inflammatory phenotypes such as $CD3^+CD4^+Foxp3^+$ Tregs and $CD11b^+Ly6C^-Ly6G^-MHCII^+$ tissue-resident macrophages (Lee Y. et al., 2020; Xiao et al., 2021).

In recent years, metal-based engineered nanomaterials have shown advantageous properties in medicine. A classical case is that gold NPs (AuNPs) can induce M2 macrophage polarization, leading to inhibition of pro-inflammatory cytokines and increasing IL-10, Arg1, and PPARγ with decreased iNOS (Taratummarat et al., 2018). In addition, lines of evidence have demonstrated that nonmetal-based nanomaterials are capable of regulating macrophage phenotype. For example, graphene quantum dots (GQDs) could effectively alleviate intestinal inflammation and prevent tissue degeneration, *via* suppressing TH1/TH17 polarization (Lee B.-C. et al., 2020). Furthermore, GQDs can not only switch the differentiation of macrophages from pro-inflammatory M1 to anti-inflammatory M2 but also induce infiltration of intestinal Tregs (Lee B.-C. et al., 2020). A similar study also investigated the effect of nonmetal-based nanomaterials on macrophage polarization by using selenium (Se) (Zhu et al., 2017). Se is an indispensable nutrient and its deceased level may be associated with the exacerbation of IBD (Castro Aguilar-Tablada et al., 2016; Zhu et al., 2017; Ala and Kheyri, 2021). SeNPs have anti-inflammatory activity and low toxicity. Mechanistically, by inhibiting the nuclear translocation of NF-κB, SeNPs strongly suppressed M1 subsets, leading to alleviation of DSS-induced acute colitis in mice models (Zhu et al., 2017). Nanomaterials could be a promising therapeutic strategy for IBD with great potential in the near future.

5.5 Approaches Under Investigation

Inflammatory events are like a complex cascade, modulated by various cytokines and chemokines. Enhancing the pro-resolving phenotype by pharmacological modulation of the Inflammation-related molecules is of high importance to modulate macrophages in IBD. cAMP plays a key role in the modulation of inflammation-related process. Phosphodiesterase-4 (PDE4) is a critical enzyme, regulating intracellular signaling by controlling cAMP, emerging as a novel therapeutic target. Apremilast and roflumilast are two new PDE4 blockers, introduced as new regulators of intracellular signaling pathways in treating IBD patients (Spadaccini et al., 2017; Sabino et al., 2019). The intracellular cAMP level was upregulated by inhibition of PDE4, thereby blocking inflammation-related factors and increasing secretion of anti-inflammatory proteins (Mazur et al., 2015; Spadaccini et al., 2017). Moreover, a double-blind phase II trial of apremilast in patients with active UC showed significant remission of clinical symptoms, changes in endoscopic features, and decrease in markers of inflammation than in the placebo group (Danese et al., 2020).

Nuclear Enriched Abundant Transcript 1 (NEAT1), a new nuclear long non-coding RNA, anchors on particular nuclear structures and participates in various aspects of the immune process (Liu et al., 2018). It has been demonstrated that suppression of the NEAT1 can regulate the intestinal mucosal barrier and induce exosome-mediated differentiation of macrophages, thereby inhibiting the inflammatory response in IBD (Liu et al., 2018).

Targeting PDE4 or NEAT1 might represent a novel strategy in the future, but more high-quality clinical trials are still required to be carried out.

SUMMARY AND PROSPECT

IBD is a prevalent gastrointestinal inflammatory disease, characterized by chronic and excessive inflammation, alternates states of relapse and remission, and needs for lifelong medical therapy (Hazel and O'Connor, 2020). Therefore, new strategies to prevent inflammatory relapse, tissular damage, and non-responsiveness to medications, are urgently needed.

The infiltration and activation of macrophages can phagocytize pathogens, as well as produce various cytokines under certain circumstances, and cooperate with distinct immune cells in many

aspects of the pathogenesis of IBD. Disequilibrium of macrophage polarization results in the exacerbation of IBD, and the production of particular cytokines and/or chemokines relies on the ratio of the pro-inflammatory M1 and anti-inflammatory M2 subsets. Therefore, targeted therapy of macrophages is a novel option to modulate the immune microenvironment and remodel intestinal tissue.

In recent years, remarkable advances have been achieved in the understanding of intestinal macrophage immunobiology. The advancement of single-cell sequencing technologies and fate-mapping approaches has helped to confirm new subsets and distinct transcriptional profiles of intestinal macrophages (Ginhoux et al., 2010; Yona et al., 2013; Bian et al., 2020). Emerging evidence strongly points to an accumulation in the pro-inflammatory monocyte/macrophage in the chronic relapse and remission in patients with IBD. Therefore, it is very crucial to elucidate the mechanisms of new molecules and signaling pathways that participate in the polarization and increase of pro-resolving macrophages. However, many aspects of intestinal macrophages are still under-investigated, including mechanisms underlying macrophage polarization and heterogeneity, the signaling pathways of macrophage functions, environmental cues regulating phenotypes, and interactions during the process of IBD. For instance, it will be crucial to explore the characteristics of distinct subpopulations of macrophages, particularly the tissue-resident macrophages deriving from the embryo. This will facilitate finding out differences among distinct macrophages, expanding our comprehension of the functions of macrophages. In addition, the identification of microenvironmental factors that modulate phenotypes and functions of intestinal macrophage has helped us to understand their fundamental role in homeostasis and autoimmune disease. Moreover, research on cross-talk of intestinal macrophages, gut microflora, and metabolites will be conducive to decreasing chronic inflammation. It is also crucial that future investigations probe the synergistic mechanisms of cross-talk between macrophages and other immune cells in IBD.

Nanomedicine-based approaches have emerged as novel and targeted therapeutic strategies for IBD, by improving delivery efficiency to the intestinal inflammatory sites (Hua et al., 2015; Giron et al., 2019). Even though potential application of NPs has been broadly investigated in mammalian models *in vitro* or *in vivo*, human studies are still insufficient, and some findings in humans are distinct from the ones observed in murine models to some degree (Nunes et al., 2019). In addition, since the studies of toxicity and interaction of NPs have been mainly observed in animal models, efficacy in human intestinal cells is not fully applicable. The complexity of NP design and suitable methods for administration are two main difficulties with NPs, which impede the manufacturing process. Up to now, there has been no optimal size range of NPs determined to enable effective delivery to the cells in inflamed sites. In addition, the fate of the NPs after the body's uptake is still unknown, because the majority of the studies have focused on delivery efficiency but not safety of the nanoparticle. Safety issues should be addressed first before moving toward clinical studies. Moreover, evaluation of the effectiveness of drug delivery is also critical. Using non-invasive methods, such as positron emission tomography, to image and trace macrophages in pharmacological responses is a potential option to evaluate the safety and delivery efficacy (Weissleder et al., 2014; Kim et al., 2018; Heo et al., 2019).

Many investigations have explored inventing novel medications and repurposing classical drugs, as well as searching for alternative strategies or improving the existing ones in the effort to eradicate IBD. More efforts are needed to create new strategies for treatment options, incorporating existing therapies, and adjusting the therapeutic schedules to better fit individualized treatment. Hence, future research needs to focus on translating the results from pre-clinical studies to be further explored in clinical trials, ultimately benefiting patients with IBD.

AUTHOR CONTRIBUTIONS

SM contributed significantly to complete manuscript writing. JZ and HL contributed to the constructive discussions. SL and QW contributed to the supervision of the review. All authors read and approved the submitted version.

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REFERENCES

Aggarwal, N. R., King, L. S., and D'Alessio, F. R. (2014). Diverse Macrophage Populations Mediate Acute Lung Inflammation and Resolution. *Am. J. Physiology-Lung Cell. Mol. Physiology* 306 (8), L709–L725. doi:10.1152/ajplung.00341.2013

Ala, M., and Kheyri, Z. (2021). The Rationale for Selenium Supplementation in Inflammatory Bowel Disease: A Mechanism-Based Point of View. *Nutrition* 85, 111153. doi:10.1016/j.nut.2021.111153

Allgayer, H. (2003). Review Article: Mechanisms of Action of Mesalazine in Preventing Colorectal Carcinoma in Inflammatory Bowel Disease. *Aliment. Pharmacol. Ther.* 18 (Suppl. 2), 10–14. doi:10.1046/j.1365-2036.18.s2.1.x

Ananthakrishnan, A. N. (2015). Epidemiology and Risk Factors for IBD. *Nat. Rev. Gastroenterol. Hepatol.* 12 (4), 205–217. doi:10.1038/nrgastro.2015.34

Baert, F. J., D'Haens, G. R., Peeters, M., Hiele, M. I., Schaible, T. F., Shealy, D., et al. (1999). Tumor Necrosis Factor α Antibody (Infliximab) Therapy Profoundly Down-Regulates the Inflammation in Crohn's Ileocolitis. *Gastroenterology* 116 (1), 22–28. doi:10.1016/s0016-5085(99)70224-6

Bailey, J. R., Bland, P. W., Tarlton, J. F., Peters, I., Moorghen, M., Sylvester, P. A., et al. (2012). IL-13 Promotes Collagen Accumulation in Crohn's Disease Fibrosis by Down-Regulation of Fibroblast MMP Synthesis: a Role for Innate Lymphoid Cells? *PLoS One* 7 (12), e52332. doi:10.1371/journal.pone.0052332

Bain, C. C., Bravo-Blas, A., Scott, C. L., Gomez Perdiguero, E., Geissmann, F., Henri, S., et al. (2014). Constant Replenishment from Circulating Monocytes

Maintains the Macrophage Pool in the Intestine of Adult Mice. *Nat. Immunol.* 15 (10), 929–937. doi:10.1038/ni.2967

Bain, C. C., and Mowat, A. M. (2014). Macrophages in Intestinal Homeostasis and Inflammation. *Immunol. Rev.* 260 (1), 102–117. doi:10.1111/imr.12192

Bain, C. C., Scott, C. L., Uronen-Hansson, H., Gudjonsson, S., Jansson, O., Grip, O., et al. (2013). Resident and Pro-inflammatory Macrophages in the Colon Represent Alternative Context-dependent Fates of the Same Ly6Chi Monocyte Precursors. *Mucosal Immunol.* 6 (3), 498–510. doi:10.1038/mi.2012.89

Bantel, H., Berg, C., Vieth, M., Stolte, M., Kruis, W., and Schulze-Osthoff, K. (2000). Mesalazine Inhibits Activation of Transcription Factor Nf-Kb in Inflamed Mucosa of Patients with Ulcerative Colitis. *Am. J. Gastroenterol.* 95 (12), 3452–3457. doi:10.1111/j.1572-0241.2000.03360.x

Barrera, P., Blom, A., van Lent, P. L. E. M., van Bloois, L., Beijnen, J. H., van Rooijen, N., et al. (2000). Synovial Macrophage Depletion with Clodronate-containing Liposomes in Rheumatoid Arthritis. *Arthritis & Rheumatism* 43 (9), 1951–1959. doi:10.1002/1529-0131(200009)43:9<1951::Aid-anr5>3.0.Co;2-k

Beloqui, A., Memvanga, P. B., Coco, R., Reimondez-Troitiño, S., Alhouayek, M., Muccioli, G. G., et al. (2016). A Comparative Study of Curcumin-Loaded Lipid-Based Nanocarriers in the Treatment of Inflammatory Bowel Disease. *Colloids Surfaces B Biointerfaces* 143, 327–335. doi:10.1016/j.colsurfb.2016.03.038

Berg, D. R., Colombel, J.-F., and Ungaro, R. (2019). The Role of Early Biologic Therapy in Inflammatory Bowel Disease. *Inflamm. Bowel Dis.* 25 (12), 1896–1905. doi:10.1093/ibd/izz059

Bernardo, D., Marin, A. C., Fernández-Tomé, S., Montalban-Arques, A., Carrasco, A., Tristán, E., et al. (2018). Human Intestinal Pro-inflammatory CD11chighCCR2+CX3CR1+ Macrophages, but Not Their Tolerogenic CD11c−CCR2−CX3CR1− Counterparts, Are Expanded in Inflammatory Bowel Disease. *Mucosal Immunol.* 11 (4), 1114–1126. doi:10.1038/s41385-018-0030-7

Bian, Z., Gong, Y., Huang, T., Lee, C. Z. W., Bian, L., Bai, Z., et al. (2020). Deciphering Human Macrophage Development at Single-Cell Resolution. *Nature* 582 (7813), 571–576. doi:10.1038/s41586-020-2316-7

Billmeier, U., Dieterich, W., Neurath, M. F., and Atreya, R. (2016). Molecular Mechanism of Action of Anti-tumor Necrosis Factor Antibodies in Inflammatory Bowel Diseases. *Wjg* 22 (42), 9300–9313. doi:10.3748/wjg.v22.i42.9300

Bloemendaal, F. M., Levin, A. D., Wildenberg, M. E., Koelink, P. J., McRae, B. L., Salfeld, J., et al. (2017). Anti-Tumor Necrosis Factor with a Glyco-Engineered Fc-Region Has Increased Efficacy in Mice with Colitis. *Gastroenterology* 153 (5), 1351–1362. e1354. doi:10.1053/j.gastro.2017.07.021

Boland, B. S., and Vermeire, S. (2017). Janus Kinase Antagonists and Other Novel Small Molecules for the Treatment of Crohn's Disease. *Gastroenterology Clin. N. Am.* 46 (3), 627–644. doi:10.1016/j.gtc.2017.05.015

Bouhlel, M. A., Derudas, B., Rigamonti, E., Dièvart, R., Brozek, J., Haulon, S., et al. (2007). PPARγ Activation Primes Human Monocytes into Alternative M2 Macrophages with Anti-inflammatory Properties. *Cell Metab.* 6 (2), 137–143. doi:10.1016/j.cmet.2007.06.010

Boyer, J. F., Baron, M., Constantin, A., Degboé, Y., Cantagrel, A., and Davignon, J.-L. (2016). Anti-TNF Certolizumab Pegol Induces Antioxidant Response in Human Monocytes via Reverse Signaling. *Arthritis Res. Ther.* 18, 56. doi:10.1186/s13075-016-0955-8

Bu, L., Gao, M., Qu, S., and Liu, D. (2013). Intraperitoneal Injection of Clodronate Liposomes Eliminates Visceral Adipose Macrophages and Blocks High-Fat Diet-Induced Weight Gain and Development of Insulin Resistance. *Aaps J.* 15 (4), 1001–1011. doi:10.1208/s12248-013-9501-7

Bujko, A., Atlasy, N., Landsverk, O. J. B., Richter, L., Yaqub, S., Horneland, R., et al. (2018). Transcriptional and Functional Profiling Defines Human Small Intestinal Macrophage Subsets. *J. Exp. Med.* 215 (2), 441–458. doi:10.1084/jem.20170057

Casanova, J.-L., and Abel, L. (2009). Revisiting Crohn's Disease as a Primary Immunodeficiency of Macrophages. *J. Exp. Med.* 206 (9), 1839–1843. doi:10.1084/jem.20091683

Castro Aguilar-Tablada, T., Navarro-Alarcón, M., Quesada Granados, J., Samaniego Sánchez, C., Rufián-Henares, J., and Nogueras-Lopez, F. (2016). Ulcerative Colitis and Crohn's Disease Are Associated with Decreased Serum Selenium Concentrations and Increased Cardiovascular Risk. *Nutrients* 8 (12), 780. doi:10.3390/nu8120780

Chen, F., Liu, Q., Xiong, Y., and Xu, L. (2021). Current Strategies and Potential Prospects of Nanomedicine-Mediated Therapy in Inflammatory Bowel Disease. *Ijn* 16, 4225–4237. doi:10.2147/ijn.S310952

Chistiakov, D. A., Myasoedova, V. A., Revin, V. V., Orekhov, A. N., and Bobryshev, Y. V. (2018). The Impact of Interferon-Regulatory Factors to Macrophage Differentiation and Polarization into M1 and M2. *Immunobiology* 223 (1), 101–111. doi:10.1016/j.imbio.2017.10.005

Chudy-Onwugaje, K. O., Christian, K. E., Farraye, F. A., and Cross, R. K. (2019). A State-Of-The-Art Review of New and Emerging Therapies for the Treatment of IBD. *Inflamm. Bowel Dis.* 25 (5), 820–830. doi:10.1093/ibd/izy327

Colombel, J. F., Sandborn, W. J., Reinisch, W., Mantzaris, G. J., Kornbluth, A., Rachmilewitz, D., et al. (2010). Infliximab, Azathioprine, or Combination Therapy for Crohn's Disease. *N. Engl. J. Med.* 362 (15), 1383–1395. doi:10.1056/NEJMoa0904492

Cook, M. G., and Dixon, M. F. (1973). An Analysis of the Reliability of Detection and Diagnostic Value of Various Pathological Features in Crohn's Disease and Ulcerative Colitis. *Gut* 14 (4), 255–262. doi:10.1136/gut.14.4.255

Cordes, F., Lenker, E., Spille, L. J., Weinhage, T., Bettenworth, D., Kessel, C., et al. (2020). Tofacitinib Reprograms Human Monocytes of IBD Patients and Healthy Controls toward a More Regulatory Phenotype. *Inflamm. Bowel Dis.* 26 (3), 391–406. doi:10.1093/ibd/izz213

Cosín-Roger, J., Ortiz-Masiá, D., Calatayud, S., Hernández, C., Álvarez, A., Hinojosa, J., et al. (2013). M2 Macrophages Activate WNT Signaling Pathway in Epithelial Cells: Relevance in Ulcerative Colitis. *PLoS One* 8 (10), e78128. doi:10.1371/journal.pone.0078128

Croft, N. M., Faubion, W. A., Jr., Kugathasan, S., Kierkus, J., Ruemmele, F. M., Shimizu, T., et al. (2021). Efficacy and Safety of Adalimumab in Paediatric Patients with Moderate-To-Severe Ulcerative Colitis (ENVISION I): a Randomised, Controlled, Phase 3 Study. *Lancet Gastroenterology Hepatology* 6 (8), 616–627. doi:10.1016/s2468-1253(21)00142-4

Cui, X., Bao, L., Wang, X., and Chen, C. (2020). The Nano-Intestine Interaction: Understanding the Location-Oriented Effects of Engineered Nanomaterials in the Intestine. *Small* 16 (21), 1907665. doi:10.1002/smll.201907665

D'Haens, G., Van Deventer, S., Van Hogezand, R., Chalmers, D., Kothe, C., Baert, F., et al. (1999). Endoscopic and Histological Healing with Infliximab Anti-tumor Necrosis Factor Antibodies in Crohn's Disease: A European Multicenter Trial. *Gastroenterology* 116 (5), 1029–1034. doi:10.1016/s0016-5085(99)70005-3

Danese, S., Neurath, M. F., Kopoń, A., Zakko, S. F., Simmons, T. C., Fogel, R., et al. (2020). Effects of Apremilast, an Oral Inhibitor of Phosphodiesterase 4, in a Randomized Trial of Patients with Active Ulcerative Colitis. *Clin. Gastroenterology Hepatology* 18 (11), 2526–2534. e2529. doi:10.1016/j.cgh.2019.12.032

Danese, S., Vuitton, L., and Peyrin-Biroulet, L. (2015). Biologic Agents for IBD: Practical Insights. *Nat. Rev. Gastroenterol. Hepatol.* 12 (9), 537–545. doi:10.1038/nrgastro.2015.135

Darnell, J. E., Jr., Kerr, l. M., and Stark, G. R. (1994). Jak-STAT Pathways and Transcriptional Activation in Response to IFNs and Other Extracellular Signaling Proteins. *Science* 264 (5164), 1415–1421. doi:10.1126/science.8197455

De Schepper, S., Verheijden, S., Aguilera-Lizarraga, J., Viola, M. F., Boesmans, W., Stakenborg, N., et al. (2018). Self-Maintaining Gut Macrophages Are Essential for Intestinal Homeostasis. *Cell* 175 (2), 400–415. e413. doi:10.1016/j.cell.2018.07.048

de Souza, H. S. P., and Fiocchi, C. (2016). Immunopathogenesis of IBD: Current State of the Art. *Nat. Rev. Gastroenterol. Hepatol.* 13 (1), 13–27. doi:10.1038/nrgastro.2015.186

De Vries, L. C. S., Duarte, J. M., De Krijger, M., Welting, O., Van Hamersveld, P. H. P., Van Leeuwen-Hilbers, F. W. M., et al. (2019). A JAK1 Selective Kinase Inhibitor and Tofacitinib Affect Macrophage Activation and Function. *Inflamm. Bowel Dis.* 25 (4), 647–660. doi:10.1093/ibd/izy364

Deng, F., He, S., Cui, S., Shi, Y., Tan, Y., Li, Z., et al. (2019). A Molecular Targeted Immunotherapeutic Strategy for Ulcerative Colitis via Dual-Targeting Nanoparticles Delivering miR-146b to Intestinal Macrophages. *J. Crohns Colitis* 13 (4), 482–494. doi:10.1093/ecco-jcc/jjy181

Deng, F., Yan, J., Lu, J., Luo, M., Xia, P., Liu, S., et al. (2021). M2 Macrophage-Derived Exosomal miR-590-3p Attenuates DSS-Induced Mucosal Damage and Promotes Epithelial Repair via the LATS1/YAP/ β-Catenin Signalling Axis. *J. Crohns Colitis* 15 (4), 665–677. doi:10.1093/ecco-jcc/jjaa214

Dionne, S., Duchatelier, C.-F., and Seidman, E. G. (2017). The Influence of Vitamin D on M1 and M2 Macrophages in Patients with Crohn's Disease. *Innate Immun.* 23 (6), 557–565. doi:10.1177/1753425917721965

Du, Y., Rong, L., Cong, Y., Shen, L., Zhang, N., and Wang, B. (2021). Macrophage Polarization: an Effective Approach to Targeted Therapy of Inflammatory Bowel Disease. *Expert Opin. Ther. Targets* 25 (3), 1–19. doi:10.1080/14728222.2021.1901079

Ehrchen, J., Steinmüller, L., Barczyk, K., Tenbrock, K., Nacken, W., Eisenacher, M., et al. (2007). Glucocorticoids Induce Differentiation of a Specifically Activated, Anti-inflammatory Subtype of Human Monocytes. *Blood* 109 (3), 1265–1274. doi:10.1182/blood-2006-02-001115

Elion, G. B. (1989). The Purine Path to Chemotherapy. *Science* 244 (4900), 41–47. doi:10.1126/science.2649979

Elliott, M. R., Koster, K. M., and Murphy, P. S. (2017). Efferocytosis Signaling in the Regulation of Macrophage Inflammatory Responses. *J. I.* 198 (4), 1387–1394. doi:10.4049/jimmunol.1601520

Elliott, T. R., Rayment, N. B., Hudspith, B. N., Hands, R. E., Taylor, K., Parkes, G. C., et al. (2015). Lamina Propria Macrophage Phenotypes in Relation to *Escherichia coli* in Crohn's Disease. *BMC Gastroenterol.* 15, 75. doi:10.1186/s12876-015-0305-3

Epelman, S., Lavine, K. J., and Randolph, G. J. (2014). Origin and Functions of Tissue Macrophages. *Immunity* 41 (1), 21–35. doi:10.1016/j.immuni.2014.06.013

Fadok, V. A., Bratton, D. L., Konowal, A., Freed, P. W., Westcott, J. Y., and Henson, P. M. (1998). Macrophages that Have Ingested Apoptotic Cells *In Vitro* Inhibit Proinflammatory Cytokine Production through Autocrine/paracrine Mechanisms Involving TGF-Beta, PGE2, and PAF. *J. Clin. Invest.* 101 (4), 890–898. doi:10.1172/jci1112

Feagan, B. G., Danese, S., Loftus, E. V., Jr., Vermeire, S., Schreiber, S., Ritter, T., et al. (2021). Filgotinib as Induction and Maintenance Therapy for Ulcerative Colitis (SELECTION): a Phase 2b/3 Double-Blind, Randomised, Placebo-Controlled Trial. *Lancet* 397 (10292), 2372–2384. doi:10.1016/s0140-6736(21)00666-8

Feagan, B. G., Fedorak, R. N., Irvine, E. J., Wild, G., Sutherland, L., Steinhart, A. H., et al. (2000). A Comparison of Methotrexate with Placebo for the Maintenance of Remission in Crohn's Disease. *N. Engl. J. Med.* 342 (22), 1627–1632. doi:10.1056/nejm200006013422202

Fichtner-Feigl, S., Strober, W., Kawakami, K., Puri, R. K., and Kitani, A. (2006). IL-13 Signaling through the IL-13α2 Receptor Is Involved in Induction of TGF-B1 Production and Fibrosis. *Nat. Med.* 12 (1), 99–106. doi:10.1038/nm1332

Fiocchi, C. (2008). What Is "physiological" Intestinal Inflammation and How Does it Differ from "pathological" Inflammation? *Inflamm. Bowel Dis.* 14 (Suppl. 2), S77–S78. doi:10.1002/ibd.20618

Ford, A. C., Bernstein, C. N., Khan, K. J., Abreu, M. T., Marshall, J. K., Talley, N. J., et al. (2011). Glucocorticosteroid Therapy in Inflammatory Bowel Disease: Systematic Review and Meta-Analysis. *Am. J. Gastroenterol.* 106 (4), 590–599. doi:10.1038/ajg.2011.70

Funes, S. C., Rios, M., Escobar-Vera, J., and Kalergis, A. M. (2018). Implications of Macrophage Polarization in Autoimmunity. *Immunology* 154 (2), 186–195. doi:10.1111/imm.12910

Garrett, W. S., Gordon, J. I., and Glimcher, L. H. (2010). Homeostasis and Inflammation in the Intestine. *Cell* 140 (6), 859–870. doi:10.1016/j.cell.2010.01.023

Gilbert, J. A., Blaser, M. J., Caporaso, J. G., Jansson, J. K., Lynch, S. V., and Knight, R. (2018). Current Understanding of the Human Microbiome. *Nat. Med.* 24 (4), 392–400. doi:10.1038/nm.4517

Giles, K. M., Ross, K., Rossi, A. G., Hotchin, N. A., Haslett, C., and Dransfield, I. (2001). Glucocorticoid Augmentation of Macrophage Capacity for Phagocytosis of Apoptotic Cells Is Associated with Reduced p130Cas Expression, Loss of Paxillin/pyk2 Phosphorylation, and High Levels of Active Rac. *J. Immunol.* 167 (2), 976–986. doi:10.4049/jimmunol.167.2.976

Ginhoux, F., Greter, M., Leboeuf, M., Nandi, S., See, P., Gokhan, S., et al. (2010). Fate Mapping Analysis Reveals that Adult Microglia Derive from Primitive Macrophages. *Science* 330 (6005), 841–845. doi:10.1126/science.1194637

Ginhoux, F., and Guilliams, M. (2016). Tissue-Resident Macrophage Ontogeny and Homeostasis. *Immunity* 44 (3), 439–449. doi:10.1016/j.immuni.2016.02.024

Giron, F., Pastó, A., Tasciotti, E., and Abraham, B. P. (2019). Nanotechnology in the Treatment of Inflammatory Bowel Disease. *Inflamm. Bowel Dis.* 25 (12), 1871–1880. doi:10.1093/ibd/izz205

Gordon, S. (2003). Alternative Activation of Macrophages. *Nat. Rev. Immunol.* 3 (1), 23–35. doi:10.1038/nri978

Gordon, S., and Martinez, F. O. (2010). Alternative Activation of Macrophages: Mechanism and Functions. *Immunity* 32 (5), 593–604. doi:10.1016/j.immuni.2010.05.007

Graham, D. B., and Xavier, R. J. (2020). Pathway Paradigms Revealed from the Genetics of Inflammatory Bowel Disease. *Nature* 578 (7796), 527–539. doi:10.1038/s41586-020-2025-2

Grainger, J. R., Konkel, J. E., Zangerle-Murray, T., and Shaw, T. N. (2017). Macrophages in Gastrointestinal Homeostasis and Inflammation. *Pflugers Arch. - Eur. J. Physiol.* 469 (3-4), 527–539. doi:10.1007/s00424-017-1958-2

Guilliams, M., De Kleer, I., Henri, S., Post, S., Vanhoutte, L., De Prijck, S., et al. (2013). Alveolar Macrophages Develop from Fetal Monocytes that Differentiate into Long-Lived Cells in the First Week of Life via GM-CSF. *J. Exp. Med.* 210 (10), 1977–1992. doi:10.1084/jem.20131199

Guilliams, M., and Scott, C. L. (2017). Does Niche Competition Determine the Origin of Tissue-Resident Macrophages? *Nat. Rev. Immunol.* 17 (7), 451–460. doi:10.1038/nri.2017.42

Guilliams, M., and Svedberg, F. R. (2021). Does Tissue Imprinting Restrict Macrophage Plasticity? *Nat. Immunol.* 22 (2), 118–127. doi:10.1038/s41590-020-00849-2

Hadis, U., Wahl, B., Schulz, O., Hardtke-Wolenski, M., Schippers, A., Wagner, N., et al. (2011). Intestinal Tolerance Requires Gut Homing and Expansion of FoxP3+ Regulatory T Cells in the Lamina Propria. *Immunity* 34 (2), 237–246. doi:10.1016/j.immuni.2011.01.016

Hashimoto, D., Chow, A., Noizat, C., Teo, P., Beasley, M. B., Leboeuf, M., et al. (2013). Tissue-Resident Macrophages Self-Maintain Locally throughout Adult Life with Minimal Contribution from Circulating Monocytes. *Immunity* 38 (4), 792–804. doi:10.1016/j.immuni.2013.04.004

Hayes, C. L., Dong, J., Galipeau, H. J., Jury, J., McCarville, J., Huang, X., et al. (2018). Commensal Microbiota Induces Colonic Barrier Structure and Functions that Contribute to Homeostasis. *Sci. Rep.* 8 (1), 14184. doi:10.1038/s41598-018-32366-6

Hazel, K., and O'Connor, A. (2020). Emerging Treatments for Inflammatory Bowel Disease. *Ther. Adv. Chronic Dis.* 11, 204062231989929. doi:10.1177/2040622319899297

Hedrich, C. M., and Bream, J. H. (2010). Cell Type-specific Regulation of IL-10 Expression in Inflammation and Disease. *Immunol. Res.* 47 (1-3), 185–206. doi:10.1007/s12026-009-8150-5

Heo, G. S., Kopecky, B., Sultan, D., Ou, M., Feng, G., Bajpai, G., et al. (2019). Molecular Imaging Visualizes Recruitment of Inflammatory Monocytes and Macrophages to the Injured Heart. *Circ. Res.* 124 (6), 881–890. doi:10.1161/circresaha.118.314030

Hirotani, T., Lee, P. Y., Kuwata, H., Yamamoto, M., Matsumoto, M., Kawase, I., et al. (2005). The Nuclear IκB Protein IκBNS Selectively Inhibits Lipopolysaccharide-Induced IL-6 Production in Macrophages of the Colonic Lamina Propria. *J. Immunol.* 174 (6), 3650–3657. doi:10.4049/jimmunol.174.6.3650

Hoeffel, G., Wang, Y., Greter, M., See, P., Teo, P., Malleret, B., et al. (2012). Adult Langerhans Cells Derive Predominantly from Embryonic Fetal Liver Monocytes with a Minor Contribution of Yolk Sac-Derived Macrophages. *J. Exp. Med.* 209 (6), 1167–1181. doi:10.1084/jem.20120340

Holleran, G., Lopetuso, L., Petito, V., Graziani, C., Ianiro, G., McNamara, D., et al. (2017). The Innate and Adaptive Immune System as Targets for Biologic Therapies in Inflammatory Bowel Disease. *Ijms* 18 (10), 2020. doi:10.3390/ijms18102020

Hu, X., Herrero, C., Li, W.-P., Antoniv, T. T., Falck-Pedersen, E., Koch, A. E., et al. (2002). Sensitization of IFN-γ Jak-STAT Signaling during Macrophage Activation. *Nat. Immunol.* 3 (9), 859–866. doi:10.1038/ni828

Hua, S., Marks, E., Schneider, J. J., and Keely, S. (2015). Advances in Oral Nano-Delivery Systems for Colon Targeted Drug Delivery in Inflammatory Bowel Disease: Selective Targeting to Diseased versus Healthy Tissue. *Nanomedicine Nanotechnol. Biol. Med.* 11 (5), 1117–1132. doi:10.1016/j.nano.2015.02.018

Huang, Y.-J., Hung, K.-C., Hung, H.-S., and Hsu, S.-h. (2018). Modulation of Macrophage Phenotype by Biodegradable Polyurethane Nanoparticles: Possible

Relation between Macrophage Polarization and Immune Response of Nanoparticles. *ACS Appl. Mat. Interfaces* 10 (23), 19436–19448. doi:10.1021/acsami.8b04718

Hunter, M. M., Wang, A., Parhar, K. S., Johnston, M. J. G., Van Rooijen, N., Beck, P. L., et al. (2010). In Vitro-derived Alternatively Activated Macrophages Reduce Colonic Inflammation in Mice. *Gastroenterology* 138 (4), 1395–1405. doi:10.1053/j.gastro.2009.12.041

Hunter, P. (2012). The Inflammation Theory of Disease. *EMBO Rep.* 13 (11), 968–970. doi:10.1038/embor.2012.142

Hur, S. J., Kang, S. H., Jung, H. S., Kim, S. C., Jeon, H. S., Kim, I. H., et al. (2012). Review of Natural Products Actions on Cytokines in Inflammatory Bowel Disease. *Nutr. Res.* 32 (11), 801–816. doi:10.1016/j.nutres.2012.09.013

Jakubzick, C., Gautier, E. L., Gibbings, S. L., Sojka, D. K., Schlitzer, A., Johnson, T. E., et al. (2013). Minimal Differentiation of Classical Monocytes as They Survey Steady-State Tissues and Transport Antigen to Lymph Nodes. *Immunity* 39 (3), 599–610. doi:10.1016/j.immuni.2013.08.007

Jharap, B., Seinen, M. L., de Boer, N. K. H., van Ginkel, J. R., Linskens, R. K., Kneppelhout, J. C., et al. (2010). Thiopurine Therapy in Inflammatory Bowel Disease Patients: Analyses of Two 8-year Intercept Cohorts. *Inflamm. Bowel Dis.* 16 (9), 1541–1549. doi:10.1002/ibd.21221

Kaiser, G. C., Yan, F., and Polk, D. B. (1999). Mesalamine Blocks Tumor Necrosis Factor Growth Inhibition and Nuclear Factor κB Activation in Mouse Colonocytes. *Gastroenterology* 116 (3), 602–609. doi:10.1016/s0016-5085(99)70182-4

Kany, S., Vollrath, J. T., and Relja, B. (2019). Cytokines in Inflammatory Disease. *Ijms* 20 (23), 6008. doi:10.3390/ijms20236008

Kaplan, G. G. (2015). The Global Burden of IBD: from 2015 to 2025. *Nat. Rev. Gastroenterol. Hepatol.* 12 (12), 720–727. doi:10.1038/nrgastro.2015.150

Kim, H.-Y., Li, R., Ng, T. S. C., Courties, G., Rodell, C. B., Prytyskach, M., et al. (2018). Quantitative Imaging of Tumor-Associated Macrophages and Their Response to Therapy Using 64Cu-Labeled Macrin. *ACS Nano* 12 (12), 12015–12029. doi:10.1021/acsnano.8b04338

Kobayashi, T., Siegmund, B., Le Berre, C., Wei, S. C., Ferrante, M., Shen, B., et al. (2020). Ulcerative Colitis. *Nat. Rev. Dis. Prim.* 6 (1), 74. doi:10.1038/s41572-020-0205-x

Koch, S., and Nusrat, A. (2012). The Life and Death of Epithelia during Inflammation: Lessons Learned from the Gut. *Annu. Rev. Pathol. Mech. Dis.* 7, 35–60. doi:10.1146/annurev-pathol-011811-120905

Koelink, P. J., Bloemendaal, F. M., Li, B., Westera, L., Vogels, E. W. M., van Roest, M., et al. (2020). Anti-TNF Therapy in IBD Exerts its Therapeutic Effect through Macrophage IL-10 Signalling. *Gut* 69 (6), 1053–1063. doi:10.1136/gutjnl-2019-318264

Kredel, L. I., Batra, A., Stroh, T., Kühl, A. A., Zeitz, M., Erben, U., et al. (2013). Adipokines from Local Fat Cells Shape the Macrophage Compartment of the Creeping Fat in Crohn's Disease. *Gut* 62 (6), 852–862. doi:10.1136/gutjnl-2011-301424

Kremer, J. M., Cohen, S., Wilkinson, B. E., Connell, C. A., French, J. L., Gomez-Reino, J., et al. (2012). A Phase IIb Dose-Ranging Study of the Oral JAK Inhibitor Tofacitinib (CP-690,550) versus Placebo in Combination with Background Methotrexate in Patients with Active Rheumatoid Arthritis and an Inadequate Response to Methotrexate Alone. *Arthritis & Rheumatism* 64 (4), 970–981. doi:10.1002/art.33419

Kühl, A. A., Erben, U., Kredel, L. I., and Siegmund, B. (2015). Diversity of Intestinal Macrophages in Inflammatory Bowel Diseases. *Front. Immunol.* 6, 613. doi:10.3389/fimmu.2015.00613

Lavin, Y., Winter, D., Blecher-Gonen, R., David, E., Keren-Shaul, H., Merad, M., et al. (2014). Tissue-resident Macrophage Enhancer Landscapes Are Shaped by the Local Microenvironment. *Cell* 159 (6), 1312–1326. doi:10.1016/j.cell.2014.11.018

Lawrence, T., and Natoli, G. (2011). Transcriptional Regulation of Macrophage Polarization: Enabling Diversity with Identity. *Nat. Rev. Immunol.* 11 (11), 750–761. doi:10.1038/nri3088

Lazzerini, M., Martelossi, S., Magazzù, G., Pellegrino, S., Lucanto, M. C., Barabino, A., et al. (2013). Effect of Thalidomide on Clinical Remission in Children and Adolescents with Refractory Crohn Disease. *Jama* 310 (20), 2164–2173. doi:10.1001/jama.2013.280777

Lee, B.-C., Lee, J. Y., Kim, J., Yoo, J. M., Kang, I., Kim, J.-J., et al. (2020a). Graphene Quantum Dots as Anti-inflammatory Therapy for Colitis. *Sci. Adv.* 6 (18), eaaz2630. doi:10.1126/sciadv.aaz2630

Lee, Y., Sugihara, K., Gillilland, M. G., 3rd, Jon, S., Kamada, N., and Moon, J. J. (2020b). Hyaluronic Acid-Bilirubin Nanomedicine for Targeted Modulation of Dysregulated Intestinal Barrier, Microbiome and Immune Responses in Colitis. *Nat. Mat.* 19 (1), 118–126. doi:10.1038/s41563-019-0462-9

Leonardi, I., Li, X., Semon, A., Li, D., Doron, I., Putzel, G., et al. (2018). CX3CR1 + Mononuclear Phagocytes Control Immunity to Intestinal Fungi. *Science* 359 (6372), 232–236. doi:10.1126/science.aao1503

Levin, A. D., Wildenberg, M. E., and van den Brink, G. R. (2016). Mechanism of Action of Anti-TNF Therapy in Inflammatory Bowel Disease. *Eccojc* 10 (8), 989–997. doi:10.1093/ecco-jcc/jjw053

Lissner, D., Schumann, M., Batra, A., Kredel, L.-I., Kühl, A. A., Erben, U., et al. (2015). Monocyte and M1 Macrophage-Induced Barrier Defect Contributes to Chronic Intestinal Inflammation in IBD. *Inflamm. Bowel Dis.* 21 (6), 1–1305. doi:10.1097/mib.0000000000000384

Liu, R., Tang, A., Wang, X., Chen, X., Zhao, L., Xiao, Z., et al. (2018). Inhibition of lncRNA NEAT1 Suppresses the Inflammatory Response in IBD by Modulating the Intestinal Epithelial Barrier and by Exosome-Mediated Polarization of Macrophages. *Int. J. Mol. Med.* 42 (5), 2903–2913. doi:10.3892/ijmm.2018.3829

Lloyd-Price, J., Arze, C., Arze, C., Ananthakrishnan, A. N., Schirmer, M., Avila-Pacheco, J., et al. (2019). Multi-omics of the Gut Microbial Ecosystem in Inflammatory Bowel Diseases. *Nature* 569 (7758), 655–662. doi:10.1038/s41586-019-1237-9

Locati, M., Curtale, G., and Mantovani, A. (2020). Diversity, Mechanisms, and Significance of Macrophage Plasticity. *Annu. Rev. Pathol. Mech. Dis.* 15, 123–147. doi:10.1146/annurev-pathmechdis-012418-012718

Locati, M., Mantovani, A., and Sica, A. (2013). Macrophage Activation and Polarization as an Adaptive Component of Innate Immunity. *Adv. Immunol.* 120, 163–184. doi:10.1016/b978-0-12-417028-5.00006-5

Louwe, P. A., Badiola Gomez, L., Webster, H., Perona-Wright, G., Bain, C. C., Forbes, S. J., et al. (2021). Recruited Macrophages that Colonize the Post-inflammatory Peritoneal Niche Convert into Functionally Divergent Resident Cells. *Nat. Commun.* 12 (1). doi:10.1038/s41467-021-21778-0

Lu, J., Liu, D., Tan, Y., Deng, F., and Li, R. (2021a). M1 Macrophage Exosomes MiR-21a-5p Aggravates Inflammatory Bowel Disease through Decreasing E-cadherin and Subsequent ILC2 Activation. *J. Cell Mol. Med.* 25 (6), 3041–3050. doi:10.1111/jcmm.16348

Lu, J., Liu, D., Tan, Y., Li, R., Wang, X., and Deng, F. (2021b). Thalidomide Attenuates Colitis and Is Associated with the Suppression of M1 Macrophage Polarization by Targeting the Transcription Factor IRF5. *Dig. Dis. Sci.* 66 (11), 3803–3812. doi:10.1007/s10620-021-07067-2

Lu, J., Xie, L., Liu, C., Zhang, Q., and Sun, S. (2017). PTEN/PI3k/AKT Regulates Macrophage Polarization in Emphysematous Mice. *Scand. J. Immunol.* 85 (6), 395–405. doi:10.1111/sji.12545

Luan, B., Yoon, Y.-S., Le Lay, J., Kaestner, K. H., Hedrick, S., and Montminy, M. (2015). CREB Pathway Links PGE2 Signaling with Macrophage Polarization. *Proc. Natl. Acad. Sci. U.S.A.* 112 (51), 15642–15647. doi:10.1073/pnas.1519644112

Lucas, M., Zhang, X., Prasanna, V., and Mosser, D. M. (2005). ERK Activation Following Macrophage FcγR Ligation Leads to Chromatin Modifications at the IL-10 Locus. *J. Immunol.* 175 (1), 469–477. doi:10.4049/jimmunol.175.1.469

Ma, W.-T., Gao, F., Gu, K., and Chen, D.-K. (2019). The Role of Monocytes and Macrophages in Autoimmune Diseases: A Comprehensive Review. *Front. Immunol.* 10, 1140. doi:10.3389/fimmu.2019.01140

MacDonald, K. P. A., Palmer, J. S., Cronau, S., Seppanen, E., Olver, S., Raffelt, N. C., et al. (2010). An Antibody against the Colony-Stimulating Factor 1 Receptor Depletes the Resident Subset of Monocytes and Tissue- and Tumor-Associated Macrophages but Does Not Inhibit Inflammation. *Blood* 116 (19), 3955–3963. doi:10.1182/blood-2010-02-266296

Machado, M. A. d. Á., Moura, C. S. d., Guerra, S. F., Curtis, J. R., Abrahamowicz, M., and Bernatsky, S. (2018). Effectiveness and Safety of Tofacitinib in Rheumatoid Arthritis: a Cohort Study. *Arthritis Res. Ther.* 20 (1), 60. doi:10.1186/s13075-018-1539-6

Mahida, Y. R., Patel, S., Gionchetti, P., Vaux, D., and Jewell, D. P. (1989). Macrophage Subpopulations in Lamina Propria of Normal and Inflamed Colon and Terminal Ileum. *Gut* 30 (6), 826–834. doi:10.1136/gut.30.6.826

Marinković, G., Hamers, A. A. J., de Vries, C. J. M., and de Waard, V. (2014). 6-Mercaptopurine Reduces Macrophage Activation and Gut Epithelium Proliferation through Inhibition of GTPase Rac1. *Inflamm. Bowel Dis.* 20 (9), 1487–1495. doi:10.1097/mib.0000000000000122

Maronek, M., Link, R., Ambro, L., and Gardlik, R. (2020). Phages and Their Role in Gastrointestinal Disease: Focus on Inflammatory Bowel Disease. *Cells* 9 (4), 1013. doi:10.3390/cells9041013

Martinez, F. O., Helming, L., and Gordon, S. (2009). Alternative Activation of Macrophages: an Immunologic Functional Perspective. *Annu. Rev. Immunol.* 27, 451–483. doi:10.1146/annurev.immunol.021908.132532

Mass, E., and Gentek, R. (2021). Fetal-Derived Immune Cells at the Roots of Lifelong Pathophysiology. *Front. Cell Dev. Biol.* 9. doi:10.3389/fcell.2021.648313

Mazur, M., Karczewski, J., Lodyga, M., Żaba, R., and Adamski, Z. (2015). Inhibitors of Phosphodiesterase 4 (PDE 4): A New Therapeutic Option in the Treatment of Psoriasis Vulgaris and Psoriatic Arthritis. *J. Dermatological Treat.* 26 (4), 326–328. doi:10.3109/09546634.2014.991267

Mosser, D. M., and Edwards, J. P. (2008). Exploring the Full Spectrum of Macrophage Activation. *Nat. Rev. Immunol.* 8 (12), 958–969. doi:10.1038/nri2448

Mowat, A. M. (2018). To Respond or Not to Respond - a Personal Perspective of Intestinal Tolerance. *Nat. Rev. Immunol.* 18 (6), 405–415. doi:10.1038/s41577-018-0002-x

Mowat, C., Cole, A., Windsor, A., Ahmad, T., Arnott, I., Driscoll, R., et al. (2011). Guidelines for the Management of Inflammatory Bowel Disease in Adults. *Gut* 60 (5), 571–607. doi:10.1136/gut.2010.224154

Muller, P. A., Koscsó, B., Rajani, G. M., Stevanovic, K., Berres, M.-L., Hashimoto, D., et al. (2014). Crosstalk between Muscularis Macrophages and Enteric Neurons Regulates Gastrointestinal Motility. *Cell* 158 (2), 300–313. doi:10.1016/j.cell.2014.04.050

Municio, C., Soler Palacios, B., Estrada-Capetillo, L., Benguria, A., Dopazo, A., García-Lorenzo, E., et al. (2016). Methotrexate Selectively Targets Human Proinflammatory Macrophages through a Thymidylate Synthase/p53 axis. *Ann. Rheum. Dis.* 75 (12), 2157–2165. doi:10.1136/annrheumdis-2015-208736

Murray, P. J., and Wynn, T. A. (2011). Protective and Pathogenic Functions of Macrophage Subsets. *Nat. Rev. Immunol.* 11 (11), 723–737. doi:10.1038/nri3073

Na, Y. R., Stakenborg, M., Seok, S. H., and Matteoli, G. (2019). Macrophages in Intestinal Inflammation and Resolution: a Potential Therapeutic Target in IBD. *Nat. Rev. Gastroenterol. Hepatol.* 16 (9), 531–543. doi:10.1038/s41575-019-0172-4

Nadeem, M. S., Kumar, V., Al-Abbasi, F. A., Kamal, M. A., and Anwar, F. (2020). Risk of Colorectal Cancer in Inflammatory Bowel Diseases. *Seminars Cancer Biol.* 64, 51–60. doi:10.1016/j.semcancer.2019.05.001

Nakkala, J. R., Li, Z., Ahmad, W., Wang, K., and Gao, C. (2021). Immunomodulatory Biomaterials and Their Application in Therapies for Chronic Inflammation-Related Diseases. *Acta Biomater.* 123, 1–30. doi:10.1016/j.actbio.2021.01.025

Nunes, R., Neves, J. d., and Sarmento, B. (2019). Nanoparticles for the Regulation of Intestinal Inflammation: Opportunities and Challenges. *Nanomedicine* 14 (19), 2631–2644. doi:10.2217/nnm-2019-0191

Oakley, R. H., and Cidlowski, J. A. (2013). The Biology of the Glucocorticoid Receptor: New Signaling Mechanisms in Health and Disease. *J. Allergy Clin. Immunol.* 132 (5), 1033–1044. doi:10.1016/j.jaci.2013.09.007

Odegaard, J. I., Ricardo-Gonzalez, R. R., Goforth, M. H., Morel, C. R., Subramanian, V., Mukundan, L., et al. (2007). Macrophage-specific PPARγ Controls Alternative Activation and Improves Insulin Resistance. *Nature* 447 (7148), 1116–1120. doi:10.1038/nature05894

Oeckinghaus, A., Hayden, M. S., and Ghosh, S. (2011). Crosstalk in NF-Kb Signaling Pathways. *Nat. Immunol.* 12 (8), 695–708. doi:10.1038/ni.2065

O'Garra, A., Vieira, P. L., Vieira, P., and Goldfeld, A. E. (2004). IL-10-producing and Naturally Occurring CD4+ Tregs: Limiting Collateral Damage. *J. Clin. Invest.* 114 (10), 1372–1378. doi:10.1172/jci23215

Ohmori, Y., and Hamilton, T. A. (1997). IL-4-induced STAT6 Suppresses IFN-Gamma-Stimulated STAT1-dependent Transcription in Mouse Macrophages. *J. Immunol.* 159 (11), 5474–5482.

Olesen, C. M., Coskun, M., Peyrin-Biroulet, L., and Nielsen, O. H. (2016). Mechanisms behind Efficacy of Tumor Necrosis Factor Inhibitors in Inflammatory Bowel Diseases. *Pharmacol. Ther.* 159, 110–119. doi:10.1016/j.pharmthera.2016.01.001

Palmela, C., Chevarin, C., Xu, Z., Torres, J., Sevrin, G., Hirten, R., et al. (2018). Adherent-invasive *Escherichia coli* in Inflammatory Bowel Disease. *Gut* 67 (3), 574–587. doi:10.1136/gutjnl-2017-314903

Pander, J., Heusinkveld, M., van der Straaten, T., Jordanova, E. S., Baak-Pablo, R., Gelderblom, H., et al. (2011). Activation of Tumor-Promoting Type 2 Macrophages by EGFR-Targeting Antibody Cetuximab. *Clin. Cancer Res.* 17 (17), 5668–5673. doi:10.1158/1078-0432.Ccr-11-0239

Panés, J., Sandborn, W. J., Schreiber, S., Sands, B. E., Vermeire, S., D'Haens, G., et al. (2017). Tofacitinib for Induction and Maintenance Therapy of Crohn's Disease: Results of Two Phase IIb Randomised Placebo-Controlled Trials. *Gut* 66 (6), 1049–1059. doi:10.1136/gutjnl-2016-312735

Papamichael, K., Gils, A., Rutgeerts, P., Levesque, B. G., Vermeire, S., Sandborn, W. J., et al. (2015). Role for Therapeutic Drug Monitoring during Induction Therapy with TNF Antagonists in IBD. *Inflamm. Bowel Dis.* 21 (1), 182–197. doi:10.1097/mib.0000000000000202

Parameswaran, N., and Patial, S. (2010). Tumor Necrosis Factor-α Signaling in Macrophages. *Crit. Rev. Eukar Gene Expr.* 20 (2), 87–103. doi:10.1615/critreveukargeneexpr.v20.i2.10

Paramsothy, S., Rosenstein, A. K., Mehandru, S., and Colombel, J.-F. (2018). The Current State of the Art for Biological Therapies and New Small Molecules in Inflammatory Bowel Disease. *Mucosal Immunol.* 11 (6), 1558–1570. doi:10.1038/s41385-018-0050-3

Pattison, M. J., Mackenzie, K. F., and Arthur, J. S. C. (2012). Inhibition of JAKs in Macrophages Increases Lipopolysaccharide-Induced Cytokine Production by Blocking IL-10-mediated Feedback. *J. I.* 189 (6), 2784–2792. doi:10.4049/jimmunol.1200310

Piaggio, F., Kondylis, V., Pastorino, F., Di Paolo, D., Perri, P., Cossu, I., et al. (2016). A Novel Liposomal Clodronate Depletes Tumor-Associated Macrophages in Primary and Metastatic Melanoma: Anti-angiogenic and Anti-tumor Effects. *J. Control. Release* 223, 165–177. doi:10.1016/j.jconrel.2015.12.037

Png, C. W., Lindén, S. K., Gilshenan, K. S., Zoetendal, E. G., McSweeney, C. S., Sly, L. I., et al. (2010). Mucolytic Bacteria with Increased Prevalence in IBD Mucosa Augment *In Vitro* Utilization of Mucin by Other Bacteria. *Am. J. Gastroenterol.* 105 (11), 2420–2428. doi:10.1038/ajg.2010.281

Pothoven, K. L., and Schleimer, R. P. (2017). The Barrier Hypothesis and Oncostatin M: Restoration of Epithelial Barrier Function as a Novel Therapeutic Strategy for the Treatment of Type 2 Inflammatory Disease. *Tissue Barriers* 5 (3), e1341367. doi:10.1080/21688370.2017.1341367

Rahman, F. Z., Smith, A. M., Hayee, B. H., Marks, D. J. B., Bloom, S. L., and Segal, A. W. (2010). Delayed Resolution of Acute Inflammation in Ulcerative Colitis Is Associated with Elevated Cytokine Release Downstream of TLR4. *PLoS One* 5 (3), e9891. doi:10.1371/journal.pone.0009891

Rescigno, M., Urbano, M., Valzasina, B., Francolini, M., Rotta, G., Bonasio, R., et al. (2001). Dendritic Cells Express Tight Junction Proteins and Penetrate Gut Epithelial Monolayers to Sample Bacteria. *Nat. Immunol.* 2 (4), 361–367. doi:10.1038/86373

Roda, G., Chien Ng, S., Kotze, P. G., Argollo, M., Panaccione, R., Spinelli, A., et al. (2020). Crohn's Disease. *Nat. Rev. Dis. Prim.* 6 (1), 22. doi:10.1038/s41572-020-0156-2

Roda, G., Jharap, B., Neeraj, N., and Colombel, J.-F. (2016). Loss of Response to Anti-TNFs: Definition, Epidemiology, and Management. *Clin. Transl. Gastroenterol.* 7 (1), e135. doi:10.1038/ctg.2015.63

Rogler, G. (2020). Efficacy of JAK Inhibitors in Crohn's Disease. *J. Crohns Colitis* 14 (Suppl. ment_2), S746–s754. doi:10.1093/ecco-jcc/jjz186

Rose, T. M., and Bruce, A. G. (1991). Oncostatin M Is a Member of a Cytokine Family that Includes Leukemia-Inhibitory Factor, Granulocyte Colony-Stimulating Factor, and Interleukin 6. *Proc. Natl. Acad. Sci. U.S.A.* 88 (19), 8641–8645. doi:10.1073/pnas.88.19.8641

Rőszer, T. (2015). Understanding the Mysterious M2 Macrophage through Activation Markers and Effector Mechanisms. *Mediat. Inflamm.* 2015, 1–16. doi:10.1155/2015/816460

Ruffell, D., Mourkioti, F., Gambardella, A., Kirstetter, P., Lopez, R. G., Rosenthal, N., et al. (2009). A CREB-C/EBPβ Cascade Induces M2 Macrophage-specific Gene Expression and Promotes Muscle Injury Repair. *Proc. Natl. Acad. Sci. U.S.A.* 106 (41), 17475–17480. doi:10.1073/pnas.0908641106

Rutgeerts, P., D'Haens, G., Targan, S., Vasiliauskas, E., Hanauer, S. B., Present, D. H., et al. (1999). Efficacy and Safety of Retreatment with Anti-tumor Necrosis Factor Antibody (Infliximab) to Maintain Remission in Crohn's Disease. *Gastroenterology* 117 (4), 761–769. doi:10.1016/s0016-5085(99)70332-x

Sabate, J. M., Villarejo, J., Lemann, M., Bonnet, J., Allez, M., and Modigliani, R. (2002). An Open-Label Study of Thalidomide for Maintenance Therapy in Responders to Infliximab in Chronically Active and Fistulizing Refractory Crohn's Disease. *Aliment. Pharmacol. Ther.* 16 (6), 1117–1124. doi:10.1046/j.1365-2036.2002.01273.x

Sabino, J., Verstockt, B., Vermeire, S., and Ferrante, M. (2019). New Biologics and Small Molecules in Inflammatory Bowel Disease: an Update. *Ther. Adv. Gastroenterol.* 12, 175628481985320. doi:10.1177/1756284819853208

Salas, A., Hernandez-Rocha, C., Duijvestein, M., Faubion, W., McGovern, D., Vermeire, S., et al. (2020). JAK-STAT Pathway Targeting for the Treatment of Inflammatory Bowel Disease. *Nat. Rev. Gastroenterol. Hepatol.* 17 (6), 323–337. doi:10.1038/s41575-020-0273-0

Sales-Campos, H., Basso, P. J., Alves, V. B. F., Fonseca, M. T. C., Bonfá, G., Nardini, V., et al. (2015). Classical and Recent Advances in the Treatment of Inflammatory Bowel Diseases. *Braz J. Med. Biol. Res.* 48 (2), 96–107. doi:10.1590/1414-431x20143774

Sandborn, W. J., Ghosh, S., Panes, J., Vranic, I., Wang, W., and Niezychowski, W. (2014). A Phase 2 Study of Tofacitinib, an Oral Janus Kinase Inhibitor, in Patients with Crohn's Disease. *Clin. Gastroenterol. Hepatol.* 12 (9), 1485–e1482. doi:10.1016/j.cgh.2014.01.029

Sandborn, W. J., Feagan, B. G., Danese, S., O'Brien, C. D., Ott, E., Marano, C., et al. (2021). Safety of Ustekinumab in Inflammatory Bowel Disease: Pooled Safety Analysis of Results from Phase 2/3 Studies. *Inflamm. Bowel Dis.* 27 (7), 994–1007. doi:10.1093/ibd/izaa236

Sandborn, W. J., Su, C., and Panes, J. (2017). Tofacitinib as Induction and Maintenance Therapy for Ulcerative Colitis. *N. Engl. J. Med.* 377 (5), 496–497. doi:10.1056/NEJMc1707500

Satoh, T., Takeuchi, O., Vandenbon, A., Yasuda, K., Tanaka, Y., Kumagai, Y., et al. (2010). The Jmjd3-Irf4 axis Regulates M2 Macrophage Polarization and Host Responses against Helminth Infection. *Nat. Immunol.* 11 (10), 936–944. doi:10.1038/ni.1920

Schenk, M., Bouchon, A., Birrer, S., Colonna, M., and Mueller, C. (2005). Macrophages Expressing Triggering Receptor Expressed on Myeloid Cells-1 Are Underrepresented in the Human Intestine. *J. Immunol.* 174 (1), 517–524. doi:10.4049/jimmunol.174.1.517

Schett, G., and Neurath, M. F. (2018). Resolution of Chronic Inflammatory Disease: Universal and Tissue-specific Concepts. *Nat. Commun.* 9 (1), 3261. doi:10.1038/s41467-018-05800-6

Schridde, A., Bain, C. C., Mayer, J. U., Montgomery, J., Pollet, E., Denecke, B., et al. (2017). Tissue-specific Differentiation of Colonic Macrophages Requires TGFβ Receptor-Mediated Signaling. *Mucosal Immunol.* 10 (6), 1387–1399. doi:10.1038/mi.2016.142

Schulz, C., Perdiguero, E. G., Chorro, L., Szabo-Rogers, H., Cagnard, N., Kierdorf, K., et al. (2012). A Lineage of Myeloid Cells Independent of Myb and Hematopoietic Stem Cells. *Science* 336 (6077), 86–90. doi:10.1126/science.1219179

Schwartz, D. M., Kanno, Y., Villarino, A., Ward, M., Gadina, M., and O'Shea, J. J. (2018). Erratum: JAK Inhibition as a Therapeutic Strategy for Immune and Inflammatory Diseases. *Nat. Rev. Drug Discov.* 17 (1), 78. doi:10.1038/nrd.2017.267

Sehgal, A., Donaldson, D. S., Pridans, C., Sauter, K. A., Hume, D. A., and Mabbott, N. A. (2018). The Role of CSF1R-dependent Macrophages in Control of the Intestinal Stem-Cell Niche. *Nat. Commun.* 9 (1), 1272. doi:10.1038/s41467-018-03638-6

Seyed Tabib, N. S., Madgwick, M., Sudhakar, P., Verstockt, B., Korcsmaros, T., and Vermeire, S. (2020). Big Data in IBD: Big Progress for Clinical Practice. *Gut* 69 (8), 1520–1532. doi:10.1136/gutjnl-2019-320065

Shaw, T. N., Houston, S. A., Wemyss, K., Bridgeman, H. M., Barbera, T. A., Zangerle-Murray, T., et al. (2018). Tissue-resident Macrophages in the Intestine Are Long Lived and Defined by Tim-4 and CD4 Expression. *J. Exp. Med.* 215 (6), 1507–1518. doi:10.1084/jem.20180019

Shiratori, H., Feinweber, C., Luckhardt, S., Wallner, N., Geisslinger, G., Weigert, A., et al. (2018). An *In Vitro* Test System for Compounds that Modulate Human Inflammatory Macrophage Polarization. *Eur. J. Pharmacol.* 833, 328–338. doi:10.1016/j.ejphar.2018.06.017

Shivaji, U. N., Sharratt, C. L., Thomas, T., Smith, S. C. L., Iacucci, M., Moran, G. W., et al. (2019). Review Article: Managing the Adverse Events Caused by Anti-TNF Therapy in Inflammatory Bowel Disease. *Aliment. Pharmacol. Ther.* 49 (6), 664–680. doi:10.1111/apt.15097

Siebel, C., and Lendahl, U. (2017). Notch Signaling in Development, Tissue Homeostasis, and Disease. *Physiol. Rev.* 97 (4), 1235–1294. doi:10.1152/physrev.00005.2017

Simon, J. M., Davis, J. P., Lee, S. E., Schaner, M. R., Gipson, G. R., Weiser, M., et al. (2016). Alterations to Chromatin in Intestinal Macrophages Link IL-10 Deficiency to Inappropriate Inflammatory Responses. *Eur. J. Immunol.* 46 (8), 1912–1925. doi:10.1002/eji.201546237

Singh, Y., Pawar, V. K., Meher, J. G., Raval, K., Kumar, A., Shrivastava, R., et al. (2017). Targeting Tumor Associated Macrophages (TAMs) via Nanocarriers. *J. Control. Release* 254, 92–106. doi:10.1016/j.jconrel.2017.03.395

Smith, A. M., Rahman, F. Z., Hayee, B. H., Graham, S. J., Marks, D. J. B., Sewell, G. W., et al. (2009). Disordered Macrophage Cytokine Secretion Underlies Impaired Acute Inflammation and Bacterial Clearance in Crohn's Disease. *J. Exp. Med.* 206 (9), 1883–1897. doi:10.1084/jem.20091233

Smythies, L. E., Sellers, M., Clements, R. H., Mosteller-Barnum, M., Meng, G., Benjamin, W. H., et al. (2005). Human Intestinal Macrophages Display Profound Inflammatory Anergy Despite Avid Phagocytic and Bacteriocidal Activity. *J. Clin. Invest.* 115 (1), 66–75. doi:10.1172/jci200519229

Sohail, M., MudassirMinhas, M. U., Minhas, M. U., Khan, S., Hussain, Z., de Matas, M., et al. (2019). Natural and Synthetic Polymer-Based Smart Biomaterials for Management of Ulcerative Colitis: a Review of Recent Developments and Future Prospects. *Drug Deliv. Transl. Res.* 9 (2), 595–614. doi:10.1007/s13346-018-0512-x

Spadaccini, M., D'Alessio, S., Peyrin-Biroulet, L., and Danese, S. (2017). PDE4 Inhibition and Inflammatory Bowel Disease: A Novel Therapeutic Avenue. *Ijms* 18 (6), 1276. doi:10.3390/ijms18061276

Stack, W., Mann, S., Roy, A., Heath, P., Sopwith, M., Freeman, J., et al. (1997). Randomised Controlled Trial of CDP571 Antibody to Tumour Necrosis Factor-α in Crohn's Disease. *Lancet* 349 (9051), 521–524. doi:10.1016/s0140-6736(97)80083-9

Steinbach, E. C., and Plevy, S. E. (2014). The Role of Macrophages and Dendritic Cells in the Initiation of Inflammation in IBD. *Inflamm. Bowel Dis.* 20 (1), 166–175. doi:10.1097/MIB.0b013e3182a69dca

Taratummarat, S., Sangphech, N., Vu, C. T. B., Palaga, T., Ondee, T., Surawut, S., et al. (2018). Gold Nanoparticles Attenuates Bacterial Sepsis in Cecal Ligation and Puncture Mouse Model through the Induction of M2 Macrophage Polarization. *BMC Microbiol.* 18 (1), 85. doi:10.1186/s12866-018-1227-3

Targan, S. R., Hanauer, S. B., van Deventer, S. J. H., Mayer, L., Present, D. H., Braakman, T., et al. (1997). A Short-Term Study of Chimeric Monoclonal Antibody cA2 to Tumor Necrosis Factor α for Crohn's Disease. *N. Engl. J. Med.* 337 (15), 1029–1036. doi:10.1056/nejm199710093371502

Torres, J., Mehandru, S., Colombel, J.-F., and Peyrin-Biroulet, L. (2017). Crohn's Disease. *Lancet* 389 (10080), 1741–1755. doi:10.1016/s0140-6736(16)31711-1

Tripathi, K., and Feuerstein, J. D. (2019). New Developments in Ulcerative Colitis: Latest Evidence on Management, Treatment, and Maintenance. *Dic* 8, 1–11. doi:10.7573/dic.212572

Underwood, A. P., Kaakoush, N. O., Sodhi, N., Merif, J., Seah Lee, W., Riordan, S. M., et al. (2016). Campylobacter Concisus Pathotypes Are Present at Significant Levels in Patients with Gastroenteritis. *J. Med. Microbiol.* 65 (3), 219–226. doi:10.1099/jmm.0.000216

Ungaro, R., Mehandru, S., Allen, P. B., Peyrin-Biroulet, L., and Colombel, J.-F. (2017). Ulcerative Colitis. *Lancet* 389 (10080), 1756–1770. doi:10.1016/s0140-6736(16)32126-2

Vallon-Eberhard, A., Landsman, L., Yogev, N., Verrier, B., and Jung, S. (2006). Transepithelial Pathogen Uptake into the Small Intestinal Lamina Propria. *J. Immunol.* 176 (4), 2465–2469. doi:10.4049/jimmunol.176.4.2465

van Furth, R., Cohn, Z. A., Hirsch, J. G., Humphrey, J. H., Spector, W. G., and Langevoort, H. L. (1972). The Mononuclear Phagocyte System: a New Classification of Macrophages, Monocytes, and Their Precursor Cells. *Bull. World Health Organ* 46 (6), 845–852.

Vergadi, E., Ieronymaki, E., Lyroni, K., Vaporidi, K., and Tsatsanis, C. (2017). Akt Signaling Pathway in Macrophage Activation and M1/M2 Polarization. *J. I.* 198 (3), 1006–1014. doi:10.4049/jimmunol.1601515

Vermeire, S., Schreiber, S., Petryka, R., Kuehbacher, T., Hebuterne, X., Roblin, X., et al. (2017). Clinical Remission in Patients with Moderate-To-Severe Crohn's Disease Treated with Filgotinib (The FITZROY Study): Results from a Phase 2, Double-Blind, Randomised, Placebo-Controlled Trial. *Lancet* 389 (10066), 266–275. doi:10.1016/s0140-6736(16)32537-5

Villarino, A. V., Kanno, Y., and O'Shea, J. J. (2017). Mechanisms and Consequences of Jak-STAT Signaling in the Immune System. *Nat. Immunol.* 18 (4), 374–384. doi:10.1038/ni.3691

Viola, M. F., and Boeckxstaens, G. (2021). Niche-specific Functional Heterogeneity of Intestinal Resident Macrophages. *Gut* 70 (7), 1383–1395. doi:10.1136/gutjnl-2020-323121

Vos, A. C. W., Wildenberg, M. E., Arijs, I., Duijvestein, M., Verhaar, A. P., de Hertogh, G., et al. (2012). Regulatory Macrophages Induced by Infliximab Are Involved in Healing *In Vivo* and *In Vitro*. *Inflamm. Bowel Dis.* 18 (3), 401–408. doi:10.1002/ibd.21818

Vos, A. C. W., Wildenberg, M. E., Duijvestein, M., Verhaar, A. P., van den Brink, G. R., and Hommes, D. W. (2011). Anti-Tumor Necrosis Factor-α Antibodies Induce Regulatory Macrophages in an Fc Region-dependent Manner. *Gastroenterology* 140 (1), 221–230. doi:10.1053/j.gastro.2010.10.008

Wallace, K. L., Zheng, L. B., Kanazawa, Y., and Shih, D. Q. (2014). Immunopathology of Inflammatory Bowel Disease. *Wjg* 20 (1), 6–21. doi:10.3748/wjg.v20.i1.6

Wang, L., Tang, L., Feng, Y., Zhao, S., Han, M., Zhang, C., et al. (2020). A Purified Membrane Protein from Akkermansia Muciniphila or the Pasteurised Bacterium Blunts Colitis Associated Tumourigenesis by Modulation of CD8+ T Cells in Mice. *Gut* 69 (11), 1988–1997. doi:10.1136/gutjnl-2019-320105

Wang, L. x., Zhang, S. x., Wu, H. j., Rong, X. l., and Guo, J. (2019). M2b Macrophage Polarization and its Roles in Diseases. *J. Leukoc. Biol.* 106 (2), 345–358. doi:10.1002/jlb.3ru1018-378rr

Weissleder, R., Nahrendorf, M., and Pittet, M. J. (2014). Imaging Macrophages with Nanoparticles. *Nat. Mater* 13 (2), 125–138. doi:10.1038/nmat3780

Wells, J. M., Rossi, O., Meijerink, M., and van Baarlen, P. (2011). Epithelial Crosstalk at the Microbiota-Mucosal Interface. *Proc. Natl. Acad. Sci. U.S.A.* 108 (Suppl. 1Suppl 1), 4607–4614. doi:10.1073/pnas.1000092107

West, N. R., Hegazy, A. N., Hegazy, A. N., Owens, B. M. J., Bullers, S. J., Linggi, B., et al. (2017). Oncostatin M Drives Intestinal Inflammation and Predicts Response to Tumor Necrosis Factor-Neutralizing Therapy in Patients with Inflammatory Bowel Disease. *Nat. Med.* 23 (5), 579–589. doi:10.1038/nm.4307

Windsor, J. W., and Kaplan, G. G. (2019). Evolving Epidemiology of IBD. *Curr. Gastroenterol. Rep.* 21 (8), 40. doi:10.1007/s11894-019-0705-6

Wu, Y., and Hirschi, K. K. (2020). Tissue-Resident Macrophage Development and Function. *Front. Cell Dev. Biol.* 8, 617879. doi:10.3389/fcell.2020.617879

Wynn, T. A., and Vannella, K. M. (2016). Macrophages in Tissue Repair, Regeneration, and Fibrosis. *Immunity* 44 (3), 450–462. doi:10.1016/j.immuni.2016.02.015

Xiao, Q., Li, X., Li, Y., Wu, Z., Xu, C., Chen, Z., et al. (2021). Biological Drug and Drug Delivery-Mediated Immunotherapy. *Acta Pharm. Sin. B* 11 (4), 941–960. doi:10.1016/j.apsb.2020.12.018

Xu, H., Zhu, J., Smith, S., Foldi, J., Zhao, B., Chung, A. Y., et al. (2012). Notch-RBP-J Signaling Regulates the Transcription Factor IRF8 to Promote Inflammatory Macrophage Polarization. *Nat. Immunol.* 13 (7), 642–650. doi:10.1038/ni.2304

Yakymenko, O., Schoultz, I., Gullberg, E., Ström, M., Almer, S., Wallon, C., et al. (2018). Infliximab Restores Colonic Barrier to Adherent-Invasive *E. coli* in Crohn's Disease via Effects on Epithelial Lipid Rafts. *Scand. J. Gastroenterology* 53 (6), 677–684. doi:10.1080/00365521.2018.1458146

Yamagata, M., Mikami, T., Tsuruta, T., Yokoyama, K., Sada, M., Kobayashi, K., et al. (2011). Submucosal Fibrosis and Basic-Fibroblast Growth Factor-Positive Neutrophils Correlate with Colonic Stenosis in Cases of Ulcerative Colitis. *Digestion* 84 (1), 12–21. doi:10.1159/000320773

Yip, J. L. K., Balasuriya, G. K., Spencer, S. J., and Hill-Yardin, E. L. (2021). The Role of Intestinal Macrophages in Gastrointestinal Homeostasis: Heterogeneity and Implications in Disease. *Cell. Mol. Gastroenterology Hepatology* 12, 1701–1718. doi:10.1016/j.jcmgh.2021.08.021

Yona, S., Kim, K.-W., Wolf, Y., Mildner, A., Varol, D., Breker, M., et al. (2013). Fate Mapping Reveals Origins and Dynamics of Monocytes and Tissue Macrophages under Homeostasis. *Immunity* 38 (1), 79–91. doi:10.1016/j.immuni.2012.12.001

You, Y., Zhou, C., Li, D., Cao, Z.-L., Shen, W., Li, W.-Z., et al. (2016). Sorting Nexin 10 Acting as a Novel Regulator of Macrophage Polarization Mediates Inflammatory Response in Experimental Mouse Colitis. *Sci. Rep.* 6, 20630. doi:10.1038/srep20630

Zhang, H., Wu, X., Wang, G., Liu, P., Qin, S., Xu, K., et al. (2018). Macrophage Polarization, Inflammatory Signaling, and NF-Kb Activation in Response to Chemically Modified Titanium Surfaces. *Colloids Surfaces B Biointerfaces* 166, 269–276. doi:10.1016/j.colsurfb.2018.03.029

Zhang, L., Lee, H., Grimm, M. C., Riordan, S. M., Day, A. S., and Lemberg, D. A. (2014). Campylobacter Concisusand Inflammatory Bowel Disease. *Wjg* 20 (5), 1259–1267. doi:10.3748/wjg.v20.i5.1259

Zhu, C., Zhang, S., Song, C., Zhang, Y., Ling, Q., Hoffmann, P. R., et al. (2017). Selenium Nanoparticles Decorated with Ulva Lactuca Polysaccharide Potentially Attenuate Colitis by Inhibiting NF-Kb Mediated Hyper Inflammation. *J. Nanobiotechnol* 15 (1), 20. doi:10.1186/s12951-017-0252-y

Zhu, W., Yu, J., Nie, Y., Shi, X., Liu, Y., Li, F., et al. (2014). Disequilibrium of M1 and M2 Macrophages Correlates with the Development of Experimental Inflammatory Bowel Diseases. *Immunol. Investig.* 43 (7), 638–652. doi:10.3109/08820139.2014.909456

Zigmond, E., Bernshtein, B., Friedlander, G., Walker, C. R., Yona, S., Kim, K.-W., et al. (2014). Macrophage-restricted Interleukin-10 Receptor Deficiency, but Not IL-10 Deficiency, Causes Severe Spontaneous Colitis. *Immunity* 40 (5), 720–733. doi:10.1016/j.immuni.2014.03.012

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REVIEW
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Tissue-Resident Innate Immune Cell-Based Therapy: A Cornerstone of Immunotherapy Strategies for Cancer Treatment

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Cancer immunotherapy has led to impressive advances in cancer treatment. Unfortunately, in a high percentage of patients is difficult to consistently restore immune responses to eradicate established tumors. It is well accepted that adaptive immune cells, such as B lymphocytes, $CD4^+$ helper T lymphocytes, and $CD8^+$ cytotoxic T-lymphocytes (CTLs), are the most effective cells able to eliminate tumors. However, it has been recently reported that innate immune cells, including natural killer cells (NK), dendritic cells (DC), macrophages, myeloid-derived suppressor cells (MDSCs), and innate lymphoid cells (ILCs), represent important contributors to modulating the tumor microenvironment and shaping the adaptive tumor response. In fact, their role as a bridge to adaptive immunity, make them an attractive therapeutic target for cancer treatment. Here, we provide a comprehensive overview of the pleiotropic role of tissue-resident innate immune cells in different tumor contexts. In addition, we discuss how current and future therapeutic approaches targeting innate immune cells sustain the adaptive immune system in order to improve the efficacy of current tumor immunotherapies.

Keywords: innate immune cells, macrophages, innate lymphoid cells (ILC), NK cells, tissue-resident immune cells, cancer, MDSC (myeloid-derived suppressor cell)

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1 INTRODUCTION

Cancer is considered a major public health concern worldwide and is characterized by an uncontrolled division of altered cells. The human immune system recognizes tumor cells and induces a protective response to eliminate those cells. However, sustained tumors may protect themselves by developing immune escape mechanisms through multiple soluble and cellular mediators. In the last decades, the deep knowledge of tumorigenesis and the study of the complex interaction between the host and the immune system has been the goal for significant advances in anticancer therapy. Conventional anticancer therapy, such as surgical resection, radiotherapy, and cytotoxic drugs, involves multiple targeting of tumor cells. Though, the tumor tissue microenvironment can present a dysregulated, or weakened immune response which, in turn, uncovers pro-tumor activities favouring tumor expansion and progression (Dhara et al., 2022). Recently, new potential targets have been identified based on immunomodulatory therapies, with the aim to re-establish the host anti-tumoral immune response. Since the effect of cancer

immunotherapy is largely dependent on the status of the immune system in the tumor microenvironment, the choice of therapy and the development of new therapies based on the immune status in the tumor microenvironment would be predicted to be effective (Sambi et al., 2019). Tissue-resident innate immune cells could be found in different human tissues, performing a strategic role at all stages of the immune response, from maintaining homeostasis to responding to infectious challenges to the resolution of inflammation to tissue repair and finally, to initiating antitumor response (Goff and Danforth, 2021). In humans studying immune cells and responses in tissues is challenging, due to the difficult accessibility of tissue-resident innate immune cells, the biggest pieces of knowledge concerning their responses in tissues have been obtained using murine models or studying immune cells drawn from blood (Segura et al., 2010; Gray and Farber, 2022). In recent years thanks to new knowledge obtained from these studies it has emerged that not only adaptive immune cells are the only effective cells able to eliminate tumors, but also innate cells are able to do it. Indeed, it emerged that natural killer cells (NK), dendritic cells (DC), macrophages, Myeloid-derived suppressor cells (MDSC) and innate lymphoid cells (ILCs), represent important contributors to modulating the tumor microenvironment and shaping the adaptive tumor response (Wang et al., 2019b). This review provides an overview of the different types of tissue-resident innate immune cells involved in the suppressor activity of anti-tumor immunity. The deep knowledge of the mechanisms underlying these processes could significantly improve the clinical utility of tissue-resident innate immune cells in cancer and eventually can support the identification of biomarkers for cancer prognosis and the development of novel therapeutic approaches for cancer treatment.

1.1 Tissue-Resident Dendritic Cells in Tumor Immunity

Dendritic cells (DCs) represent a heterogeneous family of immune cells, consisting of various subgroups of specialized antigen-presenting cells, mainly involved in initiating and regulating innate and adaptive immune responses (Wculek et al., 2020). Together with macrophages and B cells, they are considered the three major professional antigen-presenting cells (APCs). DCs play a critical role in promoting immunity by providing immunomodulatory signals, such as the secretion of cytokines and growth factors, but can also promote tolerance by presenting antigens to T cells (Patente et al., 2018; Wculek et al., 2020). They are a sort of sentinel able to collect a broad spectrum of environmental signals or stimuli such as bacterial and viral PAMPs and/or DAMPs, processing an extensive spectrum of specific tissue responses and influencing the immunological outcome, one of the most important, promoting T cell-mediated immunity (Levings et al., 2005; Steinman, 2006). DCs originate in bone marrow from unique precursor $CD34^+$ that can differentiate into myeloid (MP) and lymphoid (LP) precursors. The first type of precursor gives rise to monocytes and DC precursors (MDP), which are further differentiated in common DC precursors (CDP), from which finally arise preclassical DC (pre-cDC) and plasmacytoid DC (pDC). In the last step of differentiation, pre-cDC will give rise to the most represented cDC subpopulations, named cDC1 and cDC2. Regarding the second differentiation way, or else LP, the ontogenic pathway is not completely elucidated, so nowadays we only know that it can give rise to pDC (Geissmann et al., 2010). DCs can be found in practically all tissue, they are a very plastic and dynamic cell population that can change its phenotype based on the tissue microenvironment in which is located. DCs represent the link between innate and adaptive immune responses, without inflammatory stimuli they are in an immature or tolerogenic state contributing to immune tolerance. Immature DCs express low levels of costimulatory molecules such as CD40, CD80, and CD86, besides they can infiltrate the tumor micro-environment performing a preponderant role in beginning antitumor immune response (Ganguly et al., 2013). The biggest knowledge concerning DC subpopulations have been obtained from the studies on murine DCs, mostly due to the wide range of tissue accessibility; unfortunately, the same type of characterization cannot be done for human DCs (Segura et al., 2010). Indeed, almost all studies on human DCs were performed mainly on peripheral blood where, among others, DCs constitute a rare cell population. Initially, DCs have been simply divided according to the cell localization, dividing them into resident lymphoid tissue DCs and migratory non-lymphoid tissue DCs (Haniffa et al., 2013). Nowadays, the development of new technologies, especially single-cell RNAseq, allowed to bring light new characteristics of this very heterogeneous cell population providing more information usable for classification criteria, including phenotypical, functional, and developmental criteria (Heath and Carbone, 2013).

Currently, DCs are divided into at least four wide groups using either functional or phenotypical characteristics. From a phenotypical point of view, all human DCs show a high expression of major histocompatibility complex (MHC) class II molecules (MHC-II) and of CD11c, which are expressed also on other cells, and many other molecules which allow their classification into various subtypes. Conversely, they lack key markers of T cells, B cells, natural killer (NK) cells, granulocytes, and monocytes. DCs subset can be classified into Conventional DC Type 1 (cDC1), Conventional DC Type 2, Plasmacytoid DC (pDC), and Monocyte-derived DCs (moDC), and each subset plays a different role within tumors and during their therapy (Kim et al., 2021).

The cDC1 are characterized by the presence of specific markers surface including thrombin receptor THRM (CD141), the chemokine receptor XCR1, C-type lectin CLEC9A and the cell adhesion molecule CADM1 (homologous of CD8α/CD103/XCR1 in mice) (Reynolds and Haniffa, 2015). The two mainly transcription factors involved in their generation are BATF3, a basic leucine Zipper ATF-Like Transcription Factor 3 and the IFN-regulatory factor 8 (IRF8). cDC1 are localized especially in peripheral blood and in lymphoid and non-lymphoid tissue, where they are specialized in cross-presentation, realizing the priming of $CD8^+$ T cells against extracellular antigens such as bacteria and viruses (Haniffa et al., 2012). Recently, using

different tumor murine models emerged that cDC1 also play a critical role in the induction of the cancer-immune cycle, exercised through the transport of antigens from tumor towards draining lymph nodes, inducing a robust activation/proliferation of $CD8^+$ T cells or transfer of antigen to resident myeloid cells (Roberts et al., 2016; Salmon et al., 2016; Gardner et al., 2020). The antitumor immune responses mediated by cDC1s are critical in the mechanism of tumor rejection and responses to immunotherapies, like the immune-checkpoint blockade and adoptive T cell therapy (Kim et al., 2021). In addition, the presence of cDC1 within human melanoma tumors correlated with improved response to anti-PD-1 therapy (Barry et al., 2018) as well as with higher $CD8^+$ T cell infiltration into tumors (Binnewies et al., 2019) which is associated with a positive prognosis across multiple tumor types (Fridman et al., 2012). In addition to PD-1/PD-L1 expression, it was also observed clustered expression of TIM-3 on cDC cells within tumors, particularly $CD103^+$ cDCs. de Mingo Pulido et al. (2018), have shown that the use of αTIM-3 antibody-induced an increase in cell death within tumors and an improvement in response to chemotherapy, suggesting a key role of TIM-3 as a target for therapy. Recently, Roberts et al. (2016) have shown that anti-tumour activity of migratory cDC1s subtype can be done through the expression of chemokine receptor CCR7; in fact, in mice with cDC1 defective for CCR7, it was observed a loss priming of T cell in lymph nodes area and a lack of antigen hand-off to resident myeloid cells, which led to a failure of immune control with consequential increased tumor growth. Moreover, an analysis of tumour-infiltrating cell populations, isolated by human melanoma biopsies, showed that only $CD141^+$ DC expressed a detectable CCR7 on the surface, demonstrating that CCR7 is particularly prominent on $CD141^+$ DC in human tumors. In addition, it has been demonstrated that cross-presentation activity by cDC1 is improved by I interferon (IFN) signaling, and cells that lack IFNAR1 (IFN-α/β receptor 1) are unable to perform tumor-specific T cell priming and tumor elimination (Diamond et al., 2011; Fuertes et al., 2011).

The cDC2 are a heterogeneous subset of cells that co-express high levels of CD1c and SIRPα (CD172a) (homologous of CD11b and CD172a in mice) and a range of other markers that are tissue-specific (Shortman and Liu, 2002; Reynolds and Haniffa, 2015; Wculek et al., 2020). The transcriptional factors involved in cDC2 maturation and differentiation are mainly three, ID2 (Inhibitor of DNA binding 2), IRF8 (Interferon Regulatory Factor 8) and IRF4 (Interferon Regulatory Factor 4), which seems to have a preponderant role in CD8(+) dendritic cell differentiation (Rees et al., 1990; Li et al., 2016; Wculek et al., 2020). Using single-cell RNA-seq analysis, Villani and collaborators described two novel cDC1 subpopulations, namely cDC2 and cDC3 that show both the expression of $CD11c^+$ but diverged for the expression of other molecular markers, including CD163/CD36 and CD32B (Villani et al., 2017). cDC2 are the dominant DC subset in blood but they are also localized in lymphoid and non-lymphoid tissue, which are involved in the induction of Th1, Th2, and Th17 responses (Haniffa et al., 2013; Haniffa et al., 2012; Segura et al., 2012). Moreover, recent studies in the literature suggest that cDC2 may be involved in presenting tumor-derived antigens to $CD4^+$ T cells, which assist and support $CD8^+$ T cells in their antitumor activity. Despite their role in tumorigenesis is still not well known, it emerged that they are effective stimulators of naïve T cell proliferation, required to mount an anti-tumor response (Villani et al., 2017). Besides, Binnewies and collaborators observed, in a murine model of melanoma, that the depletion of regulatory T cells into the tumoral site induced a cDC2 increase activity in eliciting intra-tumoral $CD4^+$ T cell responses with subsequent tumor growth control (Villani et al., 2017). Similarly to the mouse model, it was observed an increase of $CD4^+$ T in patients that show cDC2 abundance to the detriment of Treg suggests that the combination of high levels of cDC2 and low levels of Treg correlate with better tumor prognosis and with clinical responsiveness to immunotherapy (including anti-PD-1 therapy), though the increase of the levels of $CD4^+$ T cell infiltration (Quezada et al., 2006; Balachandran et al., 2011; Wallin et al., 2016). It will be interesting to understand how the Treg may control cDC2 function and influence the anti-tumor $CD4^+$ T cells response, both in melanoma and in other tumors (Sato et al., 2005; Binnewies et al., 2019).

Plasmacytoid DC (pDC) are characterized by the absence of CD11c and the expression of CD123 (IL-3R), CD303 (CLEC4C), and CD304 (neuropilin) markers (homologues to $CD11c^{int}$ $CD11b^-$ $B220^+$ $SiglecH^+$ $CD317^+$ in mouse) (Dzionek et al., 2000; Collin et al., 2013). They arise in two different ways, directly from LP precursors and indirectly by CDP precursors, through the MP precursor's line. The transcriptional factors that are essential for pDC development belong to the family of E2.2 expressed in both humans and mice (Liu, 2005; Cisse et al., 2008; Cheng et al., 2015). pDCs are a subset of DC specialized in response to viral RNA and DNA infection thus, for this reason, they express very high levels of TLR7 and TLR9, the two toll-like receptors specialized in signal transduction of viral and self-nucleic acids (Lande et al., 2007; Gilliet et al., 2008). The ligation of viral antigens to their TLR7 and TLR9 induces a very strong release of type I interferon (IFN-I) together with other inflammatory cytokines, including IL6 and TNFα (Patente et al., 2018). The role of pDCs in human tumors is less known compared to the other DCs subset and the data in the literature sometimes results controversial. The state of the art of pDCs asserts that they have an inert role in anti-tumor immune responses, but it also emerged that most cancers, including breast cancer (Sisirak et al., 2012), melanoma (Gerlini et al., 2007), and ovarian carcinoma (Labidi-Galy et al., 2011) are highly infiltrated by pDCs (Le Mercier et al., 2013; Kranz et al., 2016). Le Mercier et al. (2013) reported that pDCs infiltrating human primary tumors, represent an important prognostic factor associated with poor outcomes (Treilleux et al., 2004). Other studies reported that pDCs are able to limit tumoral progression, probably through IFN-α secretion (Kranz et al., 2016). Additionally, in tumor sites, it has been identified tumor-associated pDCs (TApDC) that, compared to normal pDCS, express a partially mature phenotype and an altered IFN-α, TNF-α, and IL-6 production, able to induce an

increase in Treg expansion, are associated with tumor progression with a poor overall prognosis (Hartmann et al., 2003; Treilleux et al., 2004; Labidi-Galy et al., 2011). Conversely, it was observed, *in vivo*, that an intra-tumoral injection of a TLR7 ligand led to TApDC activation displaying a potent curative effect, suggesting that TApDC could become an efficient therapeutic target (Kranz et al., 2016). Thus, several therapeutic protocols have been developed in cancers to stimulate pDC production of IFN-α. Among these, Imiquimod, a TLR7 agonist, has notably been used in cancer therapy because of its antitumoral action associated with the activation of NF-κB, which leads to the induction of proinflammatory cytokines such as IFN-α (Schon and Schon, 2008).

Monocyte-derived DCs (moDC) are a particular subset of DCs that differentiate separately from $CD14^{hi}$ monocytes in humans (homologous to $Ly6C^{+}$ in mice), in response to inflammation conditions (Jordan et al., 2020; Wculek et al., 2020). The knowledge acquired in the last years on human DCs was obtained using differentiated monocytes by *in vitro* culture with GM-CSF and IL4 (Sallusto and Lanzavecchia, 2018). The main surface marker of MoDCs overlaps with those expressed in macrophages, cDC2s and other immune cells, including CD1c, CD11b, CD14, CD209, CD172a CD1a, and CCR2 (Guilliams et al., 2014; Wculek et al., 2020). Recently, the evaluation of the Fc receptors FcγRI and FcεRI expression on moDCs allowed a better distinction between subsets, as moDCs express high levels of activating Fc receptors for IgG (FcγRs). Even if the physiological relevance of MoDC is unclear, it was noted that moDCs are produced in response to inflammation-inducing IFN-γ by $CD4^{+}$ T cells promoting Th1 immune response. Phenotypical and functional alterations in moDCs have been identified in patients with different types of cancer, including breast cancer (Ramos et al., 2012) chronic lymphocytic leukemia (Toniolo et al., 2016), chronic myeloid leukemia (Brown et al., 2014), colorectal cancer (Orsini et al., 2013) and cervical neoplasia (Lopes et al., 2017). The most observed phenotypical alteration was reduced levels of specific markers involved in antigen presentation and lymphocytes activation, including HLA-DR, CD80, CD86, and CD83. Besides, phenotypical alterations were related to loss of function in inducing proliferation of both $CD4^{+}$ and $CD8^{+}$ T cells (Toniolo et al., 2016). The peculiarity of the mo-DCs to present the antigens in both MHC class I and class II molecules have been extensively used in the clinic, mostly as vaccines to induce anti-tumor immune responses in cancer patients. In the last decade, the use of DCs is considered a hopeful adjuvant for inducing immunity to cancer and their manipulation could represent a great potential for cancer immunotherapy (Thurner et al., 1999). A series of clinical trials on cancer therapy aimed to promote DCs activation, and consequently T cell priming against tumor antigen through the administration of specific cytokines and or adjuvant, such as FLT3L, GM-CSF and/or agents blocking a series of soluble factors released by cancer cells or specific signaling pathways that contrast DCs maturation (Saito et al., 2008; Merad et al., 2013; Johnson et al., 2018; Kerdidani et al., 2019).

1.2 Tissue-Resident Macrophages in Tumor Immunity

Tissue resident macrophages (TRMs) represent an important cell component of the innate immune system, with a wide distribution in every tissue throughout the body (Epelman et al., 2014b). Despite macrophage's origin being thought to be derived from circulating blood monocytes infiltrating the tissue and differentiating into macrophages, recent literature has demonstrated that macrophage ontology is not that simple (Locati et al., 2020). Several recent pieces of evidence showed that TRMs can have embryonic progenitors, such as liver resident Kupffer cells (KCs), lung alveolar, microglia, splenic, and peritoneal macrophages (Hashimoto et al., 2013; Yona et al., 2013). Interestingly, these cells are fully differentiated before birth and self-renew in a monocytes-independent manner. However, some classes of TRMs, such as adult cardiac and skeletal muscle, derive from yolk-sac, and foetal monocytes progenitors, thus they can be substituted by blood monocytes (Epelman et al., 2014a; Wang et al., 2020). TRMs have a key role in innate immunity, as they represent the first line of defence that our body put in place upon infection with pathogens or microbes. In addition, they function by presenting antigens to T cells thus stimulating T cell response in a different types of disease conditions (Greenhalgh et al., 2018). Furthermore, macrophages maintain tissue homeostasis, by specifically contributing to the clearance of cellular debris (Herzog et al., 2019), tissue repair and remodelling (Bosurgi et al., 2017). In order to exert their functions, macrophages get activated by different stimuli coming from the tissue microenvironment *in vivo* and *in vitro* by a specific cocktail of cytokines. For a better comprehension of macrophages phenotype, Mills and collaborators classified them as classically activated TRMs (M1) and alternatively activated ones (M2) (Mills et al., 2000). While M1 macrophages release proinflammatory cytokines (TNF-α, IL-1β, IL-6, among others) and produce reactive oxygen species (ROS) to promote inflammation and defend against external pathogens, M2 ones stimulate the secretion of IL-10 and TGF-β to inhibit inflammation and to promote tissue repair and angiogenesis. However, a defined difference between these two classes of macrophages cannot be done, as their polarization can be switched according to the tissue conditions, whether it is in a steady-state or a pathological state. Thus, despite the efforts recently done to characterize M1 and M2 macrophages, a real definition of how TRMs work in physiological conditions is far from being achieved. Given their role within organs, TRMs have an important role during tumorigenesis, as they interact directly with tumor cells during progression and metastasis. Together with monocytes-derived macrophages, they represent the most abundant cells within a tumor, representing an important component of the tumor microenvironment (TME). It has been proposed that almost 50% of the tumor mass is generally represented by tumour-associated macrophages (TAMs) (Poh and Ernst, 2018). Thus, TAMs can have then different origins, although they all exert similar functions even if with different molecular mechanisms. For instance, it has been shown in transgenic mouse models of lung adenocarcinoma that while

monocyte-derived macrophages stimulate tumor dissemination, TRMs are sufficient to induce tumor growth (Loyher et al., 2018). Similar results were obtained in TRMs-depleted transgenic mouse model ($Csf1^{op/op}$), showing that alveolar macrophages depletion did not affect the dissemination of mammary tumor cells (Qian et al., 2009). However, this is not the case of pancreatic ductal adenocarcinoma (PDAC), where the pro-tumoral role of TRMs and monocyte-derived macrophages seems to be the exact opposite (Zhu et al., 2017c). These observations clearly suggest that the contribution of each macrophage population to tumor progression is strictly correlated with the tissue they reside. On the same lines of evidence, even the response to chemotherapy is different between TRMs and monocyte-derived macrophages, as it might change according to the type of tumor. Indeed, Loyher et al. (2018) elegantly demonstrated that monocyte-derived macrophages recovered faster from cyclophosphamide treatment when compared with TRMs in a transgenic mouse model of lung cancer. TAMs, either monocyte-derived macrophages or TRMs, are associated with tumor progression and poor prognosis, because of their role in controlling tumor survival and resistance to conventional therapies. In particular, different studies support the hypothesis that tumor-promoting macrophages have a M2-like phenotype (Mantovani et al., 2002; Boutilier and Elsawa, 2021), while the M1-like one is associated with anti-tumor properties (Noy and Pollard, 2014; Ubil et al., 2018; Wanderley et al., 2018). However, as discussed above for macrophages during homeostasis, macrophage polarization is influenced by cues and stimuli by the microenvironment (Sica and Mantovani, 2012; Najafi et al., 2019; Gunassekaran et al., 2021). For instance, different studies showed that TGF-β increased in hepatocellular carcinomas the expression of TIM-3, an immune checkpoint blockade inhibitor, thus leading to 1) M1 to M2 macrophages polarization, and 2) increased tumor progression and metastatization (Yan et al., 2015). This is a very important aspect, as the induction of an M1-like polarization could be an important therapeutic strategy to generate tumor-suppressive macrophages. TAMs have a pivotal role in defining therapeutic efficiency (Zitvogel et al., 2008; Hughes et al., 2015). For instance, they inhibit T cell response *via* inhibition of cytotoxic $CD8^+$ T cells, either by expressing inhibitory immune checkpoint molecules (PD-L1 and PD-L2), blocking antigen presentation or by the secretion of immunosuppressive proteins, such as IL-10, TGF-β, and prostaglandin E_2 (Blumenthal et al., 2001; Munn and Mellor, 2003; Matlack et al., 2006). In addition, they modulate T cell exclusion from the tumor by the activation of MMPs and cathepsins, and by stimulation of fibrotic mechanisms (Nielsen et al., 2016; Zhu et al., 2017c; Quaranta et al., 2018). Interestingly, it has been shown that when TAMs are depleted, cytotoxic $CD8^+$ T cells increased their presence in the tumor context, thus improving the therapeutic outcome of the treatments (Denardo et al., 2009; Andreu et al., 2010). Along with inhibition of T cell response, TAMs are also responsible for chemotherapy and radiotherapy resistance and tumor relapse. Recent works demonstrated that standard care of treatment for several tumors determine a release of bioactive factors, such as VCAM1 and CCL2, that are involved in the increased macrophages infiltration in the tumor microenvironment (Kalbasi et al., 2017; Takahashi et al., 2020). In addition, *in vivo* studies on the prostate cancer model clearly showed that macrophages depletion further improve the docetaxel chemotherapy response by reducing tumor progression (Guan et al., 2019). Furthermore, macrophages induce resistance to chemotherapy by suppressing cancer cell apoptosis *via* release of soluble factors (colon and ovarian cancer model) (Feng et al., 2011; Au Yeung et al., 2016), or by exosomal delivery of miRNA-21 (gastric cancer cells) (Zheng et al., 2017). Finally, TAMs reduce immune checkpoint blockade therapy (ICB) *via* the expression of inhibitory immune checkpoint molecules (PD-L1, PD-L2, and TIM-3), thus blocking T cell response (Thommen et al., 2015; Anfray et al., 2019). For all these reasons, TAMs have been considered an important target in tumor immunity, although preclinical studies, as well as clinical trials, did not define in the past a proper clear-cut on possible therapies aiming at TAMs eradication. Thus, different approaches are currently understudies to target macrophages for anti-cancer therapies. Among others, therapies to deplete macrophages, inhibit monocyte-derived macrophages recruitment, and stimulate TAMs repolarization towards an M1 phenotype are currently under investigation in a preclinical stage, as well as in clinical trials. Macrophages depletion *via* CSF-1R blockade as monotherapy to affect tumor growth, despite the encouraging preliminary data in preclinical studies (Strachan et al., 2013; Quail et al., 2016), did not provide substantial benefits for the treatment of established solid tumors (O'Brien et al., 2021). On the contrary, a combination of tumor resection followed by macrophage depletion did provide a valuable reduction of melanoma recurrence and metastasis (Tham et al., 2015). Thus, combined therapies, along with the identification of the right timing of the treatment itself, might be the path to contrast tumor progression. Different approaches have been described to deplete macrophages, either in normal or tumoral tissues. For instance, liposomes loaded with clodronate have been shown to reduce tumor growth in mouse models of mammary cancer. In addition, its combination with protein kinase inhibitors, such as sorafenib, was able to drastically diminish tumor angiogenesis and metastasis in a hepatocellular carcinoma model (Zhang et al., 2010). Interestingly, different groups successfully attempted to eradicate TAMs using trabectedin, a chemotherapic used for the treatment of ovarian cancer and sarcomas. Trabectedin acts by stimulating macrophages apoptosis *via* activation of TRAIL-R2, a death receptor specifically expressed in macrophages (Allavena et al., 2005; Germano et al., 2013; Gordon et al., 2016). Thus, trabectedin is currently under analysis in combined therapies in several clinical trials. TAMs depletion can be obtained also by inhibiting CSF1/CSF-1R signaling axis, *via* monoclonal antibodies or small molecule inhibitors. In addition, targeting macrophages' surface receptors (CD52, CD206, FR-β, among others) is another approach that has been recently taken for the same purpose. In all cases, these attempts result quite encouraging in the preclinical set, and some of them are now in clinical trials in combined chemotherapies for the treatment of lymphomas and chronic lymphocytic leukemia (NCT00069238, NCT01361711, and NCT01030900). Blocking monocyte-derived

macrophages recruitment in the tumor microenvironment is another approach that has been tried to target TAMs. This type of therapy mostly relies on monoclonal antibodies aiming at the inhibition of the interaction between monocyte chemokines and their specific receptors. The most studied signaling axis in this context has been the CCL2/CCR2, as CCR2 is highly expressed in tumors and have been shown to stimulate macrophages recruitment in the tumor microenvironment (Lim et al., 2016). Also, in this case though, while the pre-clinical studies were encouraging, the clinic ones did not provide important results for the treatment of some type of prostate cancers (Pienta et al., 2013). However, the combination with anti-PD-1 immune checkpoint blockade therapy demonstrated quite efficiency for metastasis inhibition of mouse models of bladder and lung cancer (Tu et al., 2020).

Finally, TAMs repolarization towards an M1-phenotype is another valid strategy that has been developed in recent years. Macrophage re-polarization has been obtained with different methods, including treatments with TLR agonists (van Dalen et al., 2018), CSF-1R (Pyonteck et al., 2013) and PI3Kγ (Kaneda et al., 2016) inhibition. TLR agonists, including LPS and several lipoproteins, determine the activation of the NFkB signaling pathway, thus stimulating the production of pro-inflammatory cytokines typical of the M1 phenotype. For instance, Poly:IC, a synthetic molecule mimicking viral dsRNA, binds to TLR3 and induces macrophages polarization and colon cancer arrest. However, TLR agonists result cytotoxic for use in anti-cancer therapies, thus stimulating alternative methods for their *in-situ* delivery. Interestingly, TLR7/8 agonists loaded into nanoparticles induced *in vitro* and *in vivo* polarization of M1-like macrophages in different models of solid tumors, including lung and colon adenocarcinoma (Rodell et al., 2018). CSF-1/CSF-1R axis inhibition, despite being firstly considered a valid strategy for TAM depletion, is now a well-accepted method to repolarize macrophages. An elegant work published in 2013 clearly demonstrated in a glioblastoma multiforme (GBM) tumor model that CSF-1 blockade did not eradicate TAMs, but instead "re-educate" them within the tumor microenvironment by decreasing M2 macrophage gene signature, and at the same time promoting overall survival in patients with GBM (Pyonteck et al., 2013). Furthermore, CSF-1 blockade was found to stimulate TAMs polarization and improved animal survival in mouse models of hepatocellular carcinoma and PDAC (Zhu et al., 2014; Ao et al., 2017). Finally, PI3Kγ selective small molecules inhibitors nicely demonstrated their effectiveness in polarizing TAMs in mouse models of PDAC, thus promoting $CD8^+$ T cell infiltration and tumor arrest.

1.3 Tissue-Resident Myeloid-Derived Suppressor Cells in Tumor Immunity

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous group of immature myeloid cells, derived from the bone marrow hematopoietic precursor cells, which constitute one of the main suppressive cell populations of the innate immune system (Veglia et al., 2018). In physiological conditions, immature myeloid cells differentiate into the different innate immune cells, such as macrophages, granulocytes, and dendritic cells, and migrate to the corresponding tissues, exerting their normal immune functions (Groth et al., 2019). In pathological conditions, such as infectious diseases, cancers or autoimmune disorders, deregulation on myeloid differentiation occurs, which, combined with a persistent stimulation of myelopoiesis, results in the expansion of MDSCs (Consonni et al., 2019; Lim et al., 2020). This deregulated generation and expansion of immunosuppressive MDSCs is promoted by a series of cytokines, such as GM-CSF, VEGF, IL-1β, IL-6, and IL-10 (Cheng et al., 2021). Significantly increased immature myeloid cells have been observed in the bone marrow and peripheral blood of patients with cancer (Cheng et al., 2021), and the presence of enriched MDSCs have been related to poor prognosis for multiple types of cancer (Jiang et al., 2015; Tian et al., 2015; De Cicco et al., 2020). In fact, throughout the entire pathological process that leads to tumor formation, MDSCs increase up to10-fold and migrate to the periphery, exerting their suppressor activity interfering with the normal functions of circulating T and other immune cells involved in the anti-tumor immunity (Safarzadeh et al., 2019; Nakamura and Smyth, 2020; Ma et al., 2022). Unlike mice's MDSCs, where these cells have been well characterized, human MDSCs are less clearly defined. Typically, they are described as lineage cells that co-express high levels of CD33 and CD11b surface markers but lack HLA-DR. Human $CD33^+CD11b^+HLA\text{-}DR^-$ MDSCs can also be subdivided in three distinct populations of $CD14^+CD15^-$ monocytic-MDSCs (M-MDSCs), $CD15^+CD66b^+CD14^-$ granulocytic-MDSCs (G-MDSCs) and $CD14^-CD15^-$ early-MDSCs (E-MDSCs), which comprised more immature progenitors (Gabrilovich and Nagaraj, 2009; Mandruzzato et al., 2016; Ma et al., 2022) myeloid markers, such as PD-L1, CD40, CD49d, CD80, CD115, and CD124, which all mediate immunosuppression, has also been discovered to describe specific patterns of MDSCs (Gabrilovich and Nagaraj, 2009; Mandruzzato et al., 2016; Ma et al., 2022). Two functional proteins, such as CCAAT/enhancer-binding protein (c/EBPβ) and STAT3, which promote generation, differentiation, and expansion of MDSCs, despite are not surface markers, could help to define two different MDSCs subgroups ($CD11b^+HLA\text{-}DR^-c/EBP\beta^+$ and $CD33^+HLA\text{-}DR^-STAT3^+$) and could provide new diagnostic and therapeutic tools for cancer immunotherapy (Lechner et al., 2011; Wu et al., 2011; Wang et al., 2019a). MDSCs have a key role in accelerating the progression of cancer, by producing a broad range of suppressive factors that prevent immune cells' anti-tumor reactivity (Ma et al., 2022). The main mechanisms by which MDSCs act as immunosuppressive cells are oxidative stress, amino acid consumption, cytokines secretion, cell-cell interaction and exosomes release. MDSCs mediate immunosuppressive effects under oxidative stress, producing reactive oxygen species (ROS), nitric oxide (NO), and reactive nitrogen species (RNS). Increased ROS production induces damages on adaptive immune response by interfering with TCR CD3ζ expression, which acts on IFN-γ expression, hampering activation, viability and proliferation of T cells (Belikov et al., 2015; Ohl and Tenbrock, 2018; Cheng et al., 2021). MDSCs express nitrogen-oxygen synthase 2 (iNOS). The

upregulated expression of iNOS leads to NO production that suppresses T cell's function by inhibiting JAK3/STAT5 activation (Bingisser et al., 1998) and decreasing MHC class II expression (Harari and Liao, 2004). NO also induce T and NK cell apoptosis in tumor microenvironment through different mechanisms, such as impaired expression of the Bcl-2 family proteins, increased expression of the p53 tumor suppressor protein, damage of mitochondrial functions, DNA fragmentation, and activation of the caspase cascade (Umansky and Schirrmacher, 2001). Additionally, in tumor cell aggregation sites enriched with MDSCs, NO reacts with superoxide forming the RNS, namely peroxynitrite, a strong nitrifying agent. It can nitrate tyrosine residues in T cell receptor, inducing reduced IL-2 production with a consequent impaired T cell activation and proliferation (Bentz et al., 2000; Cobbs et al., 2003; Szabo et al., 2007; Gabrilovich et al., 2012; Cheng et al., 2021) (Feng et al., 2018). Besides, peroxynitrite can modify TCR conformational flexibility affecting its interaction with MHC class I molecules, causing the decreased response of cytotoxic CD8 T cells to antigen-specific stimulation (Nagaraj et al., 2007). Additionally, peroxynitrite prevents antitumor infiltration of antigen-specific CD8 T cells by nitration of CCL2 chemokine (Molon et al., 2011). The second MDSCs' immunosuppressive mechanism consists in the exhaustion of some amino acids with a key role in T cell functioning. The high production of arginase-1 (Arg-1) by MDSCs causes L-arginine deficiency in the tumor microenvironment, which either provokes the cell cycle arrest to the G0–G1 phase of T cells (Rodriguez et al., 2007) and the downregulation of TCR expression, inducing T cell dysfunction and tumor escape *in vivo* (Rodriguez et al., 2002). Another essential amino acid for T cell activation is cysteine (Levring et al., 2015). Dendritic cells (DC) and macrophages can import extracellular cysteine and export it in the tumor microenvironment, making this amino acid available for T cell utilization. MDSCs competitively import extracellular cysteine, but, contrary to DC and macrophages, MDSCs are not able to export cysteine, due to the lacked expression of cysteine transporters, preventing T cell activation (Srivastava et al., 2010). The MDSCs overexpression of indoleamine 2, 3-dioxygenase (IDO), an enzyme that, metabolizing tryptophan, has been referred to support immunosuppressive properties of these cells. Tryptophan depletion causes T cell proliferation arrest (Srivastava et al., 2010) and antigen presentation impairment (Fallarino et al., 2006). Besides, highly IDO levels produced by MDSCs promotes the differentiation and expansion of T regulatory (Treg) cells, exacerbating the inhibition of anti-tumor T cells function (Curti et al., 2007). In response to tumor microenvironment, MDSCs acquire the ability to produce multiple immunosuppressive molecules, such as cytokines, chemokines, and growth factors (Lechner et al., 2010). The tumor microenvironment is characterized by high levels of IL-10 and MDSCs are the principal producers of this cytokine (Cheng et al., 2021) IL-10, in turn, strengthens the immunosuppressive ability of MDSCs in a vicious cycle, by upregulating the expression of different immunosuppressive molecules (Xiu et al., 2015; Lamichhane et al., 2017). IL-10 produced by MDSCs induce increased expression of lymphocyte activation gene 3 (LAG3) and the consequent decreased IL-2, IL-12, and IFNγ secretion by T cells, which hampered their proliferation and anti-tumor activity (Vuk-Pavlovic et al., 2010; Li et al., 2015). As IL-10, increased TGFβ production by MDSCs has been reported in various tumor types. TGFβ is a potent immune regulator cytokine that can inhibit proliferation, activation, differentiation, and cytotoxic activity of effector T cells (Cheng et al., 2021). This cytokine blocked Th1 differentiation and activation by silencing the expression of TBET and STAT4, which are key transcription factors for the formation of this important subset of anti-tumor T cells (Gorelik et al., 2002). Moreover, acting on Smad3 signaling, TGFβ could decrease IL-2 production (McKarns et al., 2004) and downregulated the expression of granzyme B and IFNγ (Zhang and Bevan, 2012). Highly production of IL-10 and TGFβ by MDSCs can also lead to the differentiation and expansion of Treg cells, by inducing FoxP3 and CD25 expression on naïve CD4 T cells (Fu et al., 2004; Heo et al., 2010). Besides, MDSCs can produce high levels of some chemokines, such as CCL3, CCL4, and CCL5, which drive CCR5-expressing Treg cells through the tumor microenvironment, supporting the tumor growth (Schlecker et al., 2012). MDSCs could also impair T cell trafficking in tumor-bearing hosts. ADAM17 (a disintegrin and metalloproteinase domain 17) expressed on MDSCs directly cleaves the ectodomain of L-Selectin on T cells to inhibit their homing to tumor sites and peripheral lymph nodes (Li et al., 2021). Other than suppressing anti-tumor immunity, MDSCs can directly promote tumor progression and metastasis by inducing stemness of tumor cells, angiogenesis, and degradation of extracellular matrix (ECM). Many immunosuppressive factors, such as IL-10, TGFβ, and IL-6 produced by MDSCs, are able to induce stem cell properties in various tumor cells (Schlegel et al., 2015; Zhu et al., 2017a; Yang et al., 2019). MDSCs produce high levels of VEGF (Shojaei et al., 2009), the most important cytokine involved in angiogenesis, which binding its receptor (VEGFR) on epithelial cells, promoting neo-angiogenesis by activating JAK2/STAT3 pathway. MDSCs also express high levels of VEGFR2, which, activated by VEGF secreted either by tumor cells or by themselves, lead to a vicious cycle that contributes to maintaining MDSCs angiogenic activity (Min et al., 2017). Besides, MDSCs produce matrix metalloproteinases (MMPs) that, degrading the ECM, contribute to tumor metastasis (Zhang et al., 2020). Another mechanism provided by MDSCs to suppress immune response is through cell-to-cell contact. MDSCs constitutively express on their surface molecules involved in the suppression of immune cells. Among these molecules, Fas ligand (Fas-L) is highly expressed by tumor-infiltrating MDSCs and can induce apoptosis of CD8 cytotoxic T cells by activating Fas-Fas-L axis, with a consequent local immune suppression as demonstrated in mice models (Zhu et al., 2017b; Rashid et al., 2021). Besides, MDSCs in tumor microenvironment bear high levels of ligands of negative immune checkpoint regulators, such as PD-L1 and Galectin-9, which respectively binding PD-1 and TIM3, inducing T cell anergy (Cheng et al., 2021). Moreover, it has been reported that MDSCs are able to induce decreased cytotoxicity, reduced IFNγ production and downregulated

expression of NKG2D of NK cells, due to membrane-bound TGFβ in a cell-cell contact mode (Li et al., 2009). Additionally, through the cell-to-cell transfer of the metabolite methylglyoxal, MDSCs could paralyze T cells, reducing their anti-tumor activity (Baumann et al., 2020). Finally, MDSCs can also exert their immunosuppressive function by releasing exosomes. Similarly, to parental MDSCs, exosomes secreted from MDSCs contain pro-tumorigenic factors and can play a crucial role in immunosuppression, tumor growth, angiogenesis, invasion, and metastasis by distributing their contents into the tumor milieu. It has been demonstrated that MDSCs-derived exosomes contain matrix metalloproteinases (MMPs) and different cytokines, chemokines, and growth factors (CSF, VEGF, MCP, SDF1α, TNFα, and IFNγ), which establish a pro-metastatic microenvironment that allows the metastatic progression of tumor cells (Umansky et al., 2016). Moreover, MDSCs-derived exosomes can induce exhaustion and apoptosis of CD8 T cells by either increasing ROS production or inducing the activation of Fas/Fas-ligand pathway (Rashid et al., 2021). Additionally, MDSCs-derived exosomes bearing the membrane-bound PD-L1, could induce the transformation of naïve B cells into B regulatory cells, recently identified as an immunosuppressive cell population (Rosser and Mauri, 2015), thus inhibiting antitumor immune response (Lee-Chang et al., 2019). Finally, some microRNA contained in MDSCs-derived exosomes, such as miR-126a and miR9, promotes tumor angiogenesis by reprogramming endothelial cells (Baroni et al., 2016; Deng et al., 2017). The deep knowledge of the mechanisms by which MDSCs exert their powerful immunosuppressive functions and pro-tumoral activity could help to develop new effective immunotherapeutic strategies for the treatment of tumors or could intensify the effectiveness of tumor treatments already used. For these reasons, several clinical trials targeting MDSCs and their products are ongoing. Among these, the use of monoclonal antibodies (mAb) against immune checkpoints inhibitors seems to improve cancer patients' outcomes, in combination with other anti-cancer therapies. Ipilimumab is a fully humanized mAb that acts blocking CTLA-4. Ipilimumab, alone or in combination with other anti-tumoral treatments, could potentiate the anti-tumor T cell response and could lower the frequency of MDSCs in tumor microenvironment, ameliorating the outcome of patients with different kinds of solid tumors (Hodi et al., 2010; Sade-Feldman et al., 2016; Tobin et al., 2018). Pembrolizumab, a PD-1 blocking mAb, alone or in combination with BL-8040, a CXCR4 antagonist, was approved to treat unresectable or metastatic solid tumors, due to its ability to reduce MDSCs number and increase effector T cell tumor infiltration (Redman et al., 2016; Vachhani and Chen, 2016; Syn et al., 2017; Bockorny et al., 2020). Another target of immunotherapies is the blockade of MDSCs' recruitment into the tumor microenvironment, by the antagonist of some chemokine receptors highly expressed on these cells. A CCR2 antagonist, namely 747, displayed anti-cancer properties and potentiate the efficacy of sorafenib in a model of hepatocellular carcinoma (Yao et al., 2017). Other chemokine receptors antagonists, such as reparixin (anti-CXCR1/2), LY2510924 and ulocuplumab (anti-CXCR4), in association with chemotherapics agents, have shown significant results in the treatment of different solid tumors (Galsky et al., 2014; Schott et al., 2017; Ghobrial et al., 2020). Another important strategy for cancer treatment is the inhibition of MDSC activation, by inducing the transition of immature MDSCs in mature myeloid cells. All-trans retinoic acid (ATRA) and the active form of vitamin D have been recognized as ideal inducers of MDSCs differentiation and have been used for different types of both hematopoietic and solid tumors (Iclozan et al., 2013; Tobin et al., 2018; Fleet et al., 2020; Makitie et al., 2020). More recently, the use of CSF-1R inhibitors, such as GW-2580, Imatinib, and pexidartinib, due to their ability to inhibit the expansion of MDSCs, have revealed important results in different solid and hematopoietic cancer treatments (Giallongo et al., 2018; Edwards et al., 2019; Wesolowski et al., 2019). Finally, Arg-1 and iNOS, critical factors in MDSC-mediated immunosuppression, are the targets of inhibitor agents (INCB001158 for Arg-1 and L-NMMA for iNOS), which, in combination with immunotherapy or chemotherapy, have shown good results in some solid tumors treatment (Cheng et al., 2021; Chung et al., 2021).

1.4 Tissue-Resident Natural Killer and Natural Killer T Cells in Tumor Immunity

Natural Killer (NK) and Natural Killer T (NKT) cells are lymphocytes of the innate immune system playing pivotal roles in immune surveillance and response against virus-infected and tumor cells. NK and NKT cells share some common phenotypes and function such as the secretion of interleukin-2 (IL-2), interferon-γ (IFN-γ) and tumor necrosis factor α (TNFα) upon interaction with the ligand or antigen (Shimizu et al., 2020). However, they express distinct lineage development, tissue distribution, antigen recognition and regulatory mechanisms in health and cancer (Vivier et al., 2012).

1.4.1 Natural Killer Cells

Human NK cells are commonly divided into two subsets: immune-regulatory cytokine-responsive $CD56^{bright}CD16^{-}$ and cytotoxic $CD56^{dim}CD16^{+}$ with potent IFN-γ, TNF-α, and GM-CSF secretion activity upon stimulation (Cooper et al., 2001). The diatribe of whether $CD56^{dim}$ are a mature form of $CD56^{bright}$ (Romagnani et al., 2007) or a distinct subpopulation originating from a separate lineage is still a matter of debate (Cichocki et al., 2019).

NK cells infiltrate has been found in several types of cancers (Mamessier et al., 2011; Ali et al., 2014). Interestingly $CD56^{bright}$ more efficiently traffic to the TME as they respond to the chemokines produced within the tumor bed. For example, IFN-γ stimulates tumor-infiltrating immune cells to release CXCL9-11, which is known to recruit $CD56^{bright}$ NK cells (Wendel et al., 2008). By contrast, highly cytotoxic $CD56^{dim}$ usually express receptors for chemokines produced at low levels and their traffic within the tumor site is often insufficient (Berahovich et al., 2006). NK cells vary expression of chemokine receptors following cytokine stimulation and therefore the composition of the NK cells in the TME changes accordingly (Pachynski et al., 2012). For example, $CD56^{bright}$ NK

cells respond to IL-15 by upregulating the expression of the chemokine receptor CCR5 and induce migration to the TME. Conversely, the same cytokine inhibits infiltration of cytotoxic $CD56^{dim}$ cells by dampening expression of CXCR4 and CX3CR1 receptors (Sechler et al., 2004). In patients with advanced melanoma, an inverse correlation between the abundance of circulating $CD56^{bright}$ NK cells and patients' survival was found, pointing out a role of these cells in the modulation of cancer response (de Jonge et al., 2019). Likewise, the proportion of $CD56^{bright}$ cells and production of IFNγ have been reported to be significantly lower in patients with prostate cancer than in controls (Koo et al., 2013).

Recently, by single-cell profiling a population of $CD56^{bright}CD127^{+}CD160^{-}CD52^{+}$ cells, NK0 was identified from human bone marrow and represent the precursors of conventional NK2/$CD56^{bright}CD160^{+}CD52^{-}$ cells and NK1/$CD56^{dim}Perforin^{high}$ cells (Crinier et al., 2021). In patients with acute myeloid leukemia, transcriptomic analysis of the bone marrow revealed inhibition of the NK cell effector function and, importantly, patients with a good prognosis exhibited increased levels in the population of $CD160^{+}$ NK cells (Crinier et al., 2021). Differently to T cells, NK cells recognize their target with a mechanism known as "missing self," where the specificity for a given antigen is dispensable. NK cell function is modulated by a dynamic balance of activating and inhibitory signals and adhesion receptors in response to "altered self" cells such as tumor cells. When the inhibitory receptors do not recognize their target *via* the interaction with "self-identifier" (HLA) molecules, NK cells kill their target (Elliott and Yokoyama, 2011). NK inhibitory receptors are killer cell Ig-like receptors (KIRs) which engage with HLA types A, B or C (Harel-Bellan et al., 1986; Marsh et al., 2003), and NKG2A which binds to the highly conserved HLA-E (Sivori et al., 1996; Islam et al., 2021). More lately, other inhibitory molecules have been added to the list such as CD161, KLRG1, SIGLEC7, SIGLEC9, PD-1, TIGIT, LAG3, and TIM3 (Cozar et al., 2021). Conversely, activation receptors include CD16, NKp30, NKp44, NKp46, NKG2D, NKG2C, and activating KIRs. NK-cell activity is also enhanced by co-receptors like DNAX accessory molecule 1 (DNAM1) (Sanchez-Correa et al., 2012), NKp80 and 2B4 (Freud et al., 2017). For many years, it has been believed that NK cells uniformly recirculate. However, alongside conventional NK (cNK) cells, NK cells resident in the peripheral tissues, termed tissue-resident NK (trNK) cells have been reported in liver, kidney, skin uterus, salivary glands, and adipose tissue (Sojka et al., 2014; Fan and Rudensky, 2016; Sun et al., 2019). These tissue-resident lymphocytes do not recirculate in the blood or lymphatic system and have a distinct phenotype, like for example, the tissue-resident $EOMES^{-}T\text{-}bet^{+}CD49a^{+}$ NK cells in the human liver (Sun et al., 2019). NK cells present immune-regulatory functions as they promote the activation of other innate and adaptive immune cells both by releasing cytokines and chemokines, or through direct cell–cell contact (Vivier et al., 2011). NK cells shape adaptive immune responses through the cross-talk with other cells such as T cells, B cells, and dendritic cells (DCs) (Malmberg et al., 2017). By inducing maturation of DC, NK cells trigger T cells mediated response; by producing IFN-γ, NK cells promote Th1 polarization (Moretta et al., 2008). Additionally, it has been proposed that NK cells can increase the function of cytotoxic $CD8^{+}$ T cells by suppressing their state of exhaustion (Zheng et al., 2016) and, importantly, NK cell play a fundamental role in checkpoint blockade therapy by exacerbating antitumor or antiviral function of $CD8^{+}$ T cells (Zhang et al., 2018). NK cells rapidly kill newly arising tumors or metastases, but their anti-tumor potency is less efficient against established solid formations. This is the result of the many strategies developed by tumors to escape immune surveillance, accompanied by a scarce capacity of NK cell to infiltrate the tumor site, as they tend to accumulate at the margins (Platonova et al., 2011). The tumor microenvironment (TME) is hostile to immune cells. NK cell effector function is limited by mechanisms of defense such as hypoxia, where tumor cells release abundant H2O2, thus limiting infiltration of $CD56^{bright}$ NK cells (Izawa et al., 2011; Terrén et al., 2019). Additionally, excessive production of the metabolic enzyme Indoleamine 2, 3-dioxygenase 1 (IDO1) causes immunosuppression and NK and T-cell (Pietra et al., 2012). Likewise, Prostaglandin-E2 (PGE2) produced by cancer cells decreases NK-cell cytotoxicity (Galland et al., 2017). Additionally, tumor cells directly inhibit the expression of NK cell markers such as NKp30, NKp44, NKp46, and NKG2D by releasing soluble factors such as TGFβ (Lee et al., 2004; Sconocchia et al., 2012; Close et al., 2020). NK cells show impaired cytotoxic function hampered by cancer cells and upregulation of inhibitory receptors like NKG2A (Mamessier et al., 2011). The TME is also populated by different cell types that orchestrate suppression of the anti-tumor immune response. These include stromal cells, regulatory T cells (Treg), fibroblasts and myeloid-derived suppressor cells (MDSC). Inhibition of $CD8^{+}$ T and NK cells is exerted by cell-to-cell contact or *via* release of TGFβ and IL10 or production of nitric oxide (Ghiringhelli et al., 2005). The TME negatively influences NK cell immune response. The phenotype, metabolism and function of intra-tumor NK cells dynamically change during the different stages of tumor occurrence and progression (Liu et al., 2021). In the early stages of breast cancer development, NK cells have cytotoxic functions, which are lost at a later phase, thus promoting tumor progression. NK cells have impaired functions and are exhausted in advanced cancers and are characterized by increased glucose and lipid metabolism (Liu et al., 2021). Downregulation of cytotoxicity genes was reported in biopsies of chemotherapy-resistant breast cancer samples by microarray expression assay (Garcia-Chagollan et al., 2018). In gastric cancer, the overall phenotype of NK cells did not differ between the population of circulating and infiltrating NK cells. What was reported, however, was impaired function in tumor infiltrating NK cells, with reduced IFNγ, TNFα, and proliferation (Peng et al., 2017). Likewise, in HCC tissues, a population of tumor infiltrating $CD11b^{-}CD27^{-}$ double negative NK cells was found to be characterized by poor effector functions and cytokine release (Zhang et al., 2017). In the TME, prolonged exposure to NKG2D ligands is associated with a reduction NKG2D expression, which causes NK cell function impairment and evasion of immune surveillance (Thompson et al., 2017). For example, in ovarian cancer, a reduction of the membrane-bound

MICA/B proteins has been underpinned as an immune escape strategy adopted by tumor cells. MICA/B molecules bind to the receptor NKG2D which is expressed by NK cells, but also $\gamma\delta^+$ and $CD8^+$ T cells (Xie et al., 2014). NKG2D engagement corresponds to the inhibition of NK cell cytotoxic function and tumor progression (Xie et al., 2014). The metalloproteinases ADAM 10 and 17 are expressed by tumor cells and are implicated in immune surveillance escape. These mediate the shedding of B7-H6, ligand of NKp30. Increases of the B7-H6 in its soluble form determine drop in NKp30 expression and loss of NK effector function in different cancers (Schlecker et al., 2014; Pesce et al., 2015; Semeraro et al., 2015; Mantovani et al., 2019). PD-1 is an exhaustion marker on both T cells and NK cells (Liu et al., 2017b; Pesce et al., 2017). Importantly, NK mediated tumor response can be restored by blocking the immune checkpoint PD-1/PD-L1 axes (Liu et al., 2017b). Additionally to PD1, co-expression of a second exhaustion marker like TIM-3 has been associated to impaired NK cell function in many cancers, with reduced release of Granzyme B and IFN-γ and inhibited cytotoxicity (Seo et al., 2017). An interesting immune checkpoint is represented by the inhibitory receptor NKG2A. Its ligand, HLA-E is expressed by many different tumor cells (Zhen et al., 2013; Sun et al., 2017). Blockade of NKG2A/HLA-E axes with the antibody Monalizumab, for example, has proofed to unlock both NK cell and T cells function and corroborate anti-tumor immunity (André et al., 2018). Several studies have tried to unravel the role of NK cells in tumor immunity highlighting discrepancies mainly based on the type of tumor (Rezaeifard et al., 2021). Divergent results on the rate of NK cells infiltration in solid tumors have highlighted the urge to find reliable markers to address this question. The NK Cells Receptor (NCR) NKp46 has been recently identified as a robust biomarker to quantify tumor-infiltrating NK cells (Cózar et al., 2021). Although NK cells seem to reach the tumor bed to a lesser extent than other lymphocytes such as $CD4^+$ and $CD8^+$ T cells, their presence within the tumor correlates with a higher survival rate, as reported in head and neck squamous cell carcinoma (Weil et al., 2017; Concha-Benavente et al., 2018), colorectal cancer (Sconocchia et al., 2014), prostate tumor (Izawa et al., 2011) in gastric and esophageal cancers (Lorenzo-Herrero et al., 2019) and metastatic melanoma (Cursons et al., 2019b). Patients with metastatic skin melanoma showed better survival rates if infiltrating NK cells were detected in the tumor biopsies (Cursons et al., 2019a). Moreover, increased numbers of NK cells were correlated with a better response to anti–PD-1 immunotherapy and with an accumulation of pro-active DCs with a protective anti-cancer role at the tumor site (Barry et al., 2018). By contrast, the opposite has also been reported with inverse correlation between the advance stages of cancer and NK cells infiltrating the tumor (Vgenopoulou et al., 2003) or accumulating into the lymph nodes draining the tumor (Rezaeifard et al., 2019). Recently, a subset of $CD49a^+Eomes^+$ NK cells with known proangiogenic function has been described to accumulate at the site of liver tumor. This population has impaired cytotoxic function and reduced TNF-α release, strongly suggesting a pro-oncogenic activity in HCC (Zecca et al., 2021). NK cell infiltrate has been described in metastases, solid tumors and lymph nodes draining the tumor (Gulubova et al., 2009; Ali et al., 2014; Carrega et al., 2014). However, the extent of NK cell infiltration in the tumor greatly depends on the nature of the tumor and its localization. Some organs are more easily reached by NK cells such as the lungs, liver and kidney, while the intestinal tract appear to be less permissive (Remark et al., 2013). That said, high infiltration rate does not solely correlate with good prognostic factors. Expression of specific activating markers on NK cell surface represent a biomarker for more accurate prognosis (Schleypen et al., 2006), as described for the HLA-E receptor NKG2A and the activating marker NKG2C in endometrial cancer (Versluis et al., 2017). Tumor infiltrating NK and T cells represent a therapeutic target to tackle tumor. NK cells express the marker CD161, regulated by the gene KLRB1. CD161 expression orchestrates NK cell cytotoxic function in several cancers (Cheng et al., 2022). A good prognosis rate is observed in those cancer patients where KLRB1 is highly upregulated as infiltration of immune cells at tumor site and sensitivity to chemotherapy is KLRB1-dependent in many cancer types (Cheng et al., 2022). Nectar Therapeutics is developing an engineered IL-2 cytokine for the treatment of solid tumors. NKTR-214 is designed to sustain growth and survival of specific cancer-killing T cells and NK ells that specifically recognize a tumor target by targeting tumor infiltrating cells lymphocytes by binding to the CD122 receptor expressed by effector $CD8^+$ T cells and NK cells (Bentebibel et al., 2019). However, it has also been reported that this effect is still partial, since a portion of the patients enrolled in the study displayed a proliferation of T_{reg} cells (Sullivan, 2019).

The efficacy of this cytokine has been tested in clinical trial also in combination with checkpoint inhibitor anti-PD1 demonstrating efficacy and safety in the treatment of solid tumor like melanoma, renal cell carcinoma, and non-small cell lung cancer (Diab et al., 2020). In Soft Tissue Sarcoma (STS), compared to circulating cells, intra-tumoral NK and T cells have upregulated TIGIT, a marker of exhaustion. TIGIT+ lymphocytes are considered prognostic in STS and recently it has been proposed that TIGIT blockade may be a promising clinical strategy in STS (Judge et al., 2020). Similarly, in colon tumor biopsies, tumor-infiltrating NK cells have increased levels of TIGIT expression than circulating NK, suggesting an exhausted phenotype (Zhang et al., 2018). Finally, CD155 is the ligand of both activating receptor DNAM1 and inhibitory ligand TIGIT and is expressed in many types of cancer. DNAM1 engagement with CD155 is associated to inhibition of NK cell function (Nakai et al., 2010; Li et al., 2020). NK cells participate to response to tumors and represent potential targets for cancer immunotherapy. However, the TME negatively affects NK cell function, phenotype, survival, and rate of infiltration as a mechanism of escape. This inactivation can be however reverted by using immune checkpoints inhibitors specific, for instance, for NKG2A and TIGIT, already used in clinical trials (Galot et al., 2021). Alternatively, some studies have addressed mechanisms to increase NK cell infiltration at the tumor bed by neutralizing soluble factors that suppress NK cells function such as TGFβ, already entered in clinical trials also in combination with anti-PD1 (Dodagatta-Marri et al., 2019).

1.4.2 Natural Killer T Cells

Unlike conventional T cells, NKT cells recognize lipid antigens in a CD1d-dependent or independent manner (Vivier et al., 2012). $CD1d^+$ NKT cells are divided into Type I or Type II NKT cells. Type I NKT cells are also known as invariant-NKT (iNKT) cells and express the invariant Vα14Jα18 TCR in mouse or Vα24Jα18 in human. This TCR recognize α-Galactosydcerimide (α-GalCer) lipid antigen (Terabe and Berzofsky, 2008). Human iNKT cells develop within the thymus and can be subdivided into functional subsets based on their expression of CD4 and CD8 into $CD4^+$ iNKT cells, $CD8^+$ iNKT cells, and DN iNKT cells (Lee et al., 2002). The DN and $CD8^+$ iNKT cells have increased IFN-γ secretion and cytotoxic function upon activation while $CD4^+$ iNKT cells have a pronounced helper function with release of type 2 (Th2) cytokines, such as IL-4 and IL-13 (Lee et al., 2002). In common with NK cells, human NKT cells express markers such as 2B4, NKG2D, DNAM-1, CD94, and NKG2A (Shimizu et al., 2020).

Frequency and function of intratumor or circulating iNKT cells have been assumed to correlate with overall survival in several types of cancers (Yanagisawa et al., 2002; Fujii et al., 2003; Tachibana et al., 2005; Schneiders et al., 2012), thus implying a role for iNKT cells in tumor immune surveillance. In details, reduced iNKT-cell numbers correlated with poor overall survival in head and neck squamous cell carcinoma (Molling et al., 2007) and acute myeloid leukemia (Najera Chuc et al., 2012). Conversely, increased numbers of intratumor or circulating iNKT cells have been associated with improved prognosis in colon cancer, prostate cancer, hematologic malignancies, and neuroblastoma (Metelitsa et al., 2004; Tachibana et al., 2005; Shaulov et al., 2008).

Human iNKT cytotoxicity cells against target cells may occur *via* TCR-dependent or independent signaling. During immune-surveillance, activation of iNKT cells can occur indirectly by cross-presentation of tumor lipids by APCs (Wu et al., 2003). Sialylated glycolipids on tumor cell membranes may become targets for iNKT cells and during tumor progression might be modified, representing a mechanism of immune surveillance escape. Several types of tumors, such as melanoma, small-cell lung cancer (SCLC), sarcoma, and neuroblastoma, highly express some gangliosides in comparison with corresponding normal tissue (Lo et al., 2010). These gangliosides might activate both $CD4^+CD8^-$ iNKT and $CD4^-CD8^-$ iNKT cells to produce IL-4 (Wu et al., 2003) and, acting as NKT cell ligands, might be related to prognosis in some cancers.

Activation of iNKT cells can also occur directly, *via* presentation of self-lipids by CD1d-positive tumors. CD1d expression has been found on solid tumors, such as prostate cancer (Tahir et al., 2001; Nowak et al., 2010), renal cell carcinoma (Chong et al., 2015), breast cancer (Hix et al., 2011), and tumors of the nervous system (Dhodapkar et al., 2004; Liu et al., 2013). For instance, in myeloma patients the frequency of iNKT cells in PBMCs are inversely correlated with disease progression (Dhodapkar et al., 2003; Jiang et al., 2018). This is due to the fact that primary myeloma cells express CD1d (Dhodapkar et al., 2003); however, its expression decreases in advanced stage cancer cells (Spanoudakis et al., 2009). Furthermore, human iNKT cells have been reported to kill $CD1d^+$ osteosarcoma cells, but not $CD1d^-$ osteoblasts, confirming the CD1d restriction of iNKT cell–dependent cytotoxicity (Fallarini et al., 2012).

Interestingly, iNKT cells from cancer patients are not impaired; rather they are in an anergic state as they can be expanded and activated by stimulation with DCs loaded with α-GalCer, thus pointing out iNKT cells as potential target for anti-cancer therapy (Iyoda et al., 2018). In myeloid leukemia patients, for instance, purified PBMCs-derived iNKT cells are responsive to stimulation with α-GalCer/CD1d-tetramer with production of IFN-γ, TNF-α, IL-2, and IL-4 and cytotoxic against autologous leukemic cells, that are $CD1d^+$ (Metelitsa et al., 2003).

iNKT cells have the capacity to alter the immune tumor microenvironment thus influencing the ability of the host to limit growth of cancer cells. iNKT cells thus represent a population to be harnessed for the development of anticancer clinical therapeutics. Indeed, the identification of strong cell agonists, such as α-GalCer and its analogues, has led to the production of synthetic lipids that have shown potential in cancer vaccination and treatment (McEwen-Smith et al., 2015). Some approaches include using nanovectors/nanoparticle-based delivery systems (Ghinnagow et al., 2017) or α-GalCer loaded exosomes (Liu et al., 2017a). Moreover, vaccination with DCs pulsed with α-Gal-Cer with the aim to expand and activate iNKT cells in human cancer patients is also being evaluated (Richter et al., 2013; Shimizu et al., 2013).

To conclude, successful therapeutic treatments should aim at increasing the rate of tumor infiltrating NK and iNKT cells and rescuing their effector function.

1.5 Tissue-Resident Innate Lymphoid Cells in Tumor Immunity

Although NK cells are defined as the prototypical innate lymphocyte population, over the past decade, innate lymphoid cells (ILCs) have expanded the definition of innate immune cells. ILCs are mainly located in barrier tissues including skin, intestine and lung and are involved in multiple physiological and pathophysiologic processes (Mjosberg and Spits, 2016). Human ILCs are identified as $Lineage^-$, $CD127^+$ cells since they lack the expression of classical lymphocyte surface markers, the recombination activating gene (RAG)-rearranged antigen receptors and other surface molecules whilst express the CD127 (also known as IL-7 receptor). The lineage markers for ILCs identification in human include: CD3, CD4, CD8, CD14, CD15, CD16, CD19, CD20, CD33, CD34, CD203c, and FcERI in order to exclude macrophages, dendritic cells, red blood cells, T, B, and NK cells (Trabanelli et al., 2018). Likewise, mouse ILCs are identified as Lin^-, $CD127^+$, $CD90^+$ where the lineage mix consists of: CD3ε, CD5, CD8α, CD11c, CD11b, CD19, B220, FCRεI, TCRαβ, TCRγδ, DX5, and Ter119) (Gomez-Cadena et al., 2020). ILCs are defined as the innate counterpart of T lymphocytes subpopulations. In fact, similarly to Th1, Th2, and Th17 cells, ILCs are classified in ILC1, ILC2, and ILC3 cells mirroring the expression of transcription factors and the cytokine secretion of their adaptive counterpart (Ercolano et al.,

2020b). In particular, ILC1s express T-bet and produce IFN-γ and TNF-α, ILC2s express GATA-3 and secrete IL-4, IL-5, and IL-13 and ILC3s express RORγt and secreting IL- 17A and/or IL-22. In the human peripheral circulation, ILC3s also comprise a population of progenitor cells (referred as ILCPs) able to differentiate into all ILC subsets and natural killer (NK) cells (Lim et al., 2017). In addition, has been recently reported that ILCPs, thanks to the expression of CD62L are able to migrate to the lymph node (Bar-Ephraim et al., 2019). As tissue-resident cells, ILCs establish close interaction with other cells in the tissues contributing to the first-line defense against different threats shaping both innate and adaptive immune response by producing their prototypic, subset specific cytokine sets. The impact of ILCs in different diseases including allergy, asthma, rheumatoid arthritis, and inflammatory bowel disease, has been widely described during the last years (Pasha et al., 2019; Wu and Shen, 2020; Ercolano et al., 2022; Trabanelli et al., 2022). Nevertheless, our group and others reported a controversial role of ILCs in cancer showing both pro and anti-tumor effects according to the tumor type and the consequent cytokines and other cells that constitute the tumor microenvironment (TME) (Ercolano et al., 2019; Ghaedi and Ohashi, 2020; Jacquelot et al., 2022). Given their ability to secrete IFN-γ and TNF-α and the expression of the natural cytotoxicity receptor (NCR) NKp46, ILC1s are often teamed to NK cells and difficult to discriminate in both human and mouse (Zhang and Huang, 2017). However, at steady state these two subsets can be distinguished according to the expression of the transcription factors Tbet and Eomes (Daussy et al., 2014; Klose et al., 2014). In addition, unlike NK cells, ILC1s show a weak cytotoxic capacity due to their poor ability to secrete granzyme B and perforin (Vivier et al., 2018). Nevertheless, recent findings based on single-cell RNA sequencing and flow cytometric analysis identified an ILC1 subset with cytotoxic ability in the liver (Di Censo et al., 2021; Friedrich et al., 2021; Chen et al., 2022). Different data demonstrated that these two populations differ in both their development and distribution. In fact, while ILC1s are predominantly tissue-resident cells, NK cells are found mostly in the bloodstream and within secondary lymphoid tissues (Gao et al., 2017). Nevertheless, has been demonstrated that, under pathological conditions, including tumor development and metastasis, NK cells can acquire an ILC1-like phenotype. In particular, in a murine model of fibrosarcoma and melanoma, has been shown that NK cells were converted in ILC1s limiting the NK cell–mediated tumor immunosurveillance (Gao et al., 2017). TGF-β resulted to be the principal mediator involved in this switching of NK phenotype suggesting that the use of antibodies targeting TGF-β and its receptors may offer a promising strategy for cancer immunotherapy inhibiting the conversion of NK cells into ILC1s. In line with these findings, Salome et al. (2019) described an uncommon population of ILC1-like cells population with cytotoxic properties which were impaired in human acute myeloid leukemia (AML) by TGF-β and AhR ligands. Together with TGF-β, different cytokines present in the TME can modulate both the pro- and anti-tumoral effect of ILC1s. For instance, an IL-15 rich environment promotes the generation of a tissue-resident ILC1-like cells, characterized by a powerful cytotoxic activity towards cancer cells as demonstrated by using a MMTV-PyMT (PyMT) mammary tumor model (Dadi et al., 2016). Likewise, high levels of IL-15 have been observed in human colorectal cancer (CRC) biopsies, as well as high expression of T-bet and IFN-γ supporting the anti-tumoral role of ILC1s in this context (Mlecnik et al., 2014). In human melanoma, our own group reported an increase in the frequency of ILC1s in both peripheral blood mononuclear cells (PBMC) and tumor-infiltrated lymph nodes (TILN) of melanoma patients compared to healthy donors. Nevertheless, ILC1s from melanoma patients were functionally impaired as demonstrated by the reduced secretion of IFN-γ. By dissecting the different mediators present in the TME, we focused on adenosine and indoleamine. In fact, it is widely known that these two mediators are highly expressed in cancer and play a key role in the progression of melanoma and other types of cancer (Vijayan et al., 2017; Jennings et al., 2021). In particular, we observed that these two mediators are involved in ILC1 exhaustion leading to a reduction in type-1 cytokine secretion (Ercolano et al., 2020a). This effect was reverted by using an adenosine receptors inhibitor showing new evidence to sustain blocking these immunosuppressive pathways in melanoma patients by targeting the innate lymphoid cells arm.

ILC2s are assigned as a primarily pro-tumorigenic subset given their ability to produce type-2 cytokines, such as IL-13 and IL-5, and other immunosuppressive mediators. Different studies reported a higher frequency of tumor-infiltrating ILC2 in gastric, breast and prostate cancer. Particularly, in a first work published by Jovanovic et al. (2014), has been reported an increase of ILC2-derived IL-13 *in vivo* by using the 4T1 syngeneic murine model. Next, Salimi et al. (2018), observed an increase of ILC2s and investigated on the expression of different activatory and inhibitory receptors in tumor-infiltrating ILCs in both human breast and gastric cancer. Similarly, Trabanelli et al. (2017), found an enrichment of ILC2s in prostate cancer patients which was correlated with tumor stage and myeloid derived suppressor cells (MDSCs) frequency. Increased numbers of ILC2 have been showed also in the tumors of gastric cancer patients infected with *Helicobacter pylori* (Li et al., 2017). Furthermore, very recently, has been reported a link between ILC2 and tuft cells (a rare population of epithelial cells present at the gastrointestinal and respiratory tract). In particular, the tuft cells/ILC2 axis seems to be involved in the development of gastric cancer as demonstrated by using a murine model of intestinal metaplasia. This result was also confirmed in tumor microarrays of intestinal-type gastric cancer patients showing a unique correlation for tuft cells, ILC2s and survival in intestinal-type gastric cancer. IL-25 and IL-13 play a key role in dictating the crosstalk between tuft cells and ILC2s, in fact, the treatment with either α-IL13 or α-IL25 neutralizing antibodies significantly restrained tumor growth which coincided with a reduced frequency of both tuft cells and ILC2s in mice (O'Keefe et al., 2022). The pro- and anti-tumoral role of ILC2s is frequently associated with the overexpression of various cytokines involved in their activation including IL-33, IL-25, and TSLP commonly defined as "alarmins" (Roan et al., 2019). Our group recently

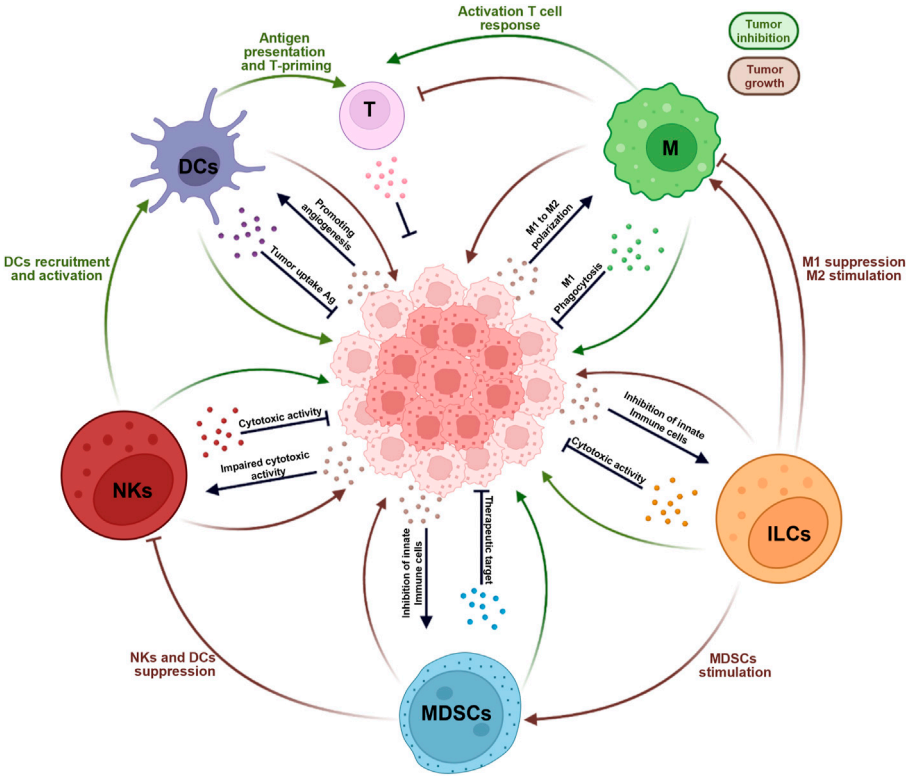

FIGURE 1 | Orchestration of cancer immune control. Within the tumor each tissue-resident innate immune cells exert both positive (tumor inhibition, in green) and negative (tumor growth, in brown) immune regulatory functions. The tissue-resident innate immune cells are important contributors in modulating the tumor microenvironment though the secretion of growth factors, chemokines, and cytokines. As show in the figure, the mainly subpopulation involved are dendritic cells (DCs), macrophages, myeloid-derived suppressor cells (MDSCs), natural killer cells (NKs), and innate lymphoid cells (ILCs).

demonstrated that IL-33 is highly expressed in tissues as well as in the serum of CRC patients. The high presence of IL-33 didn't affect the frequency of ILC2s but increased their activity in term of IL-13 and IL-5 production which in turn sustain CRC progression through the modulation of the epithelial-to-mesenchymal transition (EMT) phenomenon (Ercolano et al., 2021). Conversely, it has been recently reported that in pancreatic adenocarcinoma, IL-33 induces ILC2 expansion that was accompanied by enhanced intratumoral $CD8^+$ T cells pushing tissue-specific tumor immunity (Moral et al., 2020). Interestingly, they also reported that tumor-infiltrated ILC2s express the inhibitory checkpoint receptor PD-1 hypothesizing that PD-1 blockade could further boost ILC2 activation to enhance anti-tumor efficacy. In line with these findings, (Jacquelot et al., 2021a) demonstrated that PD-1 blockade increased ILC2 and eosinophil recruitment and enhanced anti-tumor responses in melanoma context (Jacquelot et al., 2021b). These findings suggest the need to further dissect investigation on the broad array of checkpoint inhibitors as target for ILC2 in cancer immunotherapy.

The role of ILC3s in cancer is emerging as complex and highly dependent on the tumor type as also described for their adaptive counterpart (Bruchard and Ghiringhelli, 2019). In fact, similarly to Th17 cells, ILC3s exert both pro- and anti-tumor functions showing different phenotypes according to the tissue microenvironment. Although ILC3s are generally dependent on the transcription factor RORγt, there are more complex subsets that further subdivide ILC3s in NCR^+ ILC3s and NCR– ILC3s depending on the expression of the natural cytotoxicity receptors (NCRs) NKp46 and NKp44 in both human and mice (Melo-Gonzalez and Hepworth, 2017). In addition, lymphoid-tissue inducer (LTi) cells are a further subclass of ILC3 closely related to NCR- ILC3s, involved in lymph nodes development (Spits et al., 2013). ILC3s have been associated with the pathogenesis and progression of different type of cancer. For instance, a profound reduction of ILC3 NCR^+ cells were observed also in acute myeloid leukemia (AML) patients which was completely recovered after two cycles of treatment with standard chemotherapy (represented by daunorubicin/cytarabine) (Trabanelli et al., 2015). Conversely, Liu et al. (2019) demonstrated that NCR– ILC3s were the tissue-resident cells mainly present in the liver of hepatocellular carcinoma-bearing mice. In particular, NCR- ILC3s supported the early phase of tumour development by promoting the establishment of an IL-17-rich tumor microenvironment in response to IL-23 sustaining the differentiation of other IL-17-producing cells. These findings suggest that NCR- ILC3s, as well as IL-23, could be an interesting target to exploit for the prevention of hepatocellular carcinoma progression. Given the essential role of LTi cells in generating secondary lymphoid tissues

during embryogenesis, it is possible to hypothesize an involvement of these cells in cancer context. In fact, has been demonstrated that an IL-12 enriched environment promotes the recruitment and proliferation of NKp46$^+$ LTi cells, able to inhibit tumor growth and thwart the development of lung metastases in a murine model of cutaneous melanoma (Eisenring et al., 2010). Importantly, (Jacquelot et al., 2021b) recently reviewed that LTi cells are implicated in the development of tertiary lymphoid structures that are formed in inflamed tissues (including tumor) and are composed by different immune cells driving the immune response against tumor development and progression and improving the clinical outcome (Jacquelot et al., 2021a). Moreover, in subcutaneous melanoma, it has been showed that LTi cells exert a tumoricidal effect by increasing the expression levels of adhesion molecules in the tumor which in turn promote the recruitment of adaptive immune cells and the subsequent tumor control (Eisenring et al., 2010). The ability of ILCs to foster the recruitment of other immune cells has been recently described in bladder cancer settings. In particular, Vanoni et al. (2021) demonstrated that ILCPs (the ILC3 progenitor subset present in the peripheral blood) interact with endothelial cells inducing, on one hand, the expression of adhesion molecules on the surface of endothelial cells and acquiring and, on the other, an ILC3-like phenotype sharing some phenotypical markers with LTi cells. However, in human high-grade bladder carcinoma samples, ILCPs are barely detected and their ability to upregulate adhesion molecule expression on endothelial cells was impaired (Vanoni et al., 2021). These findings could describe one of the mechanisms through which tumour cells block the infiltration of immune cells into the tumour site highlighting a new attractive target to regulate the immune infiltration and establish an antitumor immune response.

2 CONCLUDING REMARKS

Immune system evasion is a distinctive hallmark of cancer (Hanahan, 2022). In the last decades, cancer immunotherapy has experienced important clinical progresses in the treatment of different types of cancer. For instance, immune checkpoint inhibitors such as ipilimumab, nivolumab, and pembrolizumab, whose primary purpose is to unleash effector T cells response, have recast the treatment of aggressive forms of tumor including melanoma, non-small cell lung carcinoma and colorectal cancer (Bagchi et al., 2021). However, cancer relapse and recurrence occur for most patients increasing the need to find new therapeutic targets to improve cancer immunotherapy. The tumor milieu is a complex assortment of immune cells, blood vessels, connective tissue cells and extracellular matrix molecules, which all exert a remarkable influence on the cancerous cells they surround. The better understanding of this microenvironment, with particular attention focused on the function of immune cells within this region is today of particular interest. In this context, tissue-resident innate immune cells exert both positive and negative immune regulatory functions (**Figure 1**), representing important contributors in modulating the tumor microenvironment and shaping the adaptive tumor response (Wang et al., 2019b). Thus, investigate on the innate-adaptive lymphocyte crosstalk in cancer, could represent an interesting approach in order to increase the efficacy of the current immunotherapies.

AUTHOR CONTRIBUTIONS

RB, MB, EB, GZ, DM, and PC did the bibliographic research and wrote the manuscript. GE and AI critically revised the article for intellectual content. All authors contributed to the article and approved the submitted version.

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REFERENCES

Ali, T. H., Pisanti, S., Ciaglia, E., Mortarini, R., Anichini, A., Garofalo, C., et al. (2014). Enrichment of CD56dimKIR+CD57+ Highly Cytotoxic NK Cells in Tumour-Infiltrated Lymph Nodes of Melanoma Patients. *Nat. Commun.* 5, 5639. doi:10.1038/ncomms6639

Allavena, P., Signorelli, M., Chieppa, M., Erba, E., Bianchi, G., Marchesi, F., et al. (2005). Anti-inflammatory Properties of the Novel Antitumor Agent Yondelis (Trabectedin): Inhibition of Macrophage Differentiation and Cytokine Production. *Cancer Res.* 65, 2964–2971. doi:10.1158/0008-5472.can-04-4037

André, P., Denis, C., Soulas, C., Bourbon-Caillet, C., Lopez, J., Arnoux, T., et al. (2018). Anti-NKG2A mAb Is a Checkpoint Inhibitor that Promotes Anti-tumor Immunity by Unleashing Both T and NK Cells. *Cell* 175, 1731–e13. e1713. doi:10.1016/j.cell.2018.10.014

Andreu, P., Johansson, M., Affara, N. I., Pucci, F., Tan, T., Junankar, S., et al. (2010). FcRγ Activation Regulates Inflammation-Associated Squamous Carcinogenesis. *Cancer Cell* 17, 121–134. doi:10.1016/j.ccr.2009.12.019

Anfray, C., Ummarino, A., Andon, F. T., and Allavena, P. (2019). Current Strategies to Target Tumor-Associated-Macrophages to Improve Anti-tumor Immune Responses. *Cells* 9, 46. doi:10.3390/cells9010046

Ao, J.-Y., Zhu, X.-D., Chai, Z.-T., Cai, H., Zhang, Y.-Y., Zhang, K.-Z., et al. (2017). Colony-stimulating Factor 1 Receptor Blockade Inhibits Tumor Growth by Altering the Polarization of Tumor-Associated Macrophages in Hepatocellular Carcinoma. *Mol. Cancer Ther.* 16, 1544–1554. doi:10.1158/1535-7163.mct-16-0866

Au Yeung, C. L., Co, N.-N., Tsuruga, T., Yeung, T.-L., Kwan, S.-Y., Leung, C. S., et al. (2016). Exosomal Transfer of Stroma-Derived miR21 Confers Paclitaxel Resistance in Ovarian Cancer Cells through Targeting APAF1. *Nat. Commun.* 7, 11150. doi:10.1038/ncomms11150

Bagchi, S., Yuan, R., and Engleman, E. G. (2021). Immune Checkpoint Inhibitors for the Treatment of Cancer: Clinical Impact and Mechanisms of Response and Resistance. *Annu. Rev. Pathol. Mech. Dis.* 16, 223–249. doi:10.1146/annurev-pathol-042020-042741

Balachandran, V. P., Cavnar, M. J., Zeng, S., Bamboat, Z. M., Ocuin, L. M., Obaid, H., et al. (2011). Imatinib Potentiates Antitumor T Cell Responses in

Gastrointestinal Stromal Tumor through the Inhibition of Ido. *Nat. Med.* 17, 1094–1100. doi:10.1038/nm.2438

Bar-Ephraim, Y. E., Koning, J. J., Burniol Ruiz, E., Konijn, T., Mourits, V. P., Lakeman, K. A., et al. (2019). CD62L Is a Functional and Phenotypic Marker for Circulating Innate Lymphoid Cell Precursors. *J. I.* 202, 171–182. doi:10.4049/jimmunol.1701153

Baroni, S., Romero-Cordoba, S., Plantamura, I., Dugo, M., D'Ippolito, E., Cataldo, A., et al. (2016). Exosome-mediated Delivery of miR-9 Induces Cancer-Associated Fibroblast-like Properties in Human Breast Fibroblasts. *Cell Death Dis.* 7–e2312. doi:10.1038/cddis.2016.224

Barry, K. C., Hsu, J., Broz, M. L., Cueto, F. J., Binnewies, M., Combes, A. J., et al. (2018). A Natural Killer-Dendritic Cell axis Defines Checkpoint Therapy-Responsive Tumor Microenvironments. *Nat. Med.* 24, 1178–1191. doi:10.1038/s41591-018-0085-8

Baumann, T., Dunkel, A., Schmid, C., Schmitt, S., Hiltensperger, M., Lohr, K., et al. (2020). Regulatory Myeloid Cells Paralyze T Cells through Cell-Cell Transfer of the Metabolite Methylglyoxal. *Nat. Immunol.* 21, 555–566. doi:10.1038/s41590-020-0666-9

Belikov, A. V., Schraven, B., and Simeoni, L. (2015). T Cells and Reactive Oxygen Species. *J. Biomed. Sci.* 22, 85. doi:10.1186/s12929-015-0194-3

Bentebibel, S.-E., Hurwitz, M. E., Bernatchez, C., Haymaker, C., Hudgens, C. W., Kluger, H. M., et al. (2019). A First-In-Human Study and Biomarker Analysis of NKTR-214, a Novel IL2Rβγ-Biased Cytokine, in Patients with Advanced or Metastatic Solid Tumors. *Cancer Discov.* 9, 711–721. doi:10.1158/2159-8290.cd-18-1495

Bentz, B. G., Haines, G. K., 3rd, and Radosevich, J. A. (2000). Increased Protein Nitrosylation in Head and Neck Squamous Cell Carcinogenesis. *Head. Neck* 22, 64–70. doi:10.1002/(sici)1097-0347(200001)22:1<64::aid-hed10>3.0.co;2-j

Berahovich, R. D., Lai, N. L., Wei, Z., Lanier, L. L., and Schall, T. J. (2006). Evidence for NK Cell Subsets Based on Chemokine Receptor Expression. *J. Immunol.* 177, 7833–7840. doi:10.4049/jimmunol.177.11.7833

Bingisser, R. M., Tilbrook, P. A., Holt, P. G., and Kees, U. R. (1998). Macrophage-derived Nitric Oxide Regulates T Cell Activation via Reversible Disruption of the Jak3/STAT5 Signaling Pathway. *J. Immunol.* 160, 5729–5734.

Binnewies, M., Mujal, A. M., Pollack, J. L., Combes, A. J., Hardison, E. A., Barry, K. C., et al. (2019). Unleashing Type-2 Dendritic Cells to Drive Protective Antitumor CD4+ T Cell Immunity. *Cell* 177, 556–571. doi:10.1016/j.cell.2019.02.005

Blumenthal, R. L., Campbell, D. E., Hwang, P., Dekruyff, R. H., Frankel, L. R., and Umetsu, D. T. (2001). Human Alveolar Macrophages Induce Functional Inactivation in Antigen-specific CD4 T Cells. *J. Allergy Clin. Immunol.* 107, 258–264. doi:10.1067/mai.2001.112845

Bockorny, B., Semenisty, V., Macarulla, T., Borazanci, E., Wolpin, B. M., Stemmer, S. M., et al. (2020). BL-8040, a CXCR4 Antagonist, in Combination with Pembrolizumab and Chemotherapy for Pancreatic Cancer: the COMBAT Trial. *Nat. Med.* 26, 878–885. doi:10.1038/s41591-020-0880-x

Bosurgi, L., Cao, Y. G., Cabeza-Cabrerizo, M., Tucci, A., Hughes, L. D., Kong, Y., et al. (2017). Macrophage Function in Tissue Repair and Remodeling Requires IL-4 or IL-13 with Apoptotic Cells. *Science* 356, 1072–1076. doi:10.1126/science.aai8132

Boutilier, A. J., and Elsawa, S. F. (2021). Macrophage Polarization States in the Tumor Microenvironment. *Int. J. Mol. Sci.* 22. doi:10.3390/ijms22136995

Brown, S., Hutchinson, C. V., Aspinall-O'dea, M., Whetton, A. D., Johnson, S. M., Rees-Unwin, K., et al. (2014). Monocyte-derived Dendritic Cells from Chronic Myeloid Leukaemia Have Abnormal Maturation and Cytoskeletal Function that Is Associated with Defective Localisation and Signalling by Normal ABL1 Protein. *Eur. J. Haematol.* 93, 96–102. doi:10.1111/ejh.12306

Bruchard, M., and Ghiringhelli, F. (2019). Deciphering the Roles of Innate Lymphoid Cells in Cancer. *Front. Immunol.* 10, 656. doi:10.3389/fimmu.2019.00656

Carrega, P., Bonaccorsi, I., Di Carlo, E., Morandi, B., Paul, P., Rizzello, V., et al. (2014). CD56brightPerforinlow Noncytotoxic Human NK Cells Are Abundant in Both Healthy and Neoplastic Solid Tissues and Recirculate to Secondary Lymphoid Organs via Afferent Lymph. *J. I.* 192, 3805–3815. doi:10.4049/jimmunol.1301889

Chen, Y., Wang, X., Hao, X., Li, B., Tao, W., Zhu, S., et al. (2022). Ly49E Separates Liver ILC1s into Embryo-Derived and Postnatal Subsets with Different Functions. *J. Exp. Med.* 219. doi:10.1084/jem.20211805

Cheng, J.-N., Yuan, Y.-X., Zhu, B., and Jia, Q. (2021). Myeloid-Derived Suppressor Cells: A Multifaceted Accomplice in Tumor Progression. *Front. Cell Dev. Biol.* 9, 740827. doi:10.3389/fcell.2021.740827

Cheng, M., Zhang, X., Yu, H., Du, P., Plumas, J., Chaperot, L., et al. (2015). Characterization of Species-specific Genes Regulated by E2-2 in Human Plasmacytoid Dendritic Cells. *Sci. Rep.* 5, 10752. doi:10.1038/srep10752

Cheng, X., Cao, Y., Wang, X., Cheng, L., Liu, Y., Lei, J., et al. (2022). Systematic Pan-Cancer Analysis of KLRB1 with Prognostic Value and Immunological Activity across Human Tumors. *J. Immunol. Res.* 2022, 5254911. doi:10.1155/2022/5254911

Chong, T. W., Goh, F. Y., Sim, M. Y., Huang, H. H., Thike, D. A. A., Lim, W. K., et al. (2015). CD1d Expression in Renal Cell Carcinoma Is Associated with Higher Relapse Rates, Poorer Cancer-specific and Overall Survival. *J. Clin. Pathol.* 68, 200–205. doi:10.1136/jclinpath-2014-202735

Chung, A. W., Anand, K., Anselme, A. C., Chan, A. A., Gupta, N., Venta, L. A., et al. (2021). A Phase 1/2 Clinical Trial of the Nitric Oxide Synthase Inhibitor L-NMMA and Taxane for Treating Chemoresistant Triple-Negative Breast Cancer. *Sci. Transl. Med.* 13, eabj5070. doi:10.1126/scitranslmed.abj5070

Cichocki, F., Grzywacz, B., and Miller, J. S. (2019). Human NK Cell Development: One Road or Many? *Front. Immunol.* 10, 2078. doi:10.3389/fimmu.2019.02078

Cisse, B., Caton, M. L., Lehner, M., Maeda, T., Scheu, S., Locksley, R., et al. (2008). Transcription Factor E2-2 Is an Essential and Specific Regulator of Plasmacytoid Dendritic Cell Development. *Cell* 135, 37–48. doi:10.1016/j.cell.2008.09.016

Close, H. J., Stead, L. F., Nsengimana, J., Reilly, K. A., Droop, A., Wurdak, H., et al. (2020). Expression Profiling of Single Cells and Patient Cohorts Identifies Multiple Immunosuppressive Pathways and an Altered NK Cell Phenotype in Glioblastoma. *Clin. Exp. Immunol.* 200, 33–44. doi:10.1111/cei.13403

Cobbs, C. S., Whisenhunt, T. R., Wesemann, D. R., Harkins, L. E., Van Meir, E. G., and Samanta, M. (2003). Inactivation of Wild-type P53 Protein Function by Reactive Oxygen and Nitrogen Species in Malignant Glioma Cells. *Cancer Res.* 63, 8670–8673.

Collin, M., Mcgovern, N., and Haniffa, M. (2013). Human Dendritic Cell Subsets. *Immunology* 140, 22–30. doi:10.1111/imm.12117

Concha-Benavente, F., Kansy, B., Moskovitz, J., Moy, J., Chandran, U., and Ferris, R. L. (2018). PD-L1 Mediates Dysfunction in Activated PD-1+ NK Cells in Head and Neck Cancer Patients. *Cancer Immunol. Res.* 6, 1548–1560. doi:10.1158/2326-6066.cir-18-0062

Consonni, F. M., Porta, C., Marino, A., Pandolfo, C., Mola, S., Bleve, A., et al. (2019). Myeloid-Derived Suppressor Cells: Ductile Targets in Disease. *Front. Immunol.* 10, 949. doi:10.3389/fimmu.2019.00949

Cooper, M. A., Fehniger, T. A., Turner, S. C., Chen, K. S., Ghaheri, B. A., Ghayur, T., et al. (2001). Human Natural Killer Cells: a Unique Innate Immunoregulatory Role for the CD56bright Subset. *Blood, J. Am. Soc. Hematol.* 97, 3146–3151. doi:10.1182/blood.v97.10.3146

Cózar, B., Greppi, M., Carpentier, S., Narni-Mancinelli, E., Chiossone, L., and Vivier, E. (2021). Tumor-Infiltrating Natural Killer Cells. *Cancer Discov.* 11, 34–44. doi:10.1158/2159-8290.cd-20-0655

Cózar, B., Greppi, M., Carpentier, S., Narni-Mancinelli, E., Chiossone, L., and Vivier, E. (2021). Tumor-infiltrating Natural Killer Cells. *Cancer Discov.* 11, 34–44.

Crinier, A., Dumas, P.-Y., Escalière, B., Piperoglou, C., Gil, L., Villacreces, A., et al. (2021). Single-cell Profiling Reveals the Trajectories of Natural Killer Cell Differentiation in Bone Marrow and a Stress Signature Induced by Acute Myeloid Leukemia. *Cell Mol. Immunol.* 18, 1290–1304. doi:10.1038/s41423-020-00574-8

Cursons, J., Souza-Fonseca-Guimaraes, F., Foroutan, M., Anderson, A., Hollande, F., Hediyeh-Zadeh, S., et al. (2019a). A Gene Signature Predicting Natural Killer Cell Infiltration and Improved Survival in Melanoma Patients. *Cancer Immunol. Res.* 7, 1162–1174. doi:10.1158/2326-6066.cir-18-0500

Cursons, J., Souza-Fonseca-Guimaraes, F., Foroutan, M., Anderson, A., Hollande, F., Hediyeh-Zadeh, S., et al. (2019b). A Gene Signature Predicting Natural Killer Cell Infiltration and Improved Survival in Melanoma Patients. *Cancer Immunol. Res.* 7, 1162–1174. doi:10.1158/2326-6066.cir-18-0500

Curti, A., Pandolfi, S., Valzasina, B., Aluigi, M., Isidori, A., Ferri, E., et al. (2007). Modulation of Tryptophan Catabolism by Human Leukemic Cells Results in the Conversion of CD25− into CD25+ T Regulatory Cells. *Blood* 109, 2871–2877. doi:10.1182/blood-2006-07-036863

Dadi, S., Chhangawala, S., Whitlock, B. M., Franklin, R. A., Luo, C. T., Oh, S. A., et al. (2016). Cancer Immunosurveillance by Tissue-Resident Innate Lymphoid Cells and Innate-like T Cells. *Cell* 164, 365–377. doi:10.1016/j.cell.2016.01.002

Daussy, C., Faure, F., Mayol, K., Viel, S., Gasteiger, G., Charrier, E., et al. (2014). T-bet and Eomes Instruct the Development of Two Distinct Natural Killer Cell Lineages in the Liver and in the Bone Marrow. *J. Exp. Med.* 211, 563–577. doi:10.1084/jem.20131560

De Cicco, P., Ercolano, G., and Ianaro, A. (2020). The New Era of Cancer Immunotherapy: Targeting Myeloid-Derived Suppressor Cells to Overcome Immune Evasion. *Front. Immunol.* 11, 1680. doi:10.3389/fimmu.2020.01680

De Jonge, K., Ebering, A., Nassiri, S., Maby-El Hajjami, H., Ouertatani-Sakouhi, H., Baumgaertner, P., et al. (2019). Circulating CD56bright NK Cells Inversely Correlate with Survival of Melanoma Patients. *Sci. Rep.* 9, 4487. doi:10.1038/s41598-019-40933-8

De Mingo Pulido, Á., Gardner, A., Hiebler, S., Soliman, H., Rugo, H. S., Krummel, M. F., et al. (2018). TIM-3 Regulates CD103+ Dendritic Cell Function and Response to Chemotherapy in Breast Cancer. *Cancer Cell* 33, 60–74. doi:10.1016/j.ccell.2017.11.019

Denardo, D. G., Barreto, J. B., Andreu, P., Vasquez, L., Tawfik, D., Kolhatkar, N., et al. (2009). CD4+ T Cells Regulate Pulmonary Metastasis of Mammary Carcinomas by Enhancing Protumor Properties of Macrophages. *Cancer Cell* 16, 91–102. doi:10.1016/j.ccr.2009.06.018

Deng, Z., Rong, Y., Teng, Y., Zhuang, X., Samykutty, A., Mu, J., et al. (2017). Exosomes miR-126a Released from MDSC Induced by DOX Treatment Promotes Lung Metastasis. *Oncogene* 36, 639–651. doi:10.1038/onc.2016.229

Dhara, V., Shetty, S. S., De Arruda, J. A. A., Silva, T. A., Russo, R. C., Shetty, N. J., et al. (2022). Decoding the Influence of the Immune System and Immunotherapy Targets on Carcinomas: A Hidden Prism in Oral Cancer Therapy. *Disease-a-Month* 2022, 101353. doi:10.1016/j.disamonth.2022.101353

Dhodapkar, K. M., Cirignano, B., Chamian, F., Zagzag, D., Miller, D. C., Finlay, J. L., et al. (2004). Invariant Natural Killer T Cells Are Preserved in Patients with Glioma and Exhibit Antitumor Lytic Activity Following Dendritic Cell-Mediated Expansion. *Int. J. Cancer* 109, 893–899. doi:10.1002/ijc.20050

Dhodapkar, M. V., Geller, M. D., Chang, D. H., Shimizu, K., Fujii, S.-I., Dhodapkar, K. M., et al. (2003). A Reversible Defect in Natural Killer T Cell Function Characterizes the Progression of Premalignant to Malignant Multiple Myeloma. *J. Exp. Med.* 197, 1667–1676. doi:10.1084/jem.20021650

Di Censo, C., Marotel, M., Mattiola, I., Müller, L., Scarno, G., Pietropaolo, G., et al. (2021). Granzyme A and CD160 Expression Delineates ILC1 with Graded Functions in the Mouse Liver. *Eur. J. Immunol.* 51, 2568–2575. doi:10.1002/eji.202149209

Diab, A., Tannir, N. M., Bentebibel, S.-E., Hwu, P., Papadimitrakopoulou, V., Haymaker, C., et al. (2020). Bempegaldesleukin (NKTR-214) Plus Nivolumab in Patients with Advanced Solid Tumors: Phase I Dose-Escalation Study of Safety, Efficacy, and Immune Activation (PIVOT-02). *Cancer Discov.* 10, 1158–1173. doi:10.1158/2159-8290.cd-19-1510

Diamond, M. S., Kinder, M., Matsushita, H., Mashayekhi, M., Dunn, G. P., Archambault, J. M., et al. (2011). Type I Interferon Is Selectively Required by Dendritic Cells for Immune Rejection of Tumors. *J. Exp. Med.* 208, 1989–2003. doi:10.1084/jem.20101158

Dodagatta-Marri, E., Meyer, D., Reeves, M., Paniagua, R., To, M., Binnewies, M., et al. (2019). alpha-PD-1 Therapy Elevates Treg/Th Balance and Increases Tumor Cell pSmad3 that Are Both Targeted By-TGF Antibody to Promote Durable Rejection and Immunity in Squamous Cell Carcinomas. *J. Immunother. CANCER* 7. doi:10.1186/s40425-018-0493-9

Dzionek, A., Fuchs, A., Schmidt, P., Cremer, S., Zysk, M., Miltenyi, S., et al. (2000). BDCA-2, BDCA-3, and BDCA-4: Three Markers for Distinct Subsets of Dendritic Cells in Human Peripheral Blood. *J. Immunol.* 165, 6037–6046. doi:10.4049/jimmunol.165.11.6037

Edwards, D. K., Watanabe-Smith, K., Rofelty, A., Damnernsawad, A., Laderas, T., Lamble, A., et al. (2019). CSF1R Inhibitors Exhibit Antitumor Activity in Acute Myeloid Leukemia by Blocking Paracrine Signals from Support Cells. *Blood* 133, 588–599. doi:10.1182/blood-2018-03-838946

Eisenring, M., Vom Berg, J., Kristiansen, G., Saller, E., and Becher, B. (2010). IL-12 Initiates Tumor Rejection via Lymphoid Tissue-Inducer Cells Bearing the Natural Cytotoxicity Receptor NKp46. *Nat. Immunol.* 11, 1030–1038. doi:10.1038/ni.1947

Elliott, J. M., and Yokoyama, W. M. (2011). Unifying Concepts of MHC-dependent Natural Killer Cell Education. *Trends Immunol.* 32, 364–372. doi:10.1016/j.it.2011.06.001

Epelman, S., Lavine, K. J., Beaudin, A. E., Sojka, D. K., Carrero, J. A., Calderon, B., et al. (2014a). Embryonic and Adult-Derived Resident Cardiac Macrophages Are Maintained through Distinct Mechanisms at Steady State and during Inflammation. *Immunity* 40, 91–104. doi:10.1016/j.immuni.2013.11.019

Epelman, S., Lavine, K. J., and Randolph, G. J. (2014b). Origin and Functions of Tissue Macrophages. *Immunity* 41, 21–35. doi:10.1016/j.immuni.2014.06.013

Ercolano, G., Falquet, M., Vanoni, G., Trabanelli, S., and Jandus, C. (2019). ILC2s: New Actors in Tumor Immunity. *Front. Immunol.* 10, 2801. doi:10.3389/fimmu.2019.02801

Ercolano, G., Garcia-Garijo, A., Salomé, B., Gomez-Cadena, A., Vanoni, G., Mastelic-Gavillet, B., et al. (2020a). Immunosuppressive Mediators Impair Proinflammatory Innate Lymphoid Cell Function in Human Malignant Melanoma. *Cancer Immunol. Res.* 8, 556–564. doi:10.1158/2326-6066.cir-19-0504

Ercolano, G., Gomez-Cadena, A., Dumauthioz, N., Vanoni, G., Kreutzfeldt, M., Wyss, T., et al. (2021). PPARγ Drives IL-33-dependent ILC2 Pro-tumoral Functions. *Nat. Commun.* 12, 2538. doi:10.1038/s41467-021-22764-2

Ercolano, G., Trabanelli, S., Jandus, C., and Jandus, P. (2022). IL-9R Loss in Innate Lymphoid Cell Type 2 (ILC2) Reflects Treg Impairment in Mastocytosis Patients. *J. Investig. Allergol. Clin. Immunol.* 0. doi:10.18176/jiaci.0815

Ercolano, G., Wyss, T., Salomé, B., Romero, P., Trabanelli, S., and Jandus, C. (2020b). Distinct and Shared Gene Expression for Human Innate versus Adaptive Helper Lymphoid Cells. *J. Leukoc. Biol.* 108, 723–737. doi:10.1002/jlb.5ma0120-209r

Fallarini, S., Paoletti, T., Orsi Battaglini, N., and Lombardi, G. (2012). Invariant NKT Cells Increase Drug-Induced Osteosarcoma Cell Death. *Br. J. Pharmacol.* 167, 1533–1549. doi:10.1111/j.1476-5381.2012.02108.x

Fallarino, F., Grohmann, U., You, S., Mcgrath, B. C., Cavener, D. R., Vacca, C., et al. (2006). The Combined Effects of Tryptophan Starvation and Tryptophan Catabolites Down-Regulate T Cell Receptor ζ-Chain and Induce a Regulatory Phenotype in Naive T Cells. *J. Immunol.* 176, 6752–6761. doi:10.4049/jimmunol.176.11.6752

Fan, X., and Rudensky, A. Y. (2016). Hallmarks of Tissue-Resident Lymphocytes. *Cell* 164, 1198–1211. doi:10.1016/j.cell.2016.02.048

Feng, S., Cheng, X., Zhang, L., Lu, X., Chaudhary, S., Teng, R., et al. (2018). Myeloid-derived Suppressor Cells Inhibit T Cell Activation through Nitrating LCK in Mouse Cancers. *Proc. Natl. Acad. Sci. U.S.A.* 115, 10094–10099. doi:10.1073/pnas.1800695115

Feng, Y.-H., Wu, C.-L., Tsao, C.-J., Chang, J.-G., Lu, P.-J., Yeh, K.-T., et al. (2011). Deregulated Expression of Sprouty2 and microRNA-21 in Human Colon Cancer: Correlation with the Clinical Stage of the Disease. *Cancer Biol. Ther.* 11, 111–121. doi:10.4161/cbt.11.1.13965

Fleet, J. C., Burcham, G. N., Calvert, R. D., Elzey, B. D., and Ratliff, T. L. (2020). 1α, 25 Dihydroxyvitamin D (1,25(OH)2D) Inhibits the T Cell Suppressive Function of Myeloid Derived Suppressor Cells (MDSC). *J. Steroid Biochem. Mol. Biol.* 198, 105557. doi:10.1016/j.jsbmb.2019.105557

Freud, A. G., Mundy-Bosse, B. L., Yu, J., and Caligiuri, M. A. (2017). The Broad Spectrum of Human Natural Killer Cell Diversity. *Immunity* 47, 820–833. doi:10.1016/j.immuni.2017.10.008

Fridman, W. H., Pagès, F., Sautès-Fridman, C., and Galon, J. (2012). The Immune Contexture in Human Tumours: Impact on Clinical Outcome. *Nat. Rev. Cancer* 12, 298–306. doi:10.1038/nrc3245

Friedrich, C., Taggenbrock, R. L. R. E., Doucet-Ladevèze, R., Golda, G., Moenius, R., Arampatzi, P., et al. (2021). Effector Differentiation Downstream of Lineage Commitment in ILC1s Is Driven by Hobit across Tissues. *Nat. Immunol.* 22, 1256–1267. doi:10.1038/s41590-021-01013-0

Fu, S., Zhang, N., Yopp, A. C., Chen, D., Mao, M., Chen, D., et al. (2004). TGF-beta Induces Foxp3 + T-Regulatory Cells from CD4 + CD25 - Precursors. *Am. J. Transpl.* 4, 1614–1627. doi:10.1111/j.1600-6143.2004.00566.x

Fuertes, M. B., Kacha, A. K., Kline, J., Woo, S.-R., Kranz, D. M., Murphy, K. M., et al. (2011). Host Type I IFN Signals Are Required for Antitumor CD8+ T Cell Responses through CD8α+ Dendritic Cells. *J. Exp. Med.* 208, 2005–2016. doi:10.1084/jem.20101159

Fujii, S.-i., Shimizu, K., Klimek, V., Geller, M. D., Nimer, S. D., and V. Dhodapkar, M. (2003). Severe and Selective Deficiency of Interferon-γ-Producing Invariant

Natural Killer T Cells in Patients with Myelodysplastic Syndromes. *Br. J. Haematol.* 122, 617–622. doi:10.1046/j.1365-2141.2003.04465.x

Gabrilovich, D. I., and Nagaraj, S. (2009). Myeloid-derived Suppressor Cells as Regulators of the Immune System. *Nat. Rev. Immunol.* 9, 162–174. doi:10.1038/nri2506

Gabrilovich, D. I., Ostrand-Rosenberg, S., and Bronte, V. (2012). Coordinated Regulation of Myeloid Cells by Tumours. *Nat. Rev. Immunol.* 12, 253–268. doi:10.1038/nri3175

Galland, S., Vuille, J., Martin, P., Letovanec, I., Caignard, A., Fregni, G., et al. (2017). Tumor-derived Mesenchymal Stem Cells Use Distinct Mechanisms to Block the Activity of Natural Killer Cell Subsets. *Cell Rep.* 20, 2891–2905. doi:10.1016/j.celrep.2017.08.089

Galot, R., Le Tourneau, C., Saada-Bouzid, E., Daste, A., Even, C., Debruyne, P., et al. (2021). A Phase II Study of Monalizumab in Patients with Recurrent/metastatic Squamous Cell Carcinoma of the Head and Neck: The I1 Cohort of the EORTC-HNCG-1559 UPSTREAM Trial. *Eur. J. Cancer* 158, 17–26. doi:10.1016/j.ejca.2021.09.003

Galsky, M. D., Vogelzang, N. J., Conkling, P., Raddad, E., Polzer, J., Roberson, S., et al. (2014). A Phase I Trial of LY2510924, a CXCR4 Peptide Antagonist, in Patients with Advanced Cancer. *Clin. Cancer Res.* 20, 3581–3588. doi:10.1158/1078-0432.ccr-13-2686

Ganguly, D., Haak, S., Sisirak, V., and Reizis, B. (2013). The Role of Dendritic Cells in Autoimmunity. *Nat. Rev. Immunol.* 13, 566–577. doi:10.1038/nri3477

Gao, Y., Souza-Fonseca-Guimaraes, F., Bald, T., Ng, S. S., Young, A., Ngiow, S. F., et al. (2017). Tumor Immunoevasion by the Conversion of Effector NK Cells into Type 1 Innate Lymphoid Cells. *Nat. Immunol.* 18, 1004–1015. doi:10.1038/ni.3800

Garcia-Chagollan, M., Carranza-Torres, I. E., Carranza-Rosales, P., Guzmán-Delgado, N. E., Ramírez-Montoya, H., Martínez-Silva, M. G., et al. (2018). Expression of NK Cell Surface Receptors in Breast Cancer Tissue as Predictors of Resistance to Antineoplastic Treatment. *Technol. cancer Res. Treat.* 17, 1533033818764499. doi:10.1177/1533033818764499

Gardner, A., De Mingo Pulido, Á., and Ruffell, B. (2020). Dendritic Cells and Their Role in Immunotherapy. *Front. Immunol.* 11, 924. doi:10.3389/fimmu.2020.00924

Geissmann, F., Manz, M. G., Jung, S., Sieweke, M. H., Merad, M., and Ley, K. (2010). Development of Monocytes, Macrophages, and Dendritic Cells. *Science* 327, 656–661. doi:10.1126/science.1178331

Gerlini, G., Urso, C., Mariotti, G., Di Gennaro, P., Palli, D., Brandani, P., et al. (2007). Plasmacytoid Dendritic Cells Represent a Major Dendritic Cell Subset in Sentinel Lymph Nodes of Melanoma Patients and Accumulate in Metastatic Nodes. *Clin. Immunol.* 125, 184–193. doi:10.1016/j.clim.2007.07.018

Germano, G., Frapolli, R., Belgiovine, C., Anselmo, A., Pesce, S., Liguori, M., et al. (2013). Role of Macrophage Targeting in the Antitumor Activity of Trabectedin. *Cancer Cell* 23, 249–262. doi:10.1016/j.ccr.2013.01.008

Ghaedi, M., and Ohashi, P. S. (2020). ILC Transdifferentiation: Roles in Cancer Progression. *Cell Res.* 30, 562–563. doi:10.1038/s41422-020-0326-5

Ghinnagow, R., Cruz, L. J., Macho-Fernandez, E., Faveeuw, C., and Trottein, F. (2017). Enhancement of Adjuvant Functions of Natural Killer T Cells Using Nanovector Delivery Systems: Application in Anticancer Immune Therapy. *Front. Immunol.* 8, 879. doi:10.3389/fimmu.2017.00879

Ghiringhelli, F., Me´nard, C., Terme, M., Flament, C., Taieb, J., Chaput, N., et al. (2005). CD4+CD25+ Regulatory T Cells Inhibit Natural Killer Cell Functions in a Transforming Growth Factor-β-dependent Manner. *J. Exp. Med.* 202, 1075–1085. doi:10.1084/jem.20051511

Ghobrial, I. M., Liu, C.-J., Redd, R. A., Perez, R. P., Baz, R., Zavidij, O., et al. (2020). A Phase Ib/II Trial of the First-In-Class Anti-CXCR4 Antibody Ulocuplumab in Combination with Lenalidomide or Bortezomib Plus Dexamethasone in Relapsed Multiple Myeloma. *Clin. Cancer Res.* 26, 344–353. doi:10.1158/1078-0432.ccr-19-0647

Giallongo, C., Parrinello, N. L., La Cava, P., Camiolo, G., Romano, A., Scalia, M., et al. (2018). Monocytic Myeloid-Derived Suppressor Cells as Prognostic Factor in Chronic Myeloid Leukaemia Patients Treated with Dasatinib. *J. Cell Mol. Med.* 22, 1070–1080. doi:10.1111/jcmm.13326

Gilliet, M., Cao, W., and Liu, Y.-J. (2008). Plasmacytoid Dendritic Cells: Sensing Nucleic Acids in Viral Infection and Autoimmune Diseases. *Nat. Rev. Immunol.* 8, 594–606. doi:10.1038/nri2358

Goff, S. L., and Danforth, D. N. (2021). The Role of Immune Cells in Breast Tissue and Immunotherapy for the Treatment of Breast Cancer. *Clin. Breast Cancer* 21, e63–e73. doi:10.1016/j.clbc.2020.06.011

Gomez-Cadena, A., Romero, P., Trabanelli, S., and Jandus, C. (2020). Detecting and Analyzing Murine Innate Lymphoid Cells. *Methods Enzymol.* 631, 329–342. doi:10.1016/bs.mie.2019.10.022

Gordon, E. M., Sankhala, K. K., Chawla, N., and Chawla, S. P. (2016). Trabectedin for Soft Tissue Sarcoma: Current Status and Future Perspectives. *Adv. Ther.* 33, 1055–1071. doi:10.1007/s12325-016-0344-3

Gorelik, L., Constant, S., and Flavell, R. A. (2002). Mechanism of Transforming Growth Factor β-induced Inhibition of T Helper Type 1 Differentiation. *J. Exp. Med.* 195, 1499–1505. doi:10.1084/jem.20012076

Gray, J. I., and Farber, D. L. (2022). Tissue-Resident Immune Cells in Humans. *Annu. Rev. Immunol.* 40, 195–220. doi:10.1146/annurev-immunol-093019-112809

Greenhalgh, A. D., Zarruk, J. G., Healy, L. M., Baskar Jesudasan, S. J., Jhelum, P., Salmon, C. K., et al. (2018). Peripherally Derived Macrophages Modulate Microglial Function to Reduce Inflammation after CNS Injury. *PLoS Biol.* 16, e2005264. doi:10.1371/journal.pbio.2005264

Groth, C., Hu, X., Weber, R., Fleming, V., Altevogt, P., Utikal, J., et al. (2019). Immunosuppression Mediated by Myeloid-Derived Suppressor Cells (MDSCs) during Tumour Progression. *Br. J. Cancer* 120, 16–25. doi:10.1038/s41416-018-0333-1

Guan, W., Hu, J., Yang, L., Tan, P., Tang, Z., West, B. L., et al. (2019). Inhibition of TAMs Improves the Response to Docetaxel in Castration-Resistant Prostate Cancer. *Endocr. Relat. Cancer* 26, 131–140. doi:10.1530/erc-18-0284

Guilliams, M., Bruhns, P., Saeys, Y., Hammad, H., and Lambrecht, B. N. (2014). The Function of Fcγ Receptors in Dendritic Cells and Macrophages. *Nat. Rev. Immunol.* 14, 94–108. doi:10.1038/nri3582

Gulubova, M., Manolova, I., Kyurkchiev, D., Julianov, A., and Altunkova, I. (2009). Decrease in Intrahepatic CD56+ Lymphocytes in Gastric and Colorectal Cancer Patients with Liver Metastases. *Apmis* 117, 870–879. doi:10.1111/j.1600-0463.2009.02547.x

Gunassekaran, G. R., Poongkavithai Vadevoo, S. M., Baek, M.-C., and Lee, B. (2021). M1 Macrophage Exosomes Engineered to Foster M1 Polarization and Target the IL-4 Receptor Inhibit Tumor Growth by Reprogramming Tumor-Associated Macrophages into M1-like Macrophages. *Biomaterials* 278, 121137. doi:10.1016/j.biomaterials.2021.121137

Hanahan, D. (2022). Hallmarks of Cancer: New Dimensions. *Cancer Discov.* 12, 31–46. doi:10.1158/2159-8290.cd-21-1059

Haniffa, M., Collin, M., and Ginhoux, F. (2013). Ontogeny and Functional Specialization of Dendritic Cells in Human and Mouse. *Adv. Immunol.* 120, 1–49. doi:10.1016/b978-0-12-417028-5.00001-6

Haniffa, M., Shin, A., Bigley, V., Mcgovern, N., Teo, P., See, P., et al. (2012). Human Tissues Contain CD141hi Cross-Presenting Dendritic Cells with Functional Homology to Mouse CD103+ Nonlymphoid Dendritic Cells. *Immunity* 37, 60–73. doi:10.1016/j.immuni.2012.04.012

Harari, O., and Liao, J. (2004). Inhibition of MHC II Gene Transcription by Nitric Oxide and Antioxidants. *Cpd* 10, 893–898. doi:10.2174/1381612043452893

Harel-Bellan, A., Quillet, A., Marchiol, C., Demars, R., Tursz, T., and Fradelizi, D. (1986). Natural Killer Susceptibility of Human Cells May Be Regulated by Genes in the HLA Region on Chromosome 6. *Proc. Natl. Acad. Sci. U.S.A.* 83, 5688–5692. doi:10.1073/pnas.83.15.5688

Hartmann, E., Wollenberg, B., Rothenfusser, S., Wagner, M., Wellisch, D., Mack, B., et al. (2003). Identification and Functional Analysis of Tumor-Infiltrating Plasmacytoid Dendritic Cells in Head and Neck Cancer. *Cancer Res.* 63, 6478–6487.

Hashimoto, D., Chow, A., Noizat, C., Teo, P., Beasley, M. B., Leboeuf, M., et al. (2013). Tissue-resident Macrophages Self-Maintain Locally throughout Adult Life with Minimal Contribution from Circulating Monocytes. *Immunity* 38, 792–804. doi:10.1016/j.immuni.2013.04.004

Heath, W. R., and Carbone, F. R. (2013). The Skin-Resident and Migratory Immune System in Steady State and Memory: Innate Lymphocytes, Dendritic Cells and T Cells. *Nat. Immunol.* 14, 978–985. doi:10.1038/ni.2680

Heo, Y.-J., Joo, Y.-B., Oh, H.-J., Park, M.-K., Heo, Y.-M., Cho, M.-L., et al. (2010). IL-10 Suppresses Th17 Cells and Promotes Regulatory T Cells in the CD4+ T Cell Population of Rheumatoid Arthritis Patients. *Immunol. Lett.* 127, 150–156. doi:10.1016/j.imlet.2009.10.006

Herzog, C., Pons Garcia, L., Keatinge, M., Greenald, D., Moritz, C., Peri, F., et al. (2019). Rapid Clearance of Cellular Debris by Microglia Limits Secondary Neuronal Cell Death after Brain Injury *In Vivo*. *Development* 146, dev174698. doi:10.1242/dev.174698

Hix, L. M., Shi, Y. H., Brutkiewicz, R. R., Stein, P. L., Wang, C.-R., and Zhang, M. (2011). CD1d-expressing Breast Cancer Cells Modulate NKT Cell-Mediated Antitumor Immunity in a Murine Model of Breast Cancer Metastasis. *PLoS One* 6, e20702. doi:10.1371/journal.pone.0020702

Hodi, F. S., O'day, S. J., Mcdermott, D. F., Weber, R. W., Sosman, J. A., Haanen, J. B., et al. (2010). Improved Survival with Ipilimumab in Patients with Metastatic Melanoma. *N. Engl. J. Med.* 363, 711–723. doi:10.1056/nejmoa1003466

Hughes, R., Qian, B.-Z., Rowan, C., Muthana, M., Keklikoglou, I., Olson, O. C., et al. (2015). Perivascular M2 Macrophages Stimulate Tumor Relapse after Chemotherapy. *Cancer Res.* 75, 3479–3491. doi:10.1158/0008-5472.can-14-3587

Iclozan, C., Antonia, S., Chiappori, A., Chen, D.-T., and Gabrilovich, D. (2013). Therapeutic Regulation of Myeloid-Derived Suppressor Cells and Immune Response to Cancer Vaccine in Patients with Extensive Stage Small Cell Lung Cancer. *Cancer Immunol. Immunother.* 62, 909–918. doi:10.1007/s00262-013-1396-8

Islam, R., Pupovac, A., Evtimov, V., Boyd, N., Shu, R., Boyd, R., et al. (2021). Enhancing a Natural Killer: Modification of NK Cells for Cancer Immunotherapy. *Cells* 10. doi:10.3390/cells10051058

Iyoda, T., Yamasaki, S., Hidaka, M., Kawano, F., Abe, Y., Suzuki, K., et al. (2018). Amelioration of NK Cell Function Driven by Vα24 + Invariant NKT Cell Activation in Multiple Myeloma. *Clin. Immunol.* 187, 76–84. doi:10.1016/j.clim.2017.10.007

Izawa, S., Kono, K., Mimura, K., Kawaguchi, Y., Watanabe, M., Maruyama, T., et al. (2011). H2O2 Production within Tumor Microenvironment Inversely Correlated with Infiltration of CD56dim NK Cells in Gastric and Esophageal Cancer: Possible Mechanisms of NK Cell Dysfunction. *Cancer Immunol. Immunother.* 60, 1801–1810. doi:10.1007/s00262-011-1082-7

Jacquelot, N., Seillet, C., Vivier, E., and Belz, G. T. (2022). Innate Lymphoid Cells and Cancer. *Nat. Immunol.* 23, 371–379. doi:10.1038/s41590-022-01127-z

Jacquelot, N., Seillet, C., Wang, M., Pizzolla, A., Liao, Y., Hediyeh-Zadeh, S., et al. (2021b). Blockade of the Co-inhibitory Molecule PD-1 Unleashes ILC2-dependent Antitumor Immunity in Melanoma. *Nat. Immunol.* 22, 851–864. doi:10.1038/s41590-021-00943-z

Jacquelot, N., Tellier, J., Nutt, S. I., and Belz, G. T. (2021a). Tertiary Lymphoid Structures and B Lymphocytes in Cancer Prognosis and Response to Immunotherapies. *Oncoimmunology* 10, 1900508. doi:10.1080/2162402x.2021.1900508

Jennings, M. R., Munn, D., and Blazeck, J. (2021). Immunosuppressive Metabolites in Tumoral Immune Evasion: Redundancies, Clinical Efforts, and Pathways Forward. *J. Immunother. Cancer* 9. doi:10.1136/jitc-2021-003013

Jiang, F., Liu, H., Liu, Z., Yan, S., Chen, J., Shao, Q., et al. (2018). Deficient Invariant Natural Killer T Cells Had Impaired Regulation on Osteoclastogenesis in Myeloma Bone Disease. *J. Cell Mol. Med.* 22, 2706–2716. doi:10.1111/jcmm.13554

Jiang, H., Gebhardt, C., Umansky, L., Beckhove, P., Schulze, T. J., Utikal, J., et al. (2015). Elevated Chronic Inflammatory Factors and Myeloid-Derived Suppressor Cells Indicate Poor Prognosis in Advanced Melanoma Patients. *Int. J. Cancer* 136, 2352–2360. doi:10.1002/ijc.29297

Johnson, D. E., O'keefe, R. A., and Grandis, J. R. (2018). Targeting the IL-6/JAK/STAT3 Signalling axis in Cancer. *Nat. Rev. Clin. Oncol.* 15, 234–248. doi:10.1038/nrclinonc.2018.8

Jordan, V. A., Lunos, S., Sieger, G., Horvath, K. J., Cohen, S., and Misono, S. (2020). Association of Voice and Mental Health Diagnoses with Differences in Voice-related Care Utilization. *Laryngoscope* 130, 1496–1502. doi:10.1002/lary.28277

Jovanovic, I. P., Pejnovic, N. N., Radosavljevic, G. D., Pantic, J. M., Milovanovic, M. Z., Arsenijevic, N. N., et al. (2014). Interleukin-33/ST2 axis Promotes Breast Cancer Growth and Metastases by Facilitating Intratumoral Accumulation of Immunosuppressive and Innate Lymphoid Cells. *Int. J. Cancer* 134, 1669–1682. doi:10.1002/ijc.28481

Judge, S. J., Darrow, M. A., Thorpe, S. W., Gingrich, A. A., O'donnell, E. F., Bellini, A. R., et al. (2020). Analysis of Tumor-Infiltrating NK and T Cells Highlights IL-15 Stimulation and TIGIT Blockade as a Combination Immunotherapy Strategy for Soft Tissue Sarcomas. *J. Immunother. Cancer* 8. doi:10.1136/jitc-2020-001355

Kalbasi, A., Komar, C., Tooker, G. M., Liu, M., Lee, J. W., Gladney, W. L., et al. (2017). Tumor-Derived CCL2 Mediates Resistance to Radiotherapy in Pancreatic Ductal Adenocarcinoma. *Clin. Cancer Res.* 23, 137–148. doi:10.1158/1078-0432.ccr-16-0870

Kaneda, M. M., Cappello, P., Nguyen, A. V., Ralainirina, N., Hardamon, C. R., Foubert, P., et al. (2016). Macrophage PI3Kγ Drives Pancreatic Ductal Adenocarcinoma Progression. *Cancer Discov.* 6, 870–885. doi:10.1158/2159-8290.cd-15-1346

Kerdidani, D., Chouvardas, P., Arjo, A. R., Giopanou, I., Ntaliarda, G., Guo, Y. A., et al. (2019). Wnt1 Silences Chemokine Genes in Dendritic Cells and Induces Adaptive Immune Resistance in Lung Adenocarcinoma. *Nat. Commun.* 10, 1405. doi:10.1038/s41467-019-09370-z

Kim, C. W., Kim, K.-D., and Lee, H. K. (2021). The Role of Dendritic Cells in Tumor Microenvironments and Their Uses as Therapeutic Targets. *BMB Rep.* 54, 31–43. doi:10.5483/bmbrep.2021.54.1.224

Klose, C. S. N., Flach, M., Möhle, L., Rogell, L., Hoyler, T., Ebert, K., et al. (2014). Differentiation of Type 1 ILCs from a Common Progenitor to All Helper-like Innate Lymphoid Cell Lineages. *Cell* 157, 340–356. doi:10.1016/j.cell.2014.03.030

Koo, K. C., Shim, D. H., Yang, C. M., Lee, S.-B., Kim, S. M., Shin, T. Y., et al. (2013). Reduction of the CD16–CD56bright NK Cell Subset Precedes NK Cell Dysfunction in Prostate Cancer. *PLoS One* 8, e78049. doi:10.1371/journal.pone.0078049

Kranz, L. M., Diken, M., Haas, H., Kreiter, S., Loquai, C., Reuter, K. C., et al. (2016). Systemic RNA Delivery to Dendritic Cells Exploits Antiviral Defence for Cancer Immunotherapy. *Nature* 534, 396–401. doi:10.1038/nature18300

Labidi-Galy, S. I., Sisirak, V., Meeus, P., Gobert, M., Treilleux, I., Bajard, A., et al. (2011). Quantitative and Functional Alterations of Plasmacytoid Dendritic Cells Contribute to Immune Tolerance in Ovarian Cancer. *Cancer Res.* 71, 5423–5434. doi:10.1158/0008-5472.can-11-0367

Lamichhane, P., Karyampudi, L., Shreeder, B., Krempski, J., Bahr, D., Daum, J., et al. (2017). IL10 Release upon PD-1 Blockade Sustains Immunosuppression in Ovarian Cancer. *Cancer Res.* 77, 6667–6678. doi:10.1158/0008-5472.can-17-0740

Lande, R., Gregorio, J., Facchinetti, V., Chatterjee, B., Wang, Y.-H., Homey, B., et al. (2007). Plasmacytoid Dendritic Cells Sense Self-DNA Coupled with Antimicrobial Peptide. *Nature* 449, 564–569. doi:10.1038/nature06116

Le Mercier, I., Poujol, D., Sanlaville, A., Sisirak, V., Gobert, M., Durand, I., et al. (2013). Tumor Promotion by Intratumoral Plasmacytoid Dendritic Cells Is Reversed by TLR7 Ligand Treatment. *Cancer Res.* 73, 4629–4640. doi:10.1158/0008-5472.can-12-3058

Lechner, M. G., Liebertz, D. J., and Epstein, A. L. (2010). Characterization of Cytokine-Induced Myeloid-Derived Suppressor Cells from Normal Human Peripheral Blood Mononuclear Cells. *J. I.* 185, 2273–2284. doi:10.4049/jimmunol.1000901

Lechner, M. G., Megiel, C., Russell, S. M., Bingham, B., Arger, N., Woo, T., et al. (2011). Functional Characterization of Human Cd33+ and Cd11b+ Myeloid-Derived Suppressor Cell Subsets Induced from Peripheral Blood Mononuclear Cells Co-cultured with a Diverse Set of Human Tumor Cell Lines. *J. Transl. Med.* 9, 90. doi:10.1186/1479-5876-9-90

Lee, J.-C., Lee, K.-M., Kim, D.-W., and Heo, D. S. (2004). Elevated TGF-B1 Secretion and Down-Modulation of NKG2D Underlies Impaired NK Cytotoxicity in Cancer Patients. *J. Immunol.* 172, 7335–7340. doi:10.4049/jimmunol.172.12.7335

Lee, P. T., Benlagha, K., Teyton, L., and Bendelac, A. (2002). Distinct Functional Lineages of Human Vα24 Natural Killer T Cells. *J. Exp. Med.* 195, 637–641. doi:10.1084/jem.20011908

Lee-Chang, C., Rashidi, A., Miska, J., Zhang, P., Pituch, K. C., Hou, D., et al. (2019). Myeloid-Derived Suppressive Cells Promote B Cell-Mediated Immunosuppression via Transfer of PD-L1 in Glioblastoma. *Cancer Immunol. Res.* 7, 1928–1943. doi:10.1158/2326-6066.cir-19-0240

Levings, M. K., Gregori, S., Tresoldi, E., Cazzaniga, S., Bonini, C., and Roncarolo, M. G. (2005). Differentiation of Tr1 Cells by Immature Dendritic Cells Requires IL-10 but Not CD25+CD4+ Tr Cells. *Blood* 105, 1162–1169. doi:10.1182/blood-2004-03-1211

Levring, T. B., Kongsbak, M., Rode, A. K. O., Woetmann, A., Ødum, N., Bonefeld, C. M., et al. (2015). Human CD4+ T Cells Require Exogenous Cystine for Glutathione and DNA Synthesis. *Oncotarget* 6, 21853–21864. doi:10.18632/oncotarget.5213

Li, G., Wu, K., Tao, K., Lu, X., Ma, J., Mao, Z., et al. (2015). Vasoactive Intestinal Peptide Induces CD14+HLA-DR−/low Myeloid-Derived Suppressor Cells in Gastric Cancer. *Mol. Med. Rep.* 12, 760–768. doi:10.3892/mmr.2015.3374

Li, H., Han, Y., Guo, Q., Zhang, M., and Cao, X. (2009). Cancer-Expanded Myeloid-Derived Suppressor Cells Induce Anergy of NK Cells through Membrane-Bound TGF-B1. *J. Immunol.* 182, 240–249. doi:10.4049/jimmunol.182.1.240

Li, H. S., Liu, C., Xiao, Y., Chu, F., Liang, X., Peng, W., et al. (2016). Bypassing STAT3-Mediated Inhibition of the Transcriptional Regulator ID2 Improves the Antitumor Efficacy of Dendritic Cells. *Sci. Signal* 9, ra94. doi:10.1126/scisignal.aaf3957

Li, K., Shi, H., Zhang, B., Ou, X., Ma, Q., Chen, Y., et al. (2021). Myeloid-derived Suppressor Cells as Immunosuppressive Regulators and Therapeutic Targets in Cancer. *Sig Transduct. Target Ther.* 6, 362. doi:10.1038/s41392-021-00670-9

Li, R., Jiang, X. X., Zhang, L. F., Liu, X. M., Hu, T. Z., Xia, X. J., et al. (2017). Group 2 Innate Lymphoid Cells Are Involved in Skewed Type 2 Immunity of Gastric Diseases Induced by *Helicobacter pylori* Infection. *Mediat. Inflamm.* 2017, 4927964. doi:10.1155/2017/4927964

Li, Y.-C., Zhou, Q., Song, Q.-K., Wang, R.-B., Lyu, S., Guan, X., et al. (2020). Overexpression of an Immune Checkpoint (CD155) in Breast Cancer Associated with Prognostic Significance and Exhausted Tumor-Infiltrating Lymphocytes: a Cohort Study. *J. Immunol. Res.* 2020, 3948928. doi:10.1155/2020/3948928

Lim, A. I., Li, Y., Lopez-Lastra, S., Stadhouders, R., Paul, F., Casrouge, A., et al. (2017). Systemic Human ILC Precursors Provide a Substrate for Tissue ILC Differentiation. *Cell* 168, 1086–1100. doi:10.1016/j.cell.2017.02.021

Lim, H. X., Kim, T. S., and Poh, C. L. (2020). Understanding the Differentiation, Expansion, Recruitment and Suppressive Activities of Myeloid-Derived Suppressor Cells in Cancers. *Int. J. Mol. Sci.* 21. doi:10.3390/ijms21103599

Lim, S. Y., Yuzhalin, A. E., Gordon-Weeks, A. N., and Muschel, R. J. (2016). Targeting the CCL2-CCR2 Signaling axis in Cancer Metastasis. *Oncotarget* 7, 28697–28710. doi:10.18632/oncotarget.7376

Liu, D., Song, L., Brawley, V. S., Robison, N., Wei, J., Gao, X., et al. (2013). Medulloblastoma Expresses CD1d and Can Be Targeted for Immunotherapy with NKT Cells. *Clin. Immunol.* 149, 55–64. doi:10.1016/j.clim.2013.06.005

Liu, H., Chen, L., Liu, J., Meng, H., Zhang, R., Ma, L., et al. (2017a). Co-delivery of Tumor-Derived Exosomes with Alpha-Galactosylceramide on Dendritic Cell-Based Immunotherapy for Glioblastoma. *Cancer Lett.* 411, 182–190. doi:10.1016/j.canlet.2017.09.022

Liu, X., Li, L., Si, F., Huang, L., Zhao, Y., Zhang, C., et al. (2021). NK and NKT Cells Have Distinct Properties and Functions in Cancer. *Oncogene* 40, 4521–4537. doi:10.1038/s41388-021-01880-9

Liu, Y.-J. (2005). IPC: Professional Type 1 Interferon-Producing Cells and Plasmacytoid Dendritic Cell Precursors. *Annu. Rev. Immunol.* 23, 275–306. doi:10.1146/annurev.immunol.23.021704.115633

Liu, Y., Cheng, Y., Xu, Y., Wang, Z., Du, X., Li, C., et al. (2017b). Increased Expression of Programmed Cell Death Protein 1 on NK Cells Inhibits NK-Cell-Mediated Anti-tumor Function and Indicates Poor Prognosis in Digestive Cancers. *Oncogene* 36, 6143–6153. doi:10.1038/onc.2017.209

Liu, Y., Song, Y., Lin, D., Lei, L., Mei, Y., Jin, Z., et al. (2019). NCR− Group 3 Innate Lymphoid Cells Orchestrate IL-23/IL-17 axis to Promote Hepatocellular Carcinoma Development. *EBioMedicine* 41, 333–344. doi:10.1016/j.ebiom.2019.02.050

Lo, A. S. Y., Ma, Q., Liu, D. L., and Junghans, R. P. (2010). Anti-GD3 Chimeric sFv-Cd28/t-Cell Receptor ζ Designer T Cells for Treatment of Metastatic Melanoma and Other Neuroectodermal Tumors. *Clin. Cancer Res.* 16, 2769–2780. doi:10.1158/1078-0432.ccr-10-0043

Locati, M., Curtale, G., and Mantovani, A. (2020). Diversity, Mechanisms, and Significance of Macrophage Plasticity. *Annu. Rev. Pathol. Mech. Dis.* 15, 123–147. doi:10.1146/annurev-pathmechdis-012418-012718

Lopes, A. M. M., Michelin, M. A., and Murta, E. F. C. (2017). Monocyte-derived Dendritic Cells from Patients with Cervical Intraepithelial Lesions. *Oncol. Lett.* 13, 1456–1462. doi:10.3892/ol.2017.5595

Lorenzo-Herrero, S., López-Soto, A., Sordo-Bahamonde, C., Gonzalez-Rodriguez, A. P., Vitale, M., and Gonzalez, S. (2019). NK Cell-Based Immunotherapy in Cancer Metastasis. *Cancers* 11, 29.

Loyher, P.-L., Hamon, P., Laviron, M., Meghraoui-Kheddar, A., Goncalves, E., Deng, Z., et al. (2018). Macrophages of Distinct Origins Contribute to Tumor Development in the Lung. *J. Exp. Med.* 215, 2536–2553. doi:10.1084/jem.20180534

Ma, T., Renz, B. W., Ilmer, M., Koch, D., Yang, Y., Werner, J., et al. (2022). Myeloid-Derived Suppressor Cells in Solid Tumors. *Cells* 11. doi:10.3390/cells11020310

Mäkitie, A., Tuokkola, I., Laurell, G., Mäkitie, O., Olsen, K., Takes, R. P., et al. (2020). Vitamin D in Head and Neck Cancer: a Systematic Review. *Curr. Oncol. Rep.* 23, 5. doi:10.1007/s11912-020-00996-7

Malmberg, K.-J., Carlsten, M., Björklund, A., Sohlberg, E., Bryceson, Y. T., and Ljunggren, H.-G. (2017). Natural Killer Cell-Mediated Immunosurveillance of Human Cancer. *Semin. Immunol.* 2017, 20–29. doi:10.1016/j.smim.2017.08.002

Mamessier, E., Sylvain, A., Thibult, M.-L., Houvenaeghel, G., Jacquemier, J., Castellano, R., et al. (2011). Human Breast Cancer Cells Enhance Self Tolerance by Promoting Evasion from NK Cell Antitumor Immunity. *J. Clin. Invest.* 121, 3609–3622. doi:10.1172/jci45816

Mandruzzato, S., Brandau, S., Britten, C. M., Bronte, V., Damuzzo, V., Gouttefangeas, C., et al. (2016). Toward Harmonized Phenotyping of Human Myeloid-Derived Suppressor Cells by Flow Cytometry: Results from an Interim Study. *Cancer Immunol. Immunother.* 65, 161–169. doi:10.1007/s00262-015-1782-5

Mantovani, A., Sozzani, S., Locati, M., Allavena, P., and Sica, A. (2002). Macrophage Polarization: Tumor-Associated Macrophages as a Paradigm for Polarized M2 Mononuclear Phagocytes. *Trends Immunol.* 23, 549–555. doi:10.1016/s1471-4906(02)02302-5

Mantovani, S., Oliviero, B., Lombardi, A., Varchetta, S., Mele, D., Sangiovanni, A., et al. (2019). Deficient Natural Killer Cell NKp30-Mediated Function and Altered NCR3 Splice Variants in Hepatocellular Carcinoma. *Hepatology* 69, 1165–1179. doi:10.1002/hep.30235

Marsh, S. G. E., Parham, P., Dupont, B., Geraghty, D. E., Trowsdale, J., Middleton, D., et al. (2003). Killer-cell Immunoglobulin-like Receptor (KIR) Nomenclature Report, 2002. *Immunogenetics* 55, 220–226. doi:10.1007/s00251-003-0571-z

Matlack, R., Yeh, K., Rosini, L., Gonzalez, D., Taylor, J., Silberman, D., et al. (2006). Peritoneal Macrophages Suppress T-Cell Activation by Amino Acid Catabolism. *Immunology* 117, 386–395. doi:10.1111/j.1365-2567.2005.02312.x

Mcewen-Smith, R. M., Salio, M., and Cerundolo, V. (2015). The Regulatory Role of Invariant NKT Cells in Tumor Immunity. *Cancer Immunol. Res.* 3, 425–435. doi:10.1158/2326-6066.cir-15-0062

Mckarns, S. C., Schwartz, R. H., and Kaminski, N. E. (2004). Smad3 Is Essential for TGF-B1 to Suppress IL-2 Production and TCR-Induced Proliferation, but Not IL-2-Induced Proliferation. *J. Immunol.* 172, 4275–4284. doi:10.4049/jimmunol.172.7.4275

Melo-Gonzalez, F., and Hepworth, M. R. (2017). Functional and Phenotypic Heterogeneity of Group 3 Innate Lymphoid Cells. *Immunology* 150, 265–275. doi:10.1111/imm.12697

Merad, M., Sathe, P., Helft, J., Miller, J., and Mortha, A. (2013). The Dendritic Cell Lineage: Ontogeny and Function of Dendritic Cells and Their Subsets in the Steady State and the Inflamed Setting. *Annu. Rev. Immunol.* 31, 563–604. doi:10.1146/annurev-immunol-020711-074950

Metelitsa, L. S., Weinberg, K. I., Emanuel, P. D., and Seeger, R. C. (2003). Expression of CD1d by Myelomonocytic Leukemias Provides a Target for Cytotoxic NKT Cells. *Leukemia* 17, 1068–1077. doi:10.1038/sj.leu.2402943

Metelitsa, L. S., Wu, H.-W., Wang, H., Yang, Y., Warsi, Z., Asgharzadeh, S., et al. (2004). Natural Killer T Cells Infiltrate Neuroblastomas Expressing the Chemokine CCL2. *J. Exp. Med.* 199, 1213–1221. doi:10.1084/jem.20031462

Mills, C. D., Kincaid, K., Alt, J. M., Heilman, M. J., and Hill, A. M. (2000). M-1/M-2 Macrophages and the Th1/Th2 Paradigm. *J. Immunol.* 164, 6166–6173. doi:10.4049/jimmunol.164.12.6166

Min, Y., Li, J., Qu, P., and Lin, P. C. (2017). C/EBP-δ Positively Regulates MDSC Expansion and Endothelial VEGFR2 Expression in Tumor Development. *Oncotarget* 8, 50582–50593. doi:10.18632/oncotarget.16410

Mjösberg, J., and Spits, H. (2016). Human Innate Lymphoid Cells. *J. Allergy Clin. Immunol.* 138, 1265–1276. doi:10.1016/j.jaci.2016.09.009

Mlecnik, B., Bindea, G., Angell, H. K., Sasso, M. S., Obenauf, A. C., Fredriksen, T., et al. (2014). Functional Network Pipeline Reveals Genetic Determinants Associated with *In Situ* Lymphocyte Proliferation and Survival of Cancer Patients. *Sci. Transl. Med.* 6, 228ra37. doi:10.1126/scitranslmed.3007240

Molling, J. W., Langius, J. A. E., Langendijk, J. A., Leemans, C. R., Bontkes, H. J., Van Der Vliet, H. J. J., et al. (2007). Low Levels of Circulating Invariant Natural Killer T Cells Predict Poor Clinical Outcome in Patients with Head and Neck Squamous Cell Carcinoma. *Jco* 25, 862–868. doi:10.1200/jco.2006.08.5787

Molon, B., Ugel, S., Del Pozzo, F., Soldani, C., Zilio, S., Avella, D., et al. (2011). Chemokine Nitration Prevents Intratumoral Infiltration of Antigen-specific T Cells. *J. Exp. Med.* 208, 1949–1962. doi:10.1084/jem.20101956

Moral, J. A., Leung, J., Rojas, L. A., Ruan, J., Zhao, J., Sethna, Z., et al. (2020). ILC2s Amplify PD-1 Blockade by Activating Tissue-specific Cancer Immunity. *Nature* 579, 130–135. doi:10.1038/s41586-020-2015-4

Moretta, A., Marcenaro, E., Parolini, S., Ferlazzo, G., and Moretta, L. (2008). NK Cells at the Interface between Innate and Adaptive Immunity. *Cell Death Differ.* 15, 226–233. doi:10.1038/sj.cdd.4402170

Muhammad Ali Tahir, S., Cheng, O., Shaulov, A., Koezuka, Y., Bubley, G. J., Wilson, S. B., et al. (2001). Loss of IFN-γ Production by Invariant NK T Cells in Advanced Cancer. *J. Immunol.* 167, 4046–4050. doi:10.4049/jimmunol.167.7.4046

Munn, D., and Mellor, A. (2003). Macrophages and the Regulation of Self-Reactive T Cells. *Cpd* 9, 257–264. doi:10.2174/1381612033392026

Nagaraj, S., Gupta, K., Pisarev, V., Kinarsky, L., Sherman, S., Kang, L., et al. (2007). Altered Recognition of Antigen Is a Mechanism of CD8+ T Cell Tolerance in Cancer. *Nat. Med.* 13, 828–835. doi:10.1038/nm1609

Najafi, M., Hashemi Goradel, N., Farhood, B., Salehi, E., Nashtaei, M. S., Khanlarkhani, N., et al. (2019). Macrophage Polarity in Cancer: A Review. *J. Cell Biochem.* 120, 2756–2765. doi:10.1002/jcb.27646

Najera Chuc, A. E., Cervantes, L. A. M., Retiguin, F. P., Ojeda, J. V., and Maldonado, E. R. (2012). Low Number of Invariant NKT Cells Is Associated with Poor Survival in Acute Myeloid Leukemia. *J. Cancer Res. Clin. Oncol.* 138, 1427–1432. doi:10.1007/s00432-012-1251-x

Nakai, R., Maniwa, Y., Tanaka, Y., Nishio, W., Yoshimura, M., Okita, Y., et al. (2010). Overexpression of Necl-5 Correlates with Unfavorable Prognosis in Patients with Lung Adenocarcinoma. *Cancer Sci.* 101, 1326–1330. doi:10.1111/j.1349-7006.2010.01530.x

Nakamura, K., and Smyth, M. J. (2020). Myeloid Immunosuppression and Immune Checkpoints in the Tumor Microenvironment. *Cell Mol. Immunol.* 17, 1–12. doi:10.1038/s41423-019-0306-1

Nielsen, S. R., Quaranta, V., Linford, A., Emeagi, P., Rainer, C., Santos, A., et al. (2016). Macrophage-secreted Granulin Supports Pancreatic Cancer Metastasis by Inducing Liver Fibrosis. *Nat. Cell Biol.* 18, 549–560. doi:10.1038/ncb3340

Nowak, M., Arredouani, M. S., Tun-Kyi, A., Schmidt-Wolf, I., Sanda, M. G., Balk, S. P., et al. (2010). Defective NKT Cell Activation by CD1d+ TRAMP Prostate Tumor Cells Is Corrected by Interleukin-12 with Alpha-Galactosylceramide. *PLoS One* 5, e11311. doi:10.1371/journal.pone.0011311

Noy, R., and Pollard, J. W. (2014). Tumor-associated Macrophages: from Mechanisms to Therapy. *Immunity* 41, 49–61. doi:10.1016/j.immuni.2014.06.010

O'brien, S. A., Orf, J., Skrzypczynska, K. M., Tan, H., Kim, J., Devoss, J., et al. (2021). Activity of Tumor-Associated Macrophage Depletion by CSF1R Blockade Is Highly Dependent on the Tumor Model and Timing of Treatment. *Cancer Immunol. Immunother.* 70, 2401–2410. doi:10.1007/s00262-021-02861-3

Ohl, K., and Tenbrock, K. (2018). Reactive Oxygen Species as Regulators of MDSC-Mediated Immune Suppression. *Front. Immunol.* 9, 2499. doi:10.3389/fimmu.2018.02499

O'keefe, R. N., Carli, A. L. E., Baloyan, D., Afshar-Sterle, S., Eissmann, M. F., Poh, A. R., et al. (2022). *Inhibition of the Tuft cell/ILC2 axis Reduces Gastric Tumor Development in Mice.*

Orsini, G., Legitimo, A., Failli, A., Ferrari, P., Nicolini, A., Spisni, R., et al. (2013). Defective Generation and Maturation of Dendritic Cells from Monocytes in Colorectal Cancer Patients during the Course of Disease. *Ijms* 14, 22022–22041. doi:10.3390/ijms141122022

Pachynski, R. K., Zabel, B. A., Kohrt, H. E., Tejeda, N. M., Monnier, J., Swanson, C. D., et al. (2012). The Chemoattractant Chemerin Suppresses Melanoma by Recruiting Natural Killer Cell Antitumor Defenses. *J. Exp. Med.* 209, 1427–1435. doi:10.1084/jem.20112124

Pasha, M. A., Patel, G., Hopp, R., and Yang, Q. (2019). Role of Innate Lymphoid Cells in Allergic Diseases. *Allergy Asthma Proc.* 40, 138–145. doi:10.2500/aap.2019.40.4217

Patente, T. A., Pinho, M. P., Oliveira, A. A., Evangelista, G. C. M., Bergami-Santos, P. C., and Barbuto, J. A. M. (2018). Human Dendritic Cells: Their Heterogeneity and Clinical Application Potential in Cancer Immunotherapy. *Front. Immunol.* 9, 3176. doi:10.3389/fimmu.2018.03176

Peng, L.-S., Zhang, J.-Y., Teng, Y.-S., Zhao, Y.-L., Wang, T.-T., Mao, F.-Y., et al. (2017). Tumor-Associated Monocytes/Macrophages Impair NK-Cell Function via TGFβ1 in Human Gastric Cancer. *Cancer Immunol. Res.* 5, 248–256. doi:10.1158/2326-6066.cir-16-0152

Pesce, S., Greppi, M., Tabellini, G., Rampinelli, F., Parolini, S., Olive, D., et al. (2017). Identification of a Subset of Human Natural Killer Cells Expressing High Levels of Programmed Death 1: A Phenotypic and Functional Characterization. *J. Allergy Clin. Immunol.* 139, 335–346. e333. doi:10.1016/j.jaci.2016.04.025

Pesce, S., Tabellini, G., Cantoni, C., Patrizi, O., Coltrini, D., Rampinelli, F., et al. (2015). B7-H6-mediated Downregulation of NKp30 in NK Cells Contributes to Ovarian Carcinoma Immune Escape. *Oncoimmunology* 4, e1001224. doi:10.1080/2162402x.2014.1001224

Pienta, K. J., Machiels, J.-P., Schrijvers, D., Alekseev, B., Shkolnik, M., Crabb, S. J., et al. (2013). Phase 2 Study of Carlumab (CNTO 888), a Human Monoclonal Antibody against CC-Chemokine Ligand 2 (CCL2), in Metastatic Castration-Resistant Prostate Cancer. *Invest New Drugs* 31, 760–768. doi:10.1007/s10637-012-9869-8

Pietra, G., Vitale, M., Moretta, L., and Mingari, M. C. (2012). How Melanoma Cells Inactivate NK Cells. *Oncoimmunology* 1, 974–975. doi:10.4161/onci.20405

Platonova, S., Cherfils-Vicini, J., Damotte, D., Crozet, L., Vieillard, V., Validire, P., et al. (2011). Profound Coordinated Alterations of Intratumoral NK Cell Phenotype and Function in Lung Carcinoma. *Cancer Res.* 71, 5412–5422. doi:10.1158/0008-5472.can-10-4179

Poh, A. R., and Ernst, M. (2018). Targeting Macrophages in Cancer: From Bench to Bedside. *Front. Oncol.* 8, 49. doi:10.3389/fonc.2018.00049

Pyonteck, S. M., Akkari, L., Schuhmacher, A. J., Bowman, R. L., Sevenich, L., Quail, D. F., et al. (2013). CSF-1R Inhibition Alters Macrophage Polarization and Blocks Glioma Progression. *Nat. Med.* 19, 1264–1272. doi:10.1038/nm.3337

Qian, B., Deng, Y., Im, J. H., Muschel, R. J., Zou, Y., Li, J., et al. (2009). A Distinct Macrophage Population Mediates Metastatic Breast Cancer Cell Extravasation, Establishment and Growth. *PLoS One* 4, e6562. doi:10.1371/journal.pone.0006562

Quail, D. F., Bowman, R. L., Akkari, L., Quick, M. L., Schuhmacher, A. J., Huse, J. T., et al. (2016). The Tumor Microenvironment Underlies Acquired Resistance to CSF-1R Inhibition in Gliomas. *Science* 352, aad3018. doi:10.1126/science.aad3018

Quaranta, V., Rainer, C., Nielsen, S. R., Raymant, M. L., Ahmed, M. S., Engle, D. D., et al. (2018). Macrophage-Derived Granulin Drives Resistance to Immune Checkpoint Inhibition in Metastatic Pancreatic Cancer. *Cancer Res.* 78, 4253–4269. doi:10.1158/0008-5472.can-17-3876

Quezada, S. A., Peggs, K. S., Curran, M. A., and Allison, J. P. (2006). CTLA4 Blockade and GM-CSF Combination Immunotherapy Alters the Intratumor Balance of Effector and Regulatory T Cells. *J. Clin. Investigation* 116, 1935–1945. doi:10.1172/jci27745

Ramos, R. N., Chin, L. S., Dos Santos, A. P. S. A., Bergami-Santos, P. C., Laginha, F., and Barbuto, J. A. M. (2012). Monocyte-derived Dendritic Cells from Breast Cancer Patients Are Biased to Induce CD4+CD25+Foxp3+ Regulatory T Cells. *J. Leukoc. Biol.* 92, 673–682. doi:10.1189/jlb.0112048

Rashid, M., Borin, T., Ara, R., Piranlioglu, R., Achyut, B., Korkaya, H., et al. (2021). Critical Immunosuppressive Effect of MDSC-derived E-xosomes in the T-umor M-icroenvironment. *Oncol. Rep.* 45, 1171–1181. doi:10.3892/or.2021.7936

Redman, J. M., Gibney, G. T., and Atkins, M. B. (2016). Advances in Immunotherapy for Melanoma. *BMC Med.* 14, 20. doi:10.1186/s12916-016-0571-0

Rees, B., Bilwes, A., Samama, J. P., and Moras, D. (1990). CardiotoxinV4II fromNaja Mossambica Mossambica. *J. Mol. Biol.* 214, 281–297. doi:10.1016/0022-2836(90)90161-e

Remark, R., Alifano, M., Cremer, I., Lupo, A., Dieu-Nosjean, M.-C., Riquet, M., et al. (2013). Characteristics and Clinical Impacts of the Immune Environments in Colorectal and Renal Cell Carcinoma Lung Metastases: Influence of Tumor Origin. *Clin. Cancer Res.* 19, 4079–4091. doi:10.1158/1078-0432.ccr-12-3847

Reynolds, G., and Haniffa, M. (2015). Human and Mouse Mononuclear Phagocyte Networks: A Tale of Two Species? *Front. Immunol.* 6, 330. doi:10.3389/fimmu.2015.00330

Rezaeifard, S., Safaei, A., Talei, A., Faghih, Z., and Erfani, N. (2019). NK, NKT and Invariant-NKT Cells in Tumor Draining Lymph Nodes of Patients with Breast Cancer. *Iran. J. Immunol.* 16, 291–298. doi:10.22034/IJI.2019.80280

Rezaeifard, S., Talei, A., Shariat, M., and Erfani, N. (2021). Tumor Infiltrating NK Cell (TINK) Subsets and Functional Molecules in Patients with Breast Cancer. *Mol. Immunol.* 136, 161–167. doi:10.1016/j.molimm.2021.03.003

Richter, J., Neparidze, N., Zhang, L., Nair, S., Monesmith, T., Sundaram, R., et al. (2013). Clinical Regressions and Broad Immune Activation Following Combination Therapy Targeting Human NKT Cells in Myeloma. *Blood* 121, 423–430. doi:10.1182/blood-2012-06-435503

Roan, F., Obata-Ninomiya, K., and Ziegler, S. F. (2019). Epithelial Cell-Derived Cytokines: More Than Just Signaling the Alarm. *J. Clin. Invest* 129, 1441–1451. doi:10.1172/jci124606

Roberts, E. W., Broz, M. L., Binnewies, M., Headley, M. B., Nelson, A. E., Wolf, D. M., et al. (2016). Critical Role for CD103+/CD141+ Dendritic Cells Bearing CCR7 for Tumor Antigen Trafficking and Priming of T Cell Immunity in Melanoma. *Cancer Cell* 30, 324–336. doi:10.1016/j.ccell.2016.06.003

Rodell, C. B., Arlauckas, S. P., Cuccarese, M. F., Garris, C. S., Li, R., Ahmed, M. S., et al. (2018). TLR7/8-agonist-loaded Nanoparticles Promote the Polarization of Tumour-Associated Macrophages to Enhance Cancer Immunotherapy. *Nat. Biomed. Eng.* 2, 578–588. doi:10.1038/s41551-018-0236-8

Rodriguez, P. C., Quiceno, D. G., and Ochoa, A. C. (2007). L-arginine Availability Regulates T-Lymphocyte Cell-Cycle Progression. *Blood* 109, 1568–1573. doi:10.1182/blood-2006-06-031856

Rodriguez, P. C., Zea, A. H., Culotta, K. S., Zabaleta, J., Ochoa, J. B., and Ochoa, A. C. (2002). Regulation of T Cell Receptor CD3ζ Chain Expression Byl-Arginine. *J. Biol. Chem.* 277, 21123–21129. doi:10.1074/jbc.m110675200

Romagnani, C., Juelke, K., Falco, M., Morandi, B., D'Agostino, A., Costa, R., et al. (2007). CD56brightCD16− Killer Ig-like Receptor− NK Cells Display Longer Telomeres and Acquire Features of CD56dim NK Cells upon Activation. *J. Immunol.* 178, 4947–4955. doi:10.4049/jimmunol.178.8.4947

Rosser, E. C., and Mauri, C. (2015). Regulatory B Cells: Origin, Phenotype, and Function. *Immunity* 42, 607–612. doi:10.1016/j.immuni.2015.04.005

Sade-Feldman, M., Kanterman, J., Klieger, Y., Ish-Shalom, E., Olga, M., Saragovi, A., et al. (2016). Clinical Significance of Circulating CD33+CD11b+HLA-DR− Myeloid Cells in Patients with Stage IV Melanoma Treated with Ipilimumab. *Clin. Cancer Res.* 22, 5661–5672. doi:10.1158/1078-0432.ccr-15-3104

Safarzadeh, E., Hashemzadeh, S., Duijf, P. H. G., Mansoori, B., Khaze, V., Mohammadi, A., et al. (2019). Circulating Myeloid-derived Suppressor Cells: An Independent Prognostic Factor in Patients with Breast Cancer. *J. Cell. Physiology* 234, 3515–3525. doi:10.1002/jcp.26896

Saito, T., Takayama, T., Osaki, T., Nagai, S., Suzuki, T., Sato, M., et al. (2008). Combined Mobilization and Stimulation of Tumor-Infiltrating Dendritic Cells and Natural Killer Cells with Flt3 Ligand and IL-18 *In Vivo* Induces Systemic Antitumor Immunity. *Cancer Sci.* 99, 2028–2036. doi:10.1111/j.1349-7006.2008.00907.x

Salimi, M., Wang, R., Yao, X., Li, X., Wang, X., Hu, Y., et al. (2018). Activated Innate Lymphoid Cell Populations Accumulate in Human Tumour Tissues. *BMC Cancer* 18, 341. doi:10.1186/s12885-018-4262-4

Sallusto, F., and Lanzavecchia, A. (2018). Pillars Article: Efficient Presentation of Soluble Antigen by Cultured Human Dendritic Cells Is Maintained by Granulocyte/macrophage Colony-Stimulating Factor Plus Interleukin 4 and Downregulated by Tumor Necrosis Factor α. J. Exp. Med. 1994. 179:1109-1118. *J. Exp. MedJ Immunol.* 200, 887–896.

Salmon, H., Idoyaga, J., Rahman, A., Leboeuf, M., Remark, R., Jordan, S., et al. (2016). Expansion and Activation of CD103 + Dendritic Cell Progenitors at the Tumor Site Enhances Tumor Responses to Therapeutic PD-L1 and BRAF Inhibition. *Immunity* 44, 924–938. doi:10.1016/j.immuni.2016.03.012

Salomé, B., Gomez-Cadena, A., Loyon, R., Suffiotti, M., Salvestrini, V., Wyss, T., et al. (2019). CD56 as a Marker of an ILC1-like Population with NK Cell Properties that Is Functionally Impaired in AML. *Blood Adv.* 3, 3674–3687. doi:10.1182/bloodadvances.2018030478

Sambi, M., Bagheri, L., and Szewczuk, M. R. (2019). Current Challenges in Cancer Immunotherapy: Multimodal Approaches to Improve Efficacy and Patient Response Rates. *J. Oncol.* 2019, 4508794. doi:10.1155/2019/4508794

Sanchez-Correa, B., Gayoso, I., Bergua, J. M., Casado, J. G., Morgado, S., Solana, R., et al. (2012). Decreased Expression of DNAM-1 on NK Cells from Acute Myeloid Leukemia Patients. *Immunol. Cell Biol.* 90, 109–115. doi:10.1038/icb.2011.15

Sato, E., Olson, S. H., Ahn, J., Bundy, B., Nishikawa, H., Qian, F., et al. (2005). Intraepithelial CD8 + Tumor-Infiltrating Lymphocytes and a High CD8 +/regulatory T Cell Ratio Are Associated with Favorable Prognosis in Ovarian Cancer. *Proc. Natl. Acad. Sci. U.S.A.* 102, 18538–18543. doi:10.1073/pnas.0509182102

Schlecker, E., Fiegler, N., Arnold, A., Altevogt, P., Rose-John, S., Moldenhauer, G., et al. (2014). Metalloprotease-Mediated Tumor Cell Shedding of B7-H6, the Ligand of the Natural Killer Cell-Activating Receptor NKp30. *Cancer Res.* 74, 3429–3440. doi:10.1158/0008-5472.can-13-3017

Schlecker, E., Stojanovic, A., Eisen, C., Quack, C., Falk, C. S., Umansky, V., et al. (2012). Tumor-infiltrating Monocytic Myeloid-Derived Suppressor Cells Mediate CCR5-dependent Recruitment of Regulatory T Cells Favoring Tumor Growth. *J. I.* 189, 5602–5611. doi:10.4049/jimmunol.1201018

Schlegel, N. C., Von Planta, A., Widmer, D. S., Dummer, R., and Christofori, G. (2015). PI3K Signalling Is Required for a TGFβ-Induced Epithelial-mesenchymal-like Transition (EMT-like) in Human Melanoma Cells. *Exp. Dermatol* 24, 22–28. doi:10.1111/exd.12580

Schleypen, J. S., Baur, N., Kammerer, R., Nelson, P. J., Rohrmann, K., Gröne, E. F., et al. (2006). Cytotoxic Markers and Frequency Predict Functional Capacity of Natural Killer Cells Infiltrating Renal Cell Carcinoma. *Clin. Cancer Res.* 12, 718–725. doi:10.1158/1078-0432.ccr-05-0857

Schneiders, F. L., De Bruin, R. C. G., Van Den Eertwegh, A. J. M., Scheper, R. J., Leemans, C. R., Brakenhoff, R. H., et al. (2012). Circulating Invariant Natural Killer T-Cell Numbers Predict Outcome in Head and Neck Squamous Cell Carcinoma: Updated Analysis with 10-year Follow-Up. *Jco* 30, 567–570. doi:10.1200/jco.2011.38.8819

Schön, M. P., and Schön, M. (2008). TLR7 and TLR8 as Targets in Cancer Therapy. *Oncogene* 27, 190–199. doi:10.1038/sj.onc.1210913

Schott, A. F., Goldstein, L. J., Cristofanilli, M., Ruffini, P. A., Mccanna, S., Reuben, J. M., et al. (2017). Phase Ib Pilot Study to Evaluate Reparixin in Combination with Weekly Paclitaxel in Patients with HER-2-Negative Metastatic Breast Cancer. *Clin. Cancer Res.* 23, 5358–5365. doi:10.1158/1078-0432.ccr-16-2748

Sconocchia, G., Arriga, R., Tornillo, L., Terracciano, L., Ferrone, S., and Spagnoli, G. C. (2012). Melanoma Cells Inhibit NK Cell Functions-Letter. *Cancer Res.* 72, 5428–5429. doi:10.1158/0008-5472.can-12-1181

Sconocchia, G., Eppenberger, S., Spagnoli, G. C., Tornillo, L., Droeser, R., Caratelli, S., et al. (2014). NK Cells and T Cells Cooperate during the Clinical Course of Colorectal Cancer. *Oncoimmunology* 3, e952197. doi:10.4161/21624011.2014.952197

Sechler, J. M., Barlic, J., Grivel, J.-C., and Murphy, P. M. (2004). IL-15 Alters Expression and Function of the Chemokine Receptor CX3CR1 in Human NK Cells. *Cell. Immunol.* 230, 99–108. doi:10.1016/j.cellimm.2004.10.001

Segura, E., Kapp, E., Gupta, N., Wong, J., Lim, J., Ji, H., et al. (2010). Differential Expression of Pathogen-Recognition Molecules between Dendritic Cell Subsets Revealed by Plasma Membrane Proteomic Analysis. *Mol. Immunol.* 47, 1765–1773. doi:10.1016/j.molimm.2010.02.028

Segura, E., Valladeau-Guilemond, J., Donnadieu, M.-H., Sastre-Garau, X., Soumelis, V., and Amigorena, S. (2012). Characterization of Resident and Migratory Dendritic Cells in Human Lymph Nodes. *J. Exp. Med.* 209, 653–660. doi:10.1084/jem.20111457

Semeraro, M., Rusakiewicz, S., Minard-Colin, V., Delahaye, N. F., Enot, D., Vély, F., et al. (2015). Clinical Impact of the NKp30/B7-H6 axis in High-Risk Neuroblastoma Patients. *Sci. Transl. Med.* 7, 283ra55. doi:10.1126/scitranslmed.aaa2327

Seo, H., Jeon, I., Kim, B. S., Park, M., Bae, E. A., Song, B., et al. (2017). IL-21-mediated Reversal of NK Cell Exhaustion Facilitates Anti-tumour Immunity in MHC Class I-Deficient Tumours. *Nat. Commun.* 8, 15776–15814. doi:10.1038/ncomms15776

Shaulov, A., Yue, S., Wang, R., Joyce, R. M., Balk, S. P., Kim, H. T., et al. (2008). Peripheral Blood Progenitor Cell Product Contains Th1-Biased Noninvariant CD1d-Reactive Natural Killer T Cells: Implications for Posttransplant Survival. *Exp. Hematol.* 36, 464–472. doi:10.1016/j.exphem.2007.12.010

Shimizu, K., Iyoda, T., Yamasaki, S., Kadowaki, N., Tojo, A., and Fujii, A. S. (2020). NK and NKT Cell-Mediated Immune Surveillance against Hematological Malignancies. *Cancers (Basel)* 12. doi:10.3390/cancers12040817

Shimizu, K., Mizuno, T., Shinga, J., Asakura, M., Kakimi, K., Ishii, Y., et al. (2013). Vaccination with Antigen-Transfected, NKT Cell Ligand-Loaded, Human Cells Elicits Robust *In Situ* Immune Responses by Dendritic Cells. *Cancer Res.* 73, 62–73. doi:10.1158/0008-5472.can-12-0759

Shojaei, F., Wu, X., Qu, X., Kowanetz, M., Yu, L., Tan, M., et al. (2009). G-CSF-initiated Myeloid Cell Mobilization and Angiogenesis Mediate Tumor Refractoriness to Anti-VEGF Therapy in Mouse Models. *Proc. Natl. Acad. Sci. U.S.A.* 106, 6742–6747. doi:10.1073/pnas.0902280106

Shortman, K., and Liu, Y.-J. (2002). Mouse and Human Dendritic Cell Subtypes. *Nat. Rev. Immunol.* 2, 151–161. doi:10.1038/nri746

Sica, A., and Mantovani, A. (2012). Macrophage Plasticity and Polarization: *In Vivo* Veritas. *J. Clin. Invest.* 122, 787–795. doi:10.1172/jci59643

Sisirak, V., Faget, J., Gobert, M., Goutagny, N., Vey, N., Treilleux, I., et al. (2012). Impaired IFN-α Production by Plasmacytoid Dendritic Cells Favors Regulatory T-Cell Expansion that May Contribute to Breast Cancer Progression. *Cancer Res.* 72, 5188–5197. doi:10.1158/0008-5472.can-11-3468

Sivori, S., Vitale, M., Bottino, C., Marcenaro, E., Sanseverino, L., Parolini, S., et al. (1996). CD94 Functions as a Natural Killer Cell Inhibitory Receptor for Different HLA Class I Alleles: Identification of the Inhibitory Form of CD94 by the Use of Novel Monoclonal Antibodies. *Eur. J. Immunol.* 26, 2487–2492. doi:10.1002/eji.1830261032

Sojka, D. K., Tian, Z., and Yokoyama, W. M. (2014). Tissue-resident Natural Killer Cells and Their Potential Diversity. *Seminars Immunol.* 26, 127–131. doi:10.1016/j.smim.2014.01.010

Spanoudakis, E., Hu, M., Naresh, K., Terpos, E., Melo, V., Reid, A., et al. (2009). Regulation of Multiple Myeloma Survival and Progression by CD1d. *Blood* 113, 2498–2507. doi:10.1182/blood-2008-06-161281

Spits, H., Artis, D., Colonna, M., Diefenbach, A., Di Santo, J. P., Eberl, G., et al. (2013). Innate Lymphoid Cells - a Proposal for Uniform Nomenclature. *Nat. Rev. Immunol.* 13, 145–149. doi:10.1038/nri3365

Srivastava, M. K., Sinha, P., Clements, V. K., Rodriguez, P., and Ostrand-Rosenberg, S. (2010). Myeloid-derived Suppressor Cells Inhibit T-Cell Activation by Depleting Cystine and Cysteine. *Cancer Res.* 70, 68–77. doi:10.1158/0008-5472.can-09-2587

Steinman, R. M. (2006). Linking Innate to Adaptive Immunity through Dendritic Cells. *Novartis Found. Symp.* 279, 216–109.

Strachan, D. C., Ruffell, B., Oei, Y., Bissell, M. J., Coussens, L. M., Pryer, N., et al. (2013). CSF1R Inhibition Delays Cervical and Mammary Tumor Growth in Murine Models by Attenuating the Turnover of Tumor-Associated Macrophages and Enhancing Infiltration by CD8+T Cells. *Oncoimmunology* 2, e26968. doi:10.4161/onci.26968

Sullivan, R. J. (2019). Back to the Future: Rethinking and Retooling IL2 in the Immune Checkpoint Inhibitor Era. *Cancer Discov.* 9, 694–695. doi:10.1158/2159-8290.cd-19-0412

Sun, C., Xu, J., Huang, Q., Huang, M., Wen, H., Zhang, C., et al. (2017). High NKG2A Expression Contributes to NK Cell Exhaustion and Predicts a Poor Prognosis of Patients with Liver Cancer. *Oncoimmunology* 6, e1264562. doi:10.1080/2162402x.2016.1264562

Sun, H., Liu, L., Huang, Q., Liu, H., Huang, M., Wang, J., et al. (2019). Accumulation of Tumor-Infiltrating CD49a+ NK Cells Correlates with Poor Prognosis for Human Hepatocellular Carcinoma. *Cancer Immunol. Res.* 7, 1535–1546. doi:10.1158/2326-6066.cir-18-0757

Syn, N. L., Teng, M. W. L., Mok, T. S. K., and Soo, R. A. (2017). De-novo and Acquired Resistance to Immune Checkpoint Targeting. *Lancet Oncol.* 18, e731–e741. doi:10.1016/s1470-2045(17)30607-1

Szabó, C., Ischiropoulos, H., and Radi, R. (2007). Peroxynitrite: Biochemistry, Pathophysiology and Development of Therapeutics. *Nat. Rev. Drug Discov.* 6, 662–680. doi:10.1038/nrd2222

Tachibana, T., Onodera, H., Tsuruyama, T., Mori, A., Nagayama, S., Hiai, H., et al. (2005). Increased Intratumor Vα24-Positive Natural Killer T Cells: A Prognostic Factor for Primary Colorectal Carcinomas. *Clin. Cancer Res.* 11, 7322–7327. doi:10.1158/1078-0432.ccr-05-0877

Takahashi, R., Ijichi, H., Sano, M., Miyabayashi, K., Mohri, D., Kim, J., et al. (2020). Soluble VCAM-1 Promotes Gemcitabine Resistance via Macrophage Infiltration and Predicts Therapeutic Response in Pancreatic Cancer. *Sci. Rep.* 10, 21194. doi:10.1038/s41598-020-78320-3

Terabe, M., and Berzofsky, J. A. (2008). Chapter 8 the Role of NKT Cells in Tumor Immunity. *Adv. Cancer Res.* 101, 277–348. doi:10.1016/s0065-230x(08)00408-9

Terrén, I., Orrantia, A., Vitallé, J., Zenarruzabeitia, O., and Borrego, F. (2019). NK Cell Metabolism and Tumor Microenvironment. *Front. Immunol.* 10, 2278. doi:10.3389/fimmu.2019.02278

Tham, M., Khoo, K., Yeo, K. P., Kato, M., Prevost-Blondel, A., Angeli, V., et al. (2015). Macrophage Depletion Reduces Postsurgical Tumor Recurrence and Metastatic Growth in a Spontaneous Murine Model of Melanoma. *Oncotarget* 6, 22857–22868. doi:10.18632/oncotarget.3127

Thommen, D. S., Schreiner, J., Müller, P., Herzig, P., Roller, A., Belousov, A., et al. (2015). Progression of Lung Cancer Is Associated with Increased Dysfunction of T Cells Defined by Coexpression of Multiple Inhibitory Receptors. *Cancer Immunol. Res.* 3, 1344–1355. doi:10.1158/2326-6066.cir-15-0097

Thompson, T. W., Kim, A. B., Li, P. J., Wang, J., Jackson, B. T., Huang, K. T. H., et al. (2017). Endothelial Cells Express NKG2D Ligands and Desensitize Antitumor NK Responses. *Elife* 6, e30881. doi:10.7554/eLife.30881

Thurner, B., Haendle, I., Röder, C., Dieckmann, D., Keikavoussi, P., Jonuleit, H., et al. (1999). Vaccination with mage-3A1 Peptide-Pulsed Mature, Monocyte-Derived Dendritic Cells Expands Specific Cytotoxic T Cells and Induces Regression of Some Metastases in Advanced Stage IV Melanoma. *J. Exp. Med.* 190, 1669–1678. doi:10.1084/jem.190.11.1669

Tian, T., Gu, X., Zhang, B., Liu, Y., Yuan, C., Shao, L., et al. (2015). Increased Circulating CD14(+)HLA-DR-/low Myeloid-Derived Suppressor Cells Are Associated with Poor Prognosis in Patients with Small-Cell Lung Cancer. *Cbm* 15, 425–432. doi:10.3233/cbm-150473

Tobin, R. P., Jordan, K. R., Robinson, W. A., Davis, D., Borges, V. F., Gonzalez, R., et al. (2018). Targeting Myeloid-Derived Suppressor Cells Using All-Trans Retinoic Acid in Melanoma Patients Treated with Ipilimumab. *Int. Immunopharmacol.* 63, 282–291. doi:10.1016/j.intimp.2018.08.007

Toniolo, P. A., Liu, S., Yeh, J. E., Ye, D. Q., Barbuto, J. A. M., and Frank, D. A. (2016). Deregulation of SOCS5 Suppresses Dendritic Cell Function in Chronic Lymphocytic Leukemia. *Oncotarget* 7, 46301–46314. doi:10.18632/oncotarget.10093

Trabanelli, S., Chevalier, M. F., Martinez-Usatorre, A., Gomez-Cadena, A., Salomé, B., Lecciso, M., et al. (2017). Tumour-derived PGD2 and NKp30-B7h6 Engagement Drives an Immunosuppressive ILC2-MDSC axis. *Nat. Commun.* 8, 593. doi:10.1038/s41467-017-00678-2

Trabanelli, S., Curti, A., Lecciso, M., Salome, B., Riether, C., Ochsenbein, A., et al. (2015). CD127+ Innate Lymphoid Cells Are Dysregulated in Treatment Naive Acute Myeloid Leukemia Patients at Diagnosis. *Haematologica* 100, e257–e260. doi:10.3324/haematol.2014.119602

Trabanelli, S., Ercolano, G., Wyss, T., Gomez-Cadena, A., Falquet, M., Cropp, D., et al. (2022). c-Maf Enforces Cytokine Production and Promotes Memory-like Responses in Mouse and Human Type 2 Innate Lymphoid Cells. *EMBO J.* 2022, e109300. doi:10.15252/embj.2021109300

Trabanelli, S., Gomez-Cadena, A., Salomé, B., Michaud, K., Mavilio, D., Landis, B. N., et al. (2018). Human Innate Lymphoid Cells (ILCs): Toward a Uniform Immune-Phenotyping. *Cytometry* 94, 392–399. doi:10.1002/cyto.b.21614

Treilleux, I., Blay, J.-Y., Bendriss-Vermare, N., Ray-Coquard, I., Bachelot, T., Guastalla, J.-P., et al. (2004). Dendritic Cell Infiltration and Prognosis of Early Stage Breast Cancer. *Clin. Cancer Res.* 10, 7466–7474. doi:10.1158/1078-0432.ccr-04-0684

Tu, M. M., Abdel-Hafiz, H. A., Jones, R. T., Jean, A., Hoff, K. J., Duex, J. E., et al. (2020). Inhibition of the CCL2 Receptor, CCR2, Enhances Tumor Response to Immune Checkpoint Therapy. *Commun. Biol.* 3, 720. doi:10.1038/s42003-020-01441-y

Ubil, E., Caskey, L., Holtzhausen, A., Hunter, D., Story, C., and Earp, H. S. (2018). Tumor-secreted Pros1 Inhibits Macrophage M1 Polarization to Reduce Antitumor Immune Response. *J. Clin. Invest* 128, 2356–2369. doi:10.1172/jci97354

Umansky, V., Blattner, C., Gebhardt, C., and Utikal, J. (2016). The Role of Myeloid-Derived Suppressor Cells (MDSC) in Cancer Progression. *Vaccines (Basel)* 4, 36. doi:10.3390/vaccines4040036

Umansky, V., and Schirrmacher, V. (2001). Nitric Oxide-Induced Apoptosis in Tumor Cells. *Adv. Cancer Res.* 82, 107–131. doi:10.1016/s0065-230x(01)82004-2

Vachhani, P., and Chen, H. (2016). Spotlight on Pembrolizumab in Non-small Cell Lung Cancer: the Evidence to Date. *Onco Targets Ther.* 9, 5855–5866. doi:10.2147/OTT.S97746

Van Dalen, F. J., Van Stevendaal, M. H. M. E., Fennemann, F. L., Verdoes, M., and Ilina, O. (2018). Molecular Repolarisation of Tumour-Associated Macrophages. *Molecules* 24. doi:10.3390/molecules24010009

Vanoni, G., Ercolano, G., Candiani, S., Rutigliani, M., Lanata, M., Derré, L., et al. (2021). Human Primed ILCPs Support Endothelial Activation through NF-Kb Signaling. *Elife* 10. doi:10.7554/eLife.58838

Veglia, F., Perego, M., and Gabrilovich, D. (2018). Myeloid-derived Suppressor Cells Coming of Age. *Nat. Immunol.* 19, 108–119. doi:10.1038/s41590-017-0022-x

Versluis, M. A. C., Marchal, S., Plat, A., De Bock, G. H., Van Hall, T., De Bruyn, M., et al. (2017). The Prognostic Benefit of Tumour-Infiltrating Natural Killer Cells in Endometrial Cancer Is Dependent on Concurrent Overexpression of Human Leucocyte Antigen-E in the Tumour Microenvironment. *Eur. J. Cancer* 86, 285–295. doi:10.1016/j.ejca.2017.09.008

Vgenopoulou, S., Lazaris, A. C., Markopoulos, C., Boltetsou, E., Kyriakou, V., Kavantzas, N., et al. (2003). Immunohistochemical Evaluation of Immune Response in Invasive Ductal Breast Cancer of Not-otherwise-specified Type. *Breast* 12, 172–178. doi:10.1016/s0960-9776(03)00004-3

Vijayan, D., Young, A., Teng, M. W. L., and Smyth, M. J. (2017). Targeting Immunosuppressive Adenosine in Cancer. *Nat. Rev. Cancer* 17, 709–724. doi:10.1038/nrc.2017.86

Villani, A. C., Satija, R., Reynolds, G., Sarkizova, S., Shekhar, K., Fletcher, J., et al. (2017). Single-cell RNA-Seq Reveals New Types of Human Blood Dendritic Cells, Monocytes, and Progenitors. *Science* 356. doi:10.1126/science.aah4573

Vivier, E., Artis, D., Colonna, M., Diefenbach, A., Di Santo, J. P., Eberl, G., et al. (2018). Innate Lymphoid Cells: 10 Years on. *Cell* 174, 1054–1066. doi:10.1016/j.cell.2018.07.017

Vivier, E., Raulet, D. H., Moretta, A., Caligiuri, M. A., Zitvogel, L., Lanier, L. L., et al. (2011). Innate or Adaptive Immunity? the Example of Natural Killer Cells. *Science* 331, 44–49. doi:10.1126/science.1198687

Vivier, E., Ugolini, S., Blaise, D., Chabannon, C., and Brossay, L. (2012). Targeting Natural Killer Cells and Natural Killer T Cells in Cancer. *Nat. Rev. Immunol.* 12, 239–252. doi:10.1038/nri3174

Vuk-Pavlović, S., Bulur, P. A., Lin, Y., Qin, R., Szumlanski, C. L., Zhao, X., et al. (2010). Immunosuppressive CD14+HLA-DRlow/- Monocytes in Prostate Cancer. *Prostate* 70, 443–455. doi:10.1002/pros.21078

Wallin, J. J., Bendell, J. C., Funke, R., Sznol, M., Korski, K., Jones, S., et al. (2016). Atezolizumab in Combination with Bevacizumab Enhances Antigen-specific T-Cell Migration in Metastatic Renal Cell Carcinoma. *Nat. Commun.* 7, 12624. doi:10.1038/ncomms12624

Wanderley, C. W., Colón, D. F., Luiz, J. P. M., Oliveira, F. F., Viacava, P. R., Leite, C. A., et al. (2018). Paclitaxel Reduces Tumor Growth by Reprogramming Tumor-Associated Macrophages to an M1 Profile in a TLR4-dependent Manner. *Cancer Res.* 78, 5891–5900. doi:10.1158/0008-5472.CAN-17-3480

Wang, W., Xia, X., Mao, L., and Wang, S. (2019a). The CCAAT/Enhancer-Binding Protein Family: Its Roles in MDSC Expansion and Function. *Front. Immunol.* 10, 1804. doi:10.3389/fimmu.2019.01804

Wang, X., Sathe, A. A., Smith, G. R., Ruf-Zamojski, F., Nair, V., Lavine, K. J., et al. (2020). Heterogeneous Origins and Functions of Mouse Skeletal Muscle-Resident Macrophages. *Proc. Natl. Acad. Sci. U.S.A.* 117, 20729–20740. doi:10.1073/pnas.1915950117

Wang, Z., Wang, Z., Li, B., Wang, S., Chen, T., and Ye, Z. (2019b). Innate Immune Cells: A Potential and Promising Cell Population for Treating Osteosarcoma. *Front. Immunol.* 10, 1114. doi:10.3389/fimmu.2019.01114

Wculek, S. K., Cueto, F. J., Mujal, A. M., Melero, I., Krummel, M. F., and Sancho, D. (2020). Dendritic Cells in Cancer Immunology and Immunotherapy. *Nat. Rev. Immunol.* 20, 7–24. doi:10.1038/s41577-019-0210-z

Weil, S., Memmer, S., Lechner, A., Huppert, V., Giannattasio, A., Becker, T., et al. (2017). Natural Killer Group 2D Ligand Depletion Reconstitutes Natural Killer Cell Immunosurveillance of Head and Neck Squamous Cell Carcinoma. *Front. Immunol.* 8, 387. doi:10.3389/fimmu.2017.00387

Wendel, M., Galani, I. E., Suri-Payer, E., and Cerwenka, A. (2008). Natural Killer Cell Accumulation in Tumors Is Dependent on IFN-γ and CXCR3 Ligands. *Cancer Res.* 68, 8437–8445. doi:10.1158/0008-5472.can-08-1440

Wesolowski, R., Sharma, N., Reebel, L., Rodal, M. B., Peck, A., West, B. L., et al. (2019). Phase Ib Study of the Combination of Pexidartinib (PLX3397), a CSF-1R Inhibitor, and Paclitaxel in Patients with Advanced Solid Tumors. *Ther. Adv. Med. Oncol.* 11, 1758835919854238. doi:10.1177/1758835919854238

Wu, D. Y., Segal, N. H., Sidobre, S., Kronenberg, M., and Chapman, P. B. (2003). Cross-presentation of Disialoganglioside GD3 to Natural Killer T Cells. *J. Exp. Med.* 198, 173–181. doi:10.1084/jem.20030446

Wu, L., Du, H., Li, Y., Qu, P., and Yan, C. (2011). Signal Transducer and Activator of Transcription 3 (Stat3C) Promotes Myeloid-Derived Suppressor Cell Expansion and Immune Suppression during Lung Tumorigenesis. *Am. J. Pathology* 179, 2131–2141. doi:10.1016/j.ajpath.2011.06.028

Wu, Y., and Shen, J. (2020). Innate Lymphoid Cells in Crohn's Disease. *Front. Immunol.* 11, 554880. doi:10.3389/fimmu.2020.554880

Xie, J., Liu, M., Li, Y., Nie, Y., Mi, Q., and Zhao, S. (2014). Ovarian Tumor-Associated microRNA-20a Decreases Natural Killer Cell Cytotoxicity by Downregulating MICA/B Expression. *Cell Mol. Immunol.* 11, 495–502. doi:10.1038/cmi.2014.30

Xiu, B., Lin, Y., Grote, D. M., Ziesmer, S. C., Gustafson, M. P., Maas, M. L., et al. (2015). IL-10 Induces the Development of Immunosuppressive CD14+HLA-DRlow/− Monocytes in B-Cell Non-hodgkin Lymphoma. *Blood Cancer J.* 5, e328. doi:10.1038/bcj.2015.56

Yan, W., Liu, X., Ma, H., Zhang, H., Song, X., Gao, L., et al. (2015). Tim-3 Fosters HCC Development by Enhancing TGF-β-Mediated Alternative Activation of Macrophages. *Gut* 64, 1593–1604. doi:10.1136/gutjnl-2014-307671

Yanagisawa, K., Seino, K.-i., Ishikawa, Y., Nozue, M., Todoroki, T., and Fukao, K. (2002). Impaired Proliferative Response of Vα24 NKT Cells from Cancer Patients against α-Galactosylceramide. *J. Immunol.* 168, 6494–6499. doi:10.4049/jimmunol.168.12.6494

Yang, L., Dong, Y., Li, Y., Wang, D., Liu, S., Wang, D., et al. (2019). IL-10 Derived from M2 Macrophage Promotes Cancer Stemness via JAK1/STAT1/NF-κB/Notch1 Pathway in Non-small Cell Lung Cancer. *Int. J. Cancer* 145, 1099–1110. doi:10.1002/ijc.32151

Yao, W., Ba, Q., Li, X., Li, H., Zhang, S., Yuan, Y., et al. (2017). A Natural CCR2 Antagonist Relieves Tumor-Associated Macrophage-Mediated Immunosuppression to Produce a Therapeutic Effect for Liver Cancer. *EBioMedicine* 22, 58–67. doi:10.1016/j.ebiom.2017.07.014

Yona, S., Kim, K.-W., Wolf, Y., Mildner, A., Varol, D., Breker, M., et al. (2013). Fate Mapping Reveals Origins and Dynamics of Monocytes and Tissue Macrophages under Homeostasis. *Immunity* 38, 79–91. doi:10.1016/j.immuni.2012.12.001

Zecca, A., Barili, V., Rizzo, D., Olivani, A., Biasini, E., Laccabue, D., et al. (2021). Intratumor Regulatory Noncytotoxic NK Cells in Patients with Hepatocellular Carcinoma. *Cells*. doi:10.3390/cells10030614

Zhang, J., Han, X., Shi, H., Gao, Y., Qiao, X., Li, H., et al. (2020). Lung Resided Monocytic Myeloid-Derived Suppressor Cells Contribute to Premetastatic Niche Formation by Enhancing MMP-9 Expression. *Mol. Cell. Probes* 50, 101498. doi:10.1016/j.mcp.2019.101498

Zhang, N., and Bevan, M. J. (2012). TGF-β Signaling to T Cells Inhibits Autoimmunity during Lymphopenia-Driven Proliferation. *Nat. Immunol.* 13, 667–673. doi:10.1038/ni.2319

Zhang, Q.-F., Yin, W.-W., Xia, Y., Yi, Y.-Y., He, Q.-F., Wang, X., et al. (2017). Liver-infiltrating CD11b−CD27− NK Subsets Account for NK-Cell Dysfunction in Patients with Hepatocellular Carcinoma and Are Associated with Tumor Progression. *Cell Mol. Immunol.* 14, 819–829. doi:10.1038/cmi.2016.28

Zhang, Q., Bi, J., Zheng, X., Chen, Y., Wang, H., Wu, W., et al. (2018). Blockade of the Checkpoint Receptor TIGIT Prevents NK Cell Exhaustion and Elicits Potent Anti-tumor Immunity. *Nat. Immunol.* 19, 723–732. doi:10.1038/s41590-018-0132-0

Zhang, W., Zhu, X.-D., Sun, H.-C., Xiong, Y.-Q., Zhuang, P.-Y., Xu, H.-X., et al. (2010). Depletion of Tumor-Associated Macrophages Enhances the Effect of Sorafenib in Metastatic Liver Cancer Models by Antimetastatic and Antiangiogenic Effects. *Clin. Cancer Res.* 16, 3420–3430. doi:10.1158/1078-0432.ccr-09-2904

Zhang, Y., and Huang, B. (2017). The Development and Diversity of ILCs, NK Cells and Their Relevance in Health and Diseases. *Adv. Exp. Med. Biol.* 1024, 225–244. doi:10.1007/978-981-10-5987-2_11

Zhen, Z. J., Ling, J. Y., Cai, Y., Luo, W. B., and He, Y. J. (2013). Impact of HLA-E Gene Polymorphism on HLA-E Expression in Tumor Cells and Prognosis in Patients with Stage III Colorectal Cancer. *Med. Oncol.* 30, 482–489. doi:10.1007/s12032-013-0482-2

Zheng, M., Sun, R., Wei, H., and Tian, Z. (2016). NK Cells Help Induce Anti-hepatitis B Virus CD8+ T Cell Immunity in Mice. *J. I.* 196, 4122–4131. doi:10.4049/jimmunol.1500846

Zheng, P., Chen, L., Yuan, X., Luo, Q., Liu, Y., Xie, G., et al. (2017). Exosomal Transfer of Tumor-Associated Macrophage-Derived miR-21 Confers Cisplatin Resistance in Gastric Cancer Cells. *J. Exp. Clin. Cancer Res.* 36, 53. doi:10.1186/s13046-017-0528-y

Zhu, H., Gu, Y., Xue, Y., Yuan, M., Cao, X., and Liu, Q. (2017a). CXCR2+ MDSCs Promote Breast Cancer Progression by Inducing EMT and Activated T Cell Exhaustion. *Oncotarget* 8, 114554–114567. doi:10.18632/oncotarget.23020

Zhu, J., Powis De Tenbossche, C. G., Cané, S., Colau, D., Van Baren, N., Lurquin, C., et al. (2017b). Resistance to Cancer Immunotherapy Mediated by Apoptosis of Tumor-Infiltrating Lymphocytes. *Nat. Commun.* 8, 1404. doi:10.1038/s41467-017-00784-1

Zhu, Y., Herndon, J. M., Sojka, D. K., Kim, K.-W., Knolhoff, B. L., Zuo, C., et al. (2017c). Tissue-Resident Macrophages in Pancreatic Ductal Adenocarcinoma Originate from Embryonic Hematopoiesis and Promote Tumor Progression. *Immunity* 47, 323–338. doi:10.1016/j.immuni.2017.07.014

Zhu, Y., Knolhoff, B. L., Meyer, M. A., Nywening, T. M., West, B. L., Luo, J., et al. (2014). CSF1/CSF1R Blockade Reprograms Tumor-Infiltrating Macrophages and Improves Response to T-Cell Checkpoint Immunotherapy in Pancreatic Cancer Models. *Cancer Res.* 74, 5057–5069. doi:10.1158/0008-5472.can-13-3723

Zitvogel, L., Apetoh, L., Ghiringhelli, F., and Kroemer, G. (2008). Immunological Aspects of Cancer Chemotherapy. *Nat. Rev. Immunol.* 8, 59–73. doi:10.1038/nri2216

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REVIEW
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Stromal and Immune Cell Dynamics in Tumor Associated Tertiary Lymphoid Structures and Anti-Tumor Immune Responses

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Tertiary lymphoid structures (TLS) are ectopic lymphoid organs that have been observed in chronic inflammatory conditions including cancer, where they are thought to exert a positive effect on prognosis. Both immune and non-immune cells participate in the genesis of TLS by establishing complex cross-talks requiring both soluble factors and cell-to-cell contact. Several immune cell types, including T follicular helper cells (Tfh), regulatory T cells (Tregs), and myeloid cells, may accumulate in TLS, possibly promoting or inhibiting their development. In this manuscript, we propose to review the available evidence regarding specific aspects of the TLS formation in solid cancers, including 1) the role of stromal cell composition and architecture in the recruitment of specific immune subpopulations and the formation of immune cell aggregates; 2) the contribution of the myeloid compartment (macrophages and neutrophils) to the development of antibody responses and the TLS formation; 3) the immunological and metabolic mechanisms dictating recruitment, expansion and plasticity of Tregs into T follicular regulatory cells, which are potentially sensitive to immunotherapeutic strategies directed to costimulatory receptors or checkpoint molecules.

Keywords: TLS, Treg, Tfh, neutrophils, tumor stroma

Abbreviations: APC, antigen presenting cells; APRIL, proliferation-inducing ligand; BAFF, B-cell activating factor; CTLA-4, Cytotoxic T-Lymphocyte Antigen-4; DC, dendritic cells; FRC, fibroblastic reticular cell; GC, germinal center; GITR, Glucocorticoid-induced tumour necrosis factor receptor-related protein; IFNγ, interferon γ; LN, lymph node; NSCLC, non-small cell lung cancer; PD-1, Programmed Death-1; PD-L1, Programmed-Death Ligand 1; SLO, Secondary lymphoid organs; TEM, Teffector memory; Tfh, T follicular helper; Th1, T helper 1; TLS, tertiary lymphoid structures; TNFα, tumor necrosis factor α; TRM, Tissue resident memory; TME, tumor microenvironment.

INTRODUCTION

Human solid cancers have been traditionally classified into subtypes based on disease stage and histology for prognostic purposes and selection of clinical treatments. In the past decades the development of new therapeutic strategies and the accumulating knowledge on tumor biology has called for a more comprehensive classification system that takes into account the complexity of the disease in terms of qualitative heterogeneity and response to therapies. Indeed, it is now well recognized that tumors are heterogeneous entities where the stromal components (fibroblasts, blood vessels and infiltrating leukocytes) are major determinants of disease progression and can be therapeutic targets along with transformed cells. In this view, a detailed characterization of the tumor microenvironment as a whole represents a pivotal tool to fully exploit the potential of new therapies and ultimately improve patients' survival and quality of life.

Infiltrating leukocytes often outnumber transformed cells in tumor masses and can grow in organized structures resembling secondary lymphoid organs (SLO), known as tumor associated tertiary lymphoid structures (TLS). The number and qualitative composition of TLS can vary significantly among tumor types but there is now consensus about the overall positive effects they exert on prognosis and response to immunotherapy. It is therefore important to include the immune profile when characterizing tumors in order to make appropriate decisions in terms of therapy and disease clinical management. In this review the general features of tumor-associated TLS and of their formation will be described. The role of the TLS-associated cell types, mainly T and B lymphocytes subsets and myeloid cells, will also be discussed and related to the induction of tumor specific immunity and to the prognostic significance of these structures.

TERTIARY LYMPHOID STRUCTURES: GENERAL FEATURES AND PROGNOSTIC SIGNIFICANCE IN CANCER

TLS are ectopic lymphoid organs that originate within nonlymphoid tissues during chronic inflammatory conditions, such as persistent pathogen infection, autoimmune disorders, allograft rejection and cancer (Ansel and Cyster, 2001; Aloisi and Pujol-Borrell, 2006). TLS, also named tertiary lymphoid organs (TLO), develop in peripheral tissues in close proximity to

TABLE 1 | TLS features in human cancers and effects on prognosis and/or therapy.

Tumor	TLS features	Effect on prognosis and/or therapy	References
NSCLC	Mature DC (DC-LAMP$^+$) Th1 CD3$^+$ CD4$^+$ CD3$^+$CD8$^+$ T cells B cells Ki67$^+$, plasmacells Neutrophils (APC-like)	↑ OS Mature DC not detected in metastasis	(Dieu-Nosjean et al., 2008; Germain et al., 2014; Goc et al., 2014; Singhal et al., 2016)
Breast cancer	nd	Association with higher tumor grade	(Figenschau et al., 2015; Sofopoulos et al., 2019; Zhang et al., 2021)
	↑ Treg	↑ risk of recurrence and death	(Gobert et al., 2009; Miao et al., 2021; Song et al., 2019)
	↑ B cells, ↓ Treg	↑ OS	(Germain et al., 2021)
Ovarian cancer (HGSC)	Plasmacells CD8$^+$ T cells	nd	(Kroeger et al., 2016)
	Neutrophils	nd	(Montfort et al., 2017)
Prostate cancer	↑ Th1, CD8$^+$ ↓ Treg	Association with spontaneous remission	(Garcia-Hernandez et al., 2017)
Colon cancer	Tfh	nd	(Schweiger et al., 2016)
	FDC and GC B cells	↓ risk of recurrence with mature TLS	(Posch et al., 2018)
	↑ Th2 and macrophages ↑ Tfr (Bcl6$^+$)	↑ risk of recurrence	(Yamaguchi et al., 2020)
Pancreatic cancer	Intratumoral localization with Th1-Th17 signature	↑ OS and DFS	(Hiraoka et al., 2015)
Gastric cancer	↑ eTreg PD-1hi	Disease progression upon nivolumab tx	(Kamada et al., 2019)
HCC	↑ T CD8$^+$, B cells ↓ Treg	↓ risk of early tumor recurrence	(Li et al., 2020)

NSCLC, non-small cell lung cancer; OS, overall survival; DFS, disease free survival; HCC, hepatocellular carcinoma; nd, not determined; HGSC, high grade serous cancer; APC, antigen presenting cell; eTreg, effector Treg.

pathological loci, under stress conditions and as the result of persistent antigen stimulation (Sautes-Fridman et al., 2016). TLS structure resembles that of conventional secondary lymphoid organs (for example, lymph nodes) including B cell zones, T cell zones, marginal zones with activated macrophages and dendritic cells, reticular fibroblast cell networks and vasculature permissive to immune cell extravasation (Aoyama et al., 2021). However, while lymph nodes are encapsulated, TLS represent a congregation of immune and stromal cells confined within an organ or tissue (Aoyama et al., 2021). TLS form *de novo* in the microenvironment of solid tissues in response to prolonged inflammatory stimuli including cancer and may dissipate upon the resolution of inflammation (Moyron-Quiroz et al., 2004). TLS can additionally foster tumor antigen presentation, T cell activation and clonal expansion (Joshi et al., 2015; Zhu et al., 2015), activation of antigen-presenting cells (APC) (Hughes et al., 2016), germinal center (GC) formation (Silina et al., 2018), and B cell class switching (Schroder et al., 1996). Various types of innate and adaptive immune cells comprising macrophages, DCs, mast cells, as well as T and B cells, are recruited and activated by inflammatory mediators, especially cytokines, chemokines and adhesion molecules to the site of persistent inflammation and participate to the formation of TLS (Weinstein and Storkus, 2015; Helmink et al., 2020). As compared to secondary lymphoid structures, TLS exhibit a fair amount of heterogeneity in terms of cellular composition and topographical localization within tissue types.

TLS are lymphoid aggregates characterized by two distinct B and T compartments. Fibroblastic reticular cell networks, $PNAd^+$ High Endothelial Venules (HEVs) and follicular dendritic cells are found within T cell zones. On the other hand, B compartment consists of B cell follicles primarily composed of naïve B cells with evidence for class switching and reactive GC in B cell zones, and expression of the enzyme activation-induced cytidine deaminase (AID), required for the initiation of somatic hypermutation and immunoglobulin gene class switching (Muramatsu et al., 2000; Neyt et al., 2012; Dieu-Nosjean et al., 2014; Jones et al., 2016). At an early stage, TLS show a different composition and primarily consist of T and B cells without formation of follicles with evidence of GC.

Tumor-associated TLS from different tumor types vary in cellular composition and organization. In fact, they variably contain B lymphocytes with immature, naïve, activated, memory and plasma cell phenotypes (Sautes-Fridman et al., 2019). In non-small cell lung cancer (NSCLC), tumor-associated TLS include large numbers of mature dendritic cells $DC\text{-}LAMP^+$, which are not detected in lung metastatic neoplasms. The density of mature DC within primary lung tumor TLS has been found to be related to the amount of intratumoral Th1 and $CD8^+$ T cell infiltration, and positively influences the effectiveness of antitumoral cytotoxic immune response. Thus, these findings seem to indicate mature DC as possible biomarkers of clinical outcome and as predictors of long-term survival (Dieu-Nosjean et al., 2008; Dieu-Nosjean et al., 2014; Goc et al., 2014). TLS in breast cancer commonly contain T helper follicular (Tfh) cells (Gobert et al., 2009), whereas those associated with prostate and lung metastatic colon cancer contain large numbers of regulatory T cells (Tregs) (Kang et al., 2021). A high density of Tfh is commonly reported in TLS in breast cancer (Rubin and Kan, 1985), although it is also documented in those associated with prostatic adenocarcinoma (Garcia-Hernandez et al., 2017) and colorectal adenocarcinoma with pulmonary metastasis (Schweiger et al., 2016). Tfh and Treg cells have been shown to act in a complex balance in TLS formation and influence the composition of the neoplastic chronic inflammatory microenvironment. Gu-Trantien et al. demonstrated that, in breast cancer models, Treg accumulation occurs in response to antigen-induced IL-2 production and contributes to the suppression of Th1-mediated adaptive immune response. Later, the IL-2-depleted microenvironment induces the differentiation of some activated $CD4^+$ T cells into CXCL13-producing Tfh, capable of sustaining TLS formation and tumor-infiltrating B cell recruitment (Gu-Trantien et al., 2013).

Well-organized TLS have been described in several solid tumors with different topographic locations either in close contact with malignant cells and in peritumoral areas, and are generally considered a favorable prognostic factor (Sautes-Fridman et al., 2016; Sautes-Fridman et al., 2019). Several studies documented the presence of intratumoral TLS with a non-classical organization (without the evidence of discrete T- and B-cell compartments) in hepatocarcinoma (Finkin et al., 2015) and renal cell carcinoma presenting pulmonary metastasis (Remark et al., 2013). Other studies highlighted peritumoral TLS in proximity to the tumor-invasive margin (Munoz-Erazo et al., 2020).

TLS are privileged sites where the activation and the maintenance of local and systemic T and B cell response against tumor antigens occur (Heinhuis et al., 2019) and decelerate neoplastic progression (Dieu-Nosjean et al., 2016). Several studies reported their presence in NSCLC (Germain et al., 2014), colorectal cancer (McMullen et al., 2010; Di Caro et al., 2014), breast (Martinet et al., 2011), pancreatic (Hiraoka et al., 2015; Castino et al., 2016) and gastric carcinomas (Hennequin et al., 2016), oral cancer (Wirsing et al., 2018) as well as ovarian cancer (Milne et al., 2009) and melanoma (Ladanyi et al., 2007; Engelhard et al., 2018; Sautes-Fridman et al., 2019).

Numerous preclinical and clinical studies attribute varied roles to TLS in accordance with their structure or with the neoplastic characteristics related to either the site of origin or the location of the neoplasm. Indeed TLS promote a vigorous immune response intratumorally, constituting a barrier to disease progression, and also modulate immunity in the tumor microenvironment (Engelhard et al., 2018). The presence of TLS correlates to the activation of the immune response and for this reason they are considered a biomarker for stratifying untreated cancer patients' survival risk and an intriguing and promising target for predicting the efficacy of new immunotherapies (Colbeck et al., 2017a; Helmink et al., 2020). Although the prognostic impact of tumor-associated TLS has been extensively investigated, the immune response that occurs in TLS and the significance of their prognostic role remain incomplete. Several studies have indicated a significant correlation between TLS density and higher rates of disease-free survival and overall

patient survival (Sautes-Fridman et al., 2019), although there are exceptions (Figenschau et al., 2015; Finkin et al., 2015). However, it is observed that patient survival depends strictly on TLS' classical or non-classical organization and also peri- and intratumoral localization. Tumor-associated TLS and/or stromal cells (as high endothelial venules, HEVs) can be extratumoral, positioned at or outside the tumor invasive margin, or intratumoral, located within the true tumor mass or tumor nests. The importance of TLS/HEVs in enabling infiltration of T cells into the tumor has been demonstrated. Indeed, unlike intratumoral structures, extratumoral HEVs were not associated with increased tumor-infiltrating lymphocyte (TIL) frequencies (Martinet et al., 2011; Martinet et al., 2012; Bento et al., 2015). Similarly, while extratumoral TLS density was not a prognostic marker in pancreatic cancer patients, intratumoral TLS were an independent favorable prognosticator (Hiraoka et al., 2015). However, the relationship between tumor-associated TLS and patient outcome appears to depend on many parameters, including cancer type, disease stage, and quality of the immune infiltrate (**Table 1**). As discussed in detail thereafter, high frequencies of regulatory T cells in TLS can suppress antitumor immune responses (Joshi et al., 2015).

For this reason, we believe that greater knowledge about TLS development, composition, and function may offer new therapeutic opportunities to modulate antitumor immunity. In recent years the tumor microenvironment has been considered as an active niche in a state of dynamic evolution (Rosenthal et al., 2019). In fact the complex cross-talk between the neoplastic subclones and immune cells represent a crucial event for neoplastic progression (Locy et al., 2018). Although tumor-associated TLS are usually associated with higher densities of $CD8^+$ intratumoral lymphocytes, multivariate studies performed on NSCLC and colorectal cancer series have demonstrated that their prognostic value is independent of TIL density (Di Caro et al., 2014; Goc et al., 2014). Furthermore, intratumoral TLS are more significantly associated with longer patient survival than peritumoral ones in pancreatic adenocarcinoma (Hiraoka et al., 2015) and also in early stage hepatocarcinoma (Li et al., 2020). Wirsing et al. reported that oral squamous cell carcinoma patients with high TLS density tended to survive longer, although this was not statistically significant (Wirsing et al., 2014).

Bertucci et al. detected a higher expression of TLS signatures with higher sensitivity to immune checkpoint inhibitors in inflammatory breast cancer (Bertucci et al., 2021). In breast cancers, TLS were not significantly correlated with clinical variables (patient age, tumor size) but were considerably influenced by other pathological parameters (e.g., tumor differentiation grade, lymphovascular invasion status and pTNM stage), confirming their key role in the neoplastic progression (Zhang et al., 2021). Patients with $HER\text{-}2^+$ breast carcinoma featuring TLS, that were treated with chemotherapy and/or HER-2 targeted therapy, showed a good treatment response to be attributed also to active antitumor immunity of TLS (Luen et al., 2017).

Some studies highlighted a correlation between TLS and a higher frequency of lymph nodal metastasis in high grade breast carcinoma, suggesting their unfavorable prognostic value and, at the same time, the necessity of associating TLS with other prognostic parameters (Figenschau et al., 2015; Sofopoulos et al., 2019).

The prognostic value of TLS has been particularly well-explored in colorectal cancer (Schweiger et al., 2016; Posch et al., 2018; Trajkovski et al., 2018). Yamaguchi et al. performed experiments aimed to investigate the cellular composition of TLS and to assess which cytotype correlates with disease recurrence. Five types of TLS (GC-rich, B-cell rich, follicular DC rich, Th (CD4) rich, and CTL (B/Th rich) were identified in term of cellular composition, and the density of $CD4^+$ T cells and macrophages was associated with a higher rate of relapse in patients (Yamaguchi et al., 2020). On the contrary, Posch et al. delineated only three types of TLS, based on the composition of the aggregates and the functional state of a single cytotype within TLS, distinguishing the early TLS (dense aggregates with undifferentiated FDC); Primary follicle-like TLS (cluster of B cells with FDC networks but without GC); and follicle-like secondary TLS (with GC), and showing that a high percentage of mature TLS correlated with a better prognosis (Posch et al., 2018). In triple negative breast cancer, Seow et al., through a multiplex imaging analysis, identified a high density of plasma cells within TLS, suggesting a functional role for B cells in outcome (Seow et al., 2020).

Schlosser et al. demonstrated that, in esophagogastric adenocarcinoma patients, B cells within TLS produced tumor-specific antibodies, confirming their functional role in decelerating tumor growth (Schlosser et al., 2019).

CELL MEDIATED AND ANTIBODY RESPONSES IN CANCER: CONNECTION WITH TERTIARY LYMPHOID STRUCTURES

The role of adaptive immune responses in controlling tumor onset and evolution is now well established and supported by substantial preclinical and clinical evidence. Cells from nascent tumors can indeed be recognized by cytotoxic T cells since they display on their membrane peptide epitopes associated with major histocompatibility complex-class I (MHC-I) molecules that can drive tumor rejection. The intrinsic genetic instability of transformed cells is a major source of tumor antigens and, as such, the trigger of tumor specific adaptive immune responses. Nevertheless, such responses can promote the selection of poorly immunogenic tumor cell variants that escape immune control and can ultimately lead to cancer development (Mittal et al., 2014; Schumacher and Schreiber, 2015).

Animal models have highlighted the protective role of T cells and IFN-γ in tumor development. Mice lacking the adaptive arm of the immune system or unresponsive to IFN-γ (IFNGR1 and STAT1 ko) show increased susceptibility to both carcinogen-induced and spontaneous tumorigenesis (Kaplan et al., 1998; Smyth et al., 2000; Shankaran et al., 2001). In human cancers the infiltration of both $CD8^+$ and $CD4^+$ T lymphocytes is a common characteristic of tumors that have a favorable prognosis (Fridman et al., 2017; Paijens et al., 2021). $CD8^+$ T cells exert their

antitumor function both directly, by killing tumor cells expressing tumor-associated antigens on MHC-I molecules, and indirectly, by releasing proinflammatory cytokines (such as IFN-γ and TNF-α) that activate other leukocytes and sustain their antitumor activities. The infiltration of $CD8^+$ T cells with an effector memory phenotype (TEM) positively correlates with overall and disease free survival in both colorectal and breast cancer patients (Pages et al., 2005; Galon et al., 2006; Bindea et al., 2013; Ahmadvand et al., 2019). More recently, a protective role of tissue resident memory (TRM) $CD8^+$ T cells, that do not recirculate in blood, has emerged in several solid cancers. In both human and murine tumors, infiltration of $CD103^+$ $CD8^+$ T cells (a marker for TRM) is associated with improved survival (Paijens et al., 2021). Interestingly, non-tumor-specific TRM have been identified in tumor masses that can contribute to the antitumor immune response via a bystander effect (Simoni et al., 2018; Rosato et al., 2019). In mouse models of melanoma and colon carcinoma, boosting of virus-specific TRM via peptide vaccination resulted in recruitment and activation of both natural killer and dendritic cells and in upregulation of programmed death-ligand 1 (PD-L1), thus sensitizing tumors to immune checkpoint inhibitors-based immunotherapy (Rosato et al., 2019).

$CD4^+$ T cells represent a heterogeneous subset of lymphocytes with high phenotypical and functional plasticity and can be considered as the playmakers of the immune system. Several T helper cell subsets have been characterized, each one featuring a specific molecular signature, that are required for both priming of $CD8^+$ and B lymphocytes and for optimal antigen presentation by dendritic cells through the CD40L-CD40 axis. In line with their heterogeneity, $CD4^+$ T cells can play important yet opposing roles in antitumor immunity. T helper 1 (Th1) polarized $CD4^+$ T cells sustain inflammation and cytotoxic cell function and survival through production of IFN-γ and other cytokines (IL-15, IL-12) and are usually associated with a good prognosis in several cancer types (Bindea et al., 2013; Chraa et al., 2019). Likewise, detection of Tfh has been associated with improved prognosis in breast and colorectal cancers (Paijens et al., 2021). Tfh presence is indeed suggestive of TLS, and thus of antitumoral cytotoxic and antibody responses. On the other hand, Foxp3-expressing Tregs are the $CD4^+$ T cell subset devoted to maintaining immune homeostasis by suppressing the effector functions of both innate and adaptive immunity. Tregs are expanded in tumors where they promote disease progression by inhibiting antitumor immunity through several mechanisms, including release of immune-suppressive cytokines (IL-10, TGF-β) and deprivation of nutrients, growth factors (IL-2) and costimulatory signals. Treg infiltration is predictive of poor prognosis and their targeted depletion is regarded as an appealing strategy for immunotherapy, even though clinical attempts have not achieved substantial efficacy so far. On the one side, the Treg-depleting activity of anti-CTLA4 antibodies described in mouse models (Selby et al., 2013; Simpson et al., 2013) has not been replicated in human cancers (Sharma et al., 2019) and subsequent attempts of targeting tumor infiltrating Tregs by an anti-CCR4 antibody were limited by concomitant depletion of effector $CD4^+$ and $CD8^+$ T cells (Kurose et al., 2015). Recently, Campbell and coworkers proposed a CCR8-dependent depletion of tumor infiltrating Tregs based on the highly restricted expression of this receptor on tumor infiltrating Tregs as compared to both their circulating counterpart and to intratumoral effector T cells in different human tumors. Treatment of tumor bearing mice with the anti-CCR8 antibody was highly specific to tumor infiltrating Tregs, sparing both intratumoral effector T cells and Tregs in non-tumor tissues and ultimately enabling effective and long-lasting antitumor immunity (Campbell et al., 2021).

A strong endorsement to the significance of functional T cell responses in cancer control came from the licensing of immune checkpoint inhibitors (ICIs) for immunotherapy. ICIs are monoclonal antibodies targeting molecules involved in downregulation of effector T cell functions, typically CTLA-4, PD-1 and its ligand PD-L1 (Pardoll, 2012; Topalian et al., 2015). CTLA-4 and PD-1 expression is upregulated upon T cell activation and the latter has been associated with T cell functional exhaustion in both chronic viral infections and cancer (Barber et al., 2006; Keir et al., 2008). Hence, preventing PD-1 interactions with its ligands PD-L1 and PD-L2 in the tumor microenvironment is expected to restore the cytotoxic functions of exhausted tumor-specific T cells and has achieved significant success in the management of advanced stage solid tumors.

Despite having been disregarded by the majority of studies on prognosis and response to immunotherapy so far, accumulating evidence indicates that B lymphocytes can exert both anti- and pro-tumoral activities. Similarly to T cells, B cells are indeed highly versatile leukocytes endowed with both effector and modulatory functions. Besides being the source of antibodies, B cells can effectively prime $CD4^+$ T cells as professional APC (Bruno et al., 2017; Hong et al., 2018; Hua and Hou, 2020) and participate in tolerance maintenance as IL-10 producing B regulatory cells (Bregs) (Rosser and Mauri, 2015). Infiltration of B cells is often associated with a good prognosis in different tumor types and can be predictive of response to immunotherapy. Both in melanoma and soft tissue sarcoma patients, B cell abundance and the concomitant detection of TLS was associated with improved survival and to a better response rate to ICI therapy with the anti-PD-1 pembrolizumab (Helmink et al., 2020; Petitprez et al., 2020). Of note, in the sarcoma study, B cells appeared to be the strongest prognostic factor, irrespective of high or low $CD8^+$ T cells and other cytotoxic cell content (Petitprez et al., 2020). Evidence of antibody response induction has been identified in different tumor types, including melanoma and breast cancer (Garaud et al., 2019; N et al., 2021). Insights on the significance of antibody responses in cancer came from studies on breast cancer after the approval of the therapeutic antibody trastuzumab. Trastuzumab targets the HER2 receptor, overexpressed by a subtype of breast tumors, and has been shown over the years to induce both antibody-dependent cell cytotoxicity (ADCC) and antibody-dependent cell phagocytosis (ADCP) by engaging the Fcγ receptors on natural killer cells, monocytes and macrophages (Park et al., 2010; Tsao et al., 2019; Ehlers et al., 2021; Upton et al., 2021). The detection of high levels of HER2-specific autoantibodies in a cohort of breast

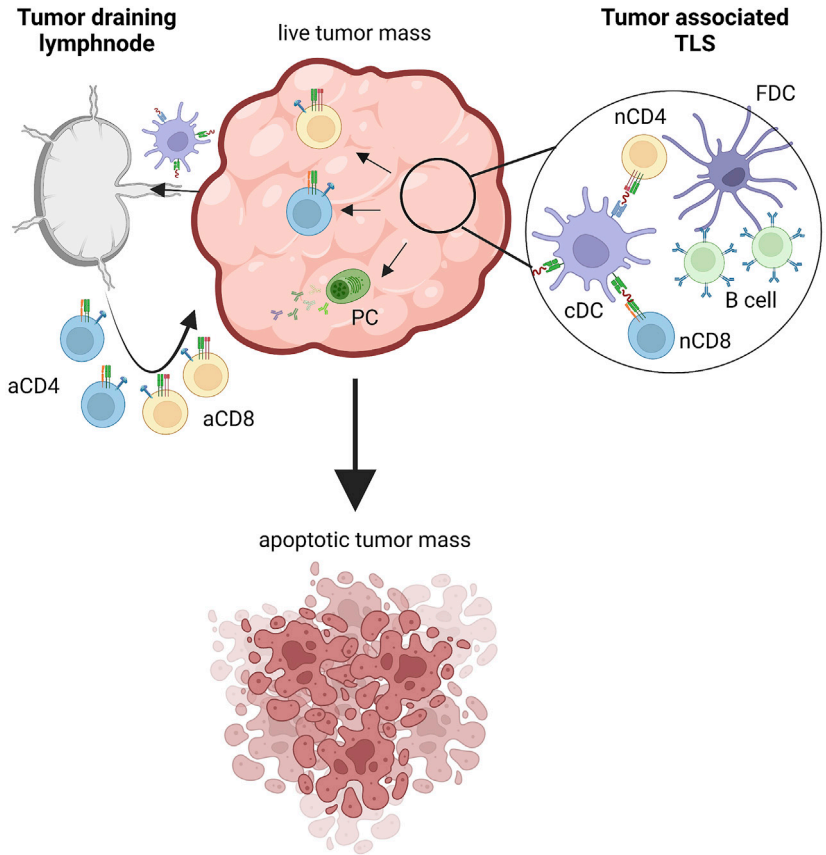

FIGURE 1 | Inductive sites of tumor-specific immune responses. Tumor cells express tumor associated antigens (TAAs) sampled by dendritic cells that migrate to tumor draining lymph nodes where adaptive immune responses are triggered. Tumors also feature tertiary lymphoid structures (TLS) where tumor specific T and B cells can be primed. In both cases, activated tumor-specific $CD4^+$ and $CD8^+$ T cells migrate within the tumor mass and attack transformed cells, ultimately inducing apoptotic cell death. Also plasma cells participate in antitumor immunity by producing tumor specific antibodies. aCD4, activated CD4 T cell; aCD8, activated CD8 T cell; nCD4, naive CD4 T cell; nCD8, naive CD8 T cell; PC, plasmacell; cDC, conventional dendritic cell; FDC, follicular dendritic cell. Created with BioRender.com.

cancer patients positively correlated with the infiltration of B cells ($CD20^+$) and of $CXCL13^+$ cells, suggestive of TLS presence (Sato et al., 2021).

The induction and persistence of functional tumor specific adaptive immune responses can be a major barrier to disease progression. It is clear from the above discussion that both T and B lymphocytes are activated in the presence of cancer cells and both arms of the adaptive immune system play a role in disease progression and response to therapy. Several retrospective studies in humans have associated the presence of TLS with good prognosis and improved responses to ICI-based immunotherapy in distinct cancer types (Fridman et al., 2017; Sautes-Fridman et al., 2019). The beneficial effect of TLS seems mostly related to their qualitative cellular composition, with Th1-polarized $CD4^+$, effector memory $CD8^+$ T cells and mature dendritic cells associated to the best outcomes in colorectal and lung cancers (Dieu-Nosjean et al., 2008; Di Caro et al., 2014; Goc et al., 2014). Whether TLS are primarily involved in induction of tumor-specific immune responses and to what extent that relates to their prognostic value has not been completely elucidated yet. The concomitant detection of dendritic cells and T and B lymphocytes in proximal vicinity may be indicative of actual antigen-specific priming occurring in TLS. In lung cancer patients, high densities of mature dendritic cells ($Lamp^+$) positively correlated with infiltration of activated $CD8^+$ T cells ($CD69^+$ $CD38^+$) and with an effector memory phenotype that localized in the tumor nest, close to target tumor cells. Notably, the concomitant detection of dendritic cells and $CD8^+$ T cells identified the group with a lower risk of death as compared to $CD8^+$ T cell infiltration alone (Goc et al., 2014). Additional studies from the same group also reported infiltration of mature DC and B cells staining positive for the proliferation marker Ki67 and the activation induced deaminase (AID), indicating ongoing germinal center reactions. Indeed, $CD138^+$ plasma cells were also detectable that specifically recognized tumor antigens in half of patients (Germain et al., 2014). As for $CD8^+$ T cells, the best clinical outcome in terms of survival was observed in patients showing concomitant infiltration of B cells and dendritic cells both in early and late stage tumors (Germain et al., 2014). In high grade ovarian cancer patients, TLS with high density of plasma cells actively synthesizing IgG were described. Again,

plasma cell infiltration correlated with cytotoxic T cells infiltration and dictated their prognostic benefit (Kroeger et al., 2016).

These studies suggest that specific and complete immune responses can be induced at the tumor site and that TLS facilitate interactions between innate and adaptive immune cells (**Figure 1**). These observations are supported by some studies that implied the occurrence of active immune responses in ectopic lymphoid structures found in autoimmune conditions and infections. For instance, splenectomized mice lacking SLO and Peyer's Patches (Lymphotoxin α −/−) can mount functional antigen-specific $CD8^+$ T cell and antibody responses upon influenza virus infection that occur at the inducible bronchus associated lymphoid tissue (iBALT) and sustain viral clearance (Moyron-Quiroz et al., 2004). Furthermore, iBALT-primed T and B cells develop a memory phenotype and provide protection against viral challenge (Moyron-Quiroz et al., 2006). Antigen-specific priming in the absence of canonical SLO has also been reported in tumor models. Schrama and colleagues grew in splenectomized $LT\alpha^{-/-}$ mice B16 derived tumors that can be specifically targeted with LTα, a well-known lymphoid tissue inducer. The authors documented delayed growth of subcutaneous tumors and complete protection from pulmonary metastasis. Importantly, $CD8^+$ T cells specific for the tumor associated antigen TRP-2 were detectable by *in situ* tetramer staining only within tumors where TLS had been induced (Schrama et al., 2008). Similarly, antitumoral activity was shown for adoptively transferred naive T cells even when lymphocyte egress from LN was blocked by FTY720 (Thompson et al., 2010; Peske et al., 2015). These results suggest that antitumor immune responses likely induced in TLS can restrain tumor growth and control disease evolution by protecting from metastatic spread. More recently, the generation of TLS has been associated with the composition of the gut microbiome, whose involvement in tumor development and immunity has raised much interest. Overacre-Delgoffe and coworkers assessed the occurrence of TLS in a carcinogen induced colorectal cancer model in the presence of the bacterial species *Helicobacter hepaticum* (Hhep). Hhep colonized mice developed smaller tumor numbers as compared to controls, survived longer and developed intratumoral TLS dominated by Tfh responses that appear to be the correlate of protection. Indeed, both TLS formation and the effect on disease progression were abrogated in mice specifically devoid of Tfh and rescued upon Hhep-specific $CD4^+$ T cell adoptive transfer (Overacre-Delgoffe et al., 2021).

STROMAL CELL COMPOSITION AND ARCHITECTURE IN TERTIARY LYMPHOID STRUCTURES FORMATION

Within the tumor microenvironment the local cross-talk between immune cells and stromal elements leads to the production of a series of pro-inflammatory cytokines and TNF receptor family components that determines the formation of TLS (Le Hir et al., 1996; Matsumoto et al., 1996; Endres et al., 1999; Forster et al., 1999; Lorenz et al., 2003; Cupedo and Mebius, 2005). Different non-hematopoietic stromal cells, namely fibroblasts, blood and lymphatic endothelial cells, pericytes, and epithelial cells, participate variably to TLS development (Buckley et al., 2015). Several studies have demonstrated a key role of the stromal elements, in particular fibroblasts, in chronic inflammation by the activation of pro-survival and retention signals. In fact, fibroblasts showed to promote the local recruitment of immune cells at the inflammatory site and support the maintenance of the inflammatory state by producing various factors, such as B-cell survival factors (BAFF) and inflammatory chemokines (e.g., IL-8, CCL5, CXCL1). However, fibroblasts can also participate in the formation of TLS through their capability to produce other chemokines, such as CXCL12, CCL21 and CXCL13 (Filer et al., 2008; Flavell et al., 2008; Barone et al., 2012). Soluble molecules produced by stromal cells have been harnessed to drive TLS formation for therapeutic purposes (Johansson-Percival and Ganss, 2021). For instance, in a neuroendocrine tumor mouse model, targeting endothelial cells and activated tumor infiltrating macrophages ($CD68^+$) with the LN-inducing cytokine LIGHT (TNFSF14) induces production of CCL21, TNFα, and IL1β that, overall, drive T/B cell recruitment and formation of mature TLS (Johansson-Percival et al., 2015; Johansson-Percival et al., 2017). In a distinct approach, genetically engineered DC overexpressing Tbet, and thus producing high levels of IFNγ, TNFα and IL36γ, have been shown to induce TLS in murine colon cancer even in the absence of peripheral LN (Weinstein et al., 2017).

In 2004 Cupedo et al. highlighted the crucial role of stromal cells in the development of TLS by performing intradermal injections of cell suspensions obtained from neonatal lymph nodes, which seemed to promote the formation of ectopic lymphocyte aggregates resembling lymph node-like follicular structures. Interestingly, the major cellular actors strictly involved in this process were the mesenchymal elements showing the capability to engage and retain host-derived T and B lymphocytes organized into two distinct compartments (Cupedo et al., 2004). Subsequently, Suematsu et al. used a mouse model and performed the implantation of stromal cell scaffolds under the kidney capsule, demonstrating the generation of lymphoid structures. These structures, described as artificial lymph nodes, recapitulated the morphological characteristics of lymph node parenchyma and proved to be able to support immune responses (Suematsu and Watanabe, 2004). Furthermore, other studies supported the pivotal contribution of stromal cells in the development of ectopic lymphoid structures during chronic inflammation. In a model of atherosclerosis, the investigators described the formation of adventitial aortic tertiary lymphoid organs, due to the activation of mouse aorta smooth muscle cells expressing both VCAM-1 and chemokines CXCL13 and CCL21 (Grabner et al., 2009).

Overall, as a consequence of persistent antigen presentation due to chronic inflammatory stimuli, stromal cells acquire lymph node-like properties that allow them to recruit, activate and prime adaptive immune cells (**Figure 2A**). A variety of inflammatory

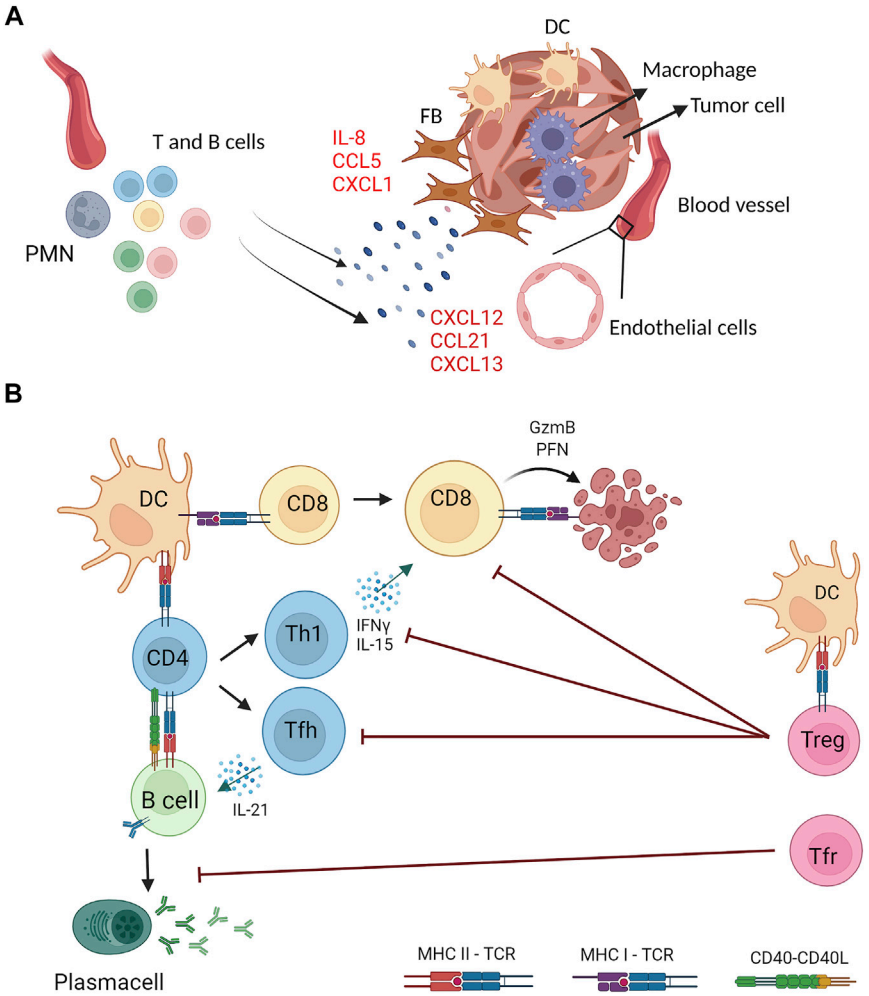

FIGURE 2 | Stromal and immune cells crosstalk in the generation and functions of tumor associated TLS. **(A)** Stromal components of tumor masses (fibroblasts, endothelial and myeloid cells) are the source of proinflammatory cytokines (e.g., IL-8) and chemokines (e.g., CXCL13) that drive recruitment and activation of lymphocytes and other myeloid cells (e.g., neutrophils) at the tumor site that generate organized lymphoid structures **(B)** Tumor associated TLS feature multiple interactions between lymphoid and myeloid cells for induction of tumor specific immune responses. Dendritic cells (DC) and B cells can present TAA on MHC class I and class II molecules to $CD8^+$ and $CD4^+$ T cells expressing the cognate T cell receptor (TCR) and, concomitantly, provide costimulatory signals by $CD40^-CD40L$ axis. Upon antigen recognition, $CD8^+$ T cells differentiate into cytotoxic cells that kill tumor targets by perforin (PFN) and Granzyme B (GzmB). $CD4^+$ T cells can differentiate into several T helper subsets, including T helper 1 (Th1) and T follicular helper (Tfh) that sustain, respectively, cytotoxic and B cell responses through the production of specific cytokines (IFNγ, IL-15, IL-21). Activated B cells differentiate into antibody secreting plasma cells. TAA-specific $CD4^+$ T cells include regulatory T cells that, once activated, counteract effector $CD4^+$ and $CD8^+$ T cells. A specialized subset of Treg with a follicular phenotype (Tfr) inhibit B cell responses. Created with BioRender.com.

stimuli, either infectious, autoimmune or tumor-related, have proved to be responsible for the formation of such antigen-specific lymphoid aggregates. Thus, in the setting of a solid neoplasm, TLS formation accounts for the promotion of a tumor-specific immune response that elicits a targeted intratumoral adaptive immunity. Knowledge of these processes helps define TLS as promising prognostic biomarkers to stratify the patient's overall survival rate and may soon contribute to the development of novel immunotherapeutic agents for the treatment of different solid neoplasms (Colbeck et al., 2017a).

Even during development, TLS stroma presents a large degree of plasticity (Barone et al., 2016). Early anlagen mesenchyme can differentiate upon specific stimuli into diverse and highly specialized stroma, which then creates functional micro domains within the TLS (Asam et al., 2021). TLS non-vascular stroma is largely represented by fibroblastic reticular cells (FRC), a fine network of canaliculi forming fibroblasts that display unique functional properties, including the ability to support the dramatic anatomical remodelling required to adapt TLS to the lymphocyte influx occurring in response to antigen stimulation (Perez-Shibayama et al., 2019). The mechanism underpinning the physical plasticity of the TLS fibroblasts has been identified in the SLO in the interaction between podoplanin, a glycoprotein broadly expressed on FRC, and its receptor CLEC2 expressed by DC, first incomers in the TLS upon antigen exposure, during inflammation or upon immunization (Acton et al., 2014). The

adaptability of the stroma during the immune response has been deemed critical to enable expansion of the B follicle required to accommodate the GC reaction upon immunization. Plasticity of the fibroblasts in TLS can therefore be defined as the most critical property required to shape an efficient immune response (Barone et al., 2016; Asam et al., 2021).

MYELOID CELLS IN TERTIARY LYMPHOID STRUCTURE FORMATION

TLS are organized formation of immune cells, which arise in tissue during chronic inflammation (e.g., autoimmunity, allograft rejection, chronic inflammation, and cancer) (Manzo et al., 2010; Thaunat et al., 2010; Neyt et al., 2012; Colbeck et al., 2017a). Similarly to lymph node and SLO, TLS displayed an inner area of $CD20^+$ B cells surrounded by $CD3^+$ T cells (Sautes-Fridman et al., 2019). Even though TLS are populated by a variety of T cells ($CD4^+$ follicular helper T cells, $CD8^+$ cytotoxic T cells, $CD4^+$ T helper 1 and regulatory T cells) (Gu-Trantien et al., 2013; Goc et al., 2014; Hennequin et al., 2016; Kroeger et al., 2016) and B cells, TLS are also populated by distinct types of myeloid cells. In particular, follicular dendritic cells (FDC), characterized by the expression of CD21, localize in the inner B zone (Bergomas et al., 2011) and dendritic cell-lysosomal associated membrane protein ($DC\text{-}LAMP^+$, also known as $CD83^+$ DC) preferentially distribute in T cell zone and activate T cell (McMullen et al., 2010). DC may also accomplish other functions in TLS organization. Indeed, it has been suggested that antigen-presenting DC, through the activation of T cells, are sufficient to TLS induction (Ludewig et al., 1998; Neyt et al., 2012). In line with this, the depletion of DC determined the loss of existing TLS, indicating the importance of DC for structural organization and maintenance of TLS, probably through the production of chemokines (e.g., CXCL13) or by the continuous activation of T cells (GeurtsvanKessel et al., 2009; Halle et al., 2009; Muniz et al., 2011). The presence of DC in TLS, as well as of other immune cell types, has been associated with better prognosis in cancer (Goc et al., 2014; Sautes-Fridman et al., 2019).

Cancer-associated TLS can also contain scattered $CD68^+$ macrophages (Dieu-Nosjean et al., 2008; Schumacher and Thommen, 2022), and the function of these macrophages has not been fully characterized. One hypothesis is that they function as scavenger cells, as it is in SLO, where T cell zone macrophages (TZM) acted as the only professional scavenger cells clearing up apoptotic cells (Baratin et al., 2017). During atherosclerosis, M1-polarized macrophages act as potential lymphoid tissue inducer cells (LTi) (Guedj et al., 2014). In this context, macrophages were identified as the inducer cells that enhanced the expression of chemokines by vascular smooth cells. Interestingly, this function in M1-polarized macrophages was independent of lymphotoxin-a1b2 (LTbR) signaling (Guedj et al., 2014). The ability of macrophages to induce TLS has been shown in another pathological context. Indeed, using a model of *Salmonella* colitis, Koscso and colleagues demonstrated that only intestinal mucosa resident $CXCR1^{hi}$ macrophages act as the APC responsible for the recruitment of $CD4^+$ T cells and B cells at the site of *Salmonella* invasion, leading to the formation of TLS and to the local pathogen specific IgA response (Koscso et al., 2020).

Neutrophils can infiltrate TLS and have been reported to infiltrate the TLS found in the omental metastasis of patients with high-grade serous ovarian cancer (HGSOC) (Montfort et al., 2017), and the TLS of patients with prostate cancer (Garcia-Hernandez et al., 2017). The function of neutrophils in this context has not been characterized. Neutrophils present in secondary lymphoid organs can influence antigen presentation and B-cell antibody response (Lok and Clatworthy, 2021). Indeed, neutrophils in the spleen provide helper signals to B cells through the production of BAFF, APRIL, and IL-21 (Puga et al., 2011). In lymph nodes, neutrophils expressing high level of major histocompatibility complex II (MHCII) are mostly located in proximity of T cells and natural killer cells, suggesting a role in $CD4^+$ T cell activation (Lok et al., 2019). In early-stage NSCLC patients, neutrophils that can acquire APC-like features and activate $CD4^+$ T cells and $CD8^+$ T cells have been identified (Singhal et al., 2016). However, the precise role of neutrophils in TLS has not been elucidated and in our opinion deserves further investigation.

REGULATORY T CELLS IN TERTIARY LYMPHOID STRUCTURES: METABOLIC ASPECTS UNDERLYING THEIR FITNESS IN THE TUMOR MICROENVIRONMENT

In accordance with their well-defined and above discussed role in tumors, a detrimental role in antitumor immunity has been documented in studies focusing on TLS-associated Tregs. In mouse models of fibrosarcoma and lung adenocarcinoma, Treg depletion triggers TLS and increases T cell activation with ensuing tumor control (Hindley et al., 2012; Colbeck et al., 2017b). In human breast and prostate cancers, the presence of $Foxp3^+$ cells in TLS is associated with adverse clinical outcomes (Gobert et al., 2009; Garcia-Hernandez et al., 2017; Germain et al., 2021). Interestingly, in a study on breast cancer the predictive value in terms of death and relapse was specifically associated to Tregs detected within lymphoid structures rather than to those infiltrating the tumor bed. Lymphoid tissue-associated Tregs show an activated phenotype, are in a proliferative state ($Ki67^+$) and in close proximity to activated DCs and T cells. This suggests that tumor antigen specific Tregs are primed in TLS, where they can directly suppress antitumor effector T cells (Gobert et al., 2009). In support of this, Joshi and colleagues have specifically demonstrated that in a mouse model of lung cancer Tregs are preferentially detected in TLS (rather than scattered in the tumor stroma) where they maintain immune quiescence. Treg depletion by diphtheria toxin administration in a transgenic model reactivates proliferation of both $CD4^+$ and $CD8^+$ T cells within TLS and results in enhanced tumor destruction (Joshi et al., 2015).

While Tregs are detected in tumor associated TLS in human cancers and murine models, their contribution in the formation of these structures is still not known. Considering the favorable prognostic value played by TLS in cancer, dissecting the processes and cell types involved in their formation may have a significant therapeutic impact. Tumor infiltrating Tregs show an activated phenotype characterized by the expression of PD-1 and OX40 (Piconese et al., 2014; Kamada et al., 2019; Polesso et al., 2019), that holds potential for modulating their abundance in the tumor microenvironment with therapeutic implications. In gastric cancer patients, effector Tregs (eTreg, Foxp3^{high} CD45RA^{-}) express high levels of PD-1 that impairs anti-PD-1 based therapy, inducing rapid disease progression. Kamada and colleagues observed hyperprogressive disease in 10% of patients after nivolumab treatment that was associated with the accumulation of proliferating intratumoral eTreg with enhanced suppressive potential (Kamada et al., 2019). In this view, combinatorial therapies exploiting the phenotypic heterogeneity of tumor infiltrating Tregs may be a backup strategy. In mouse models of melanoma and colon cancer, anti-PD-1 treatment induced the expansion of tumor Tregs with a follicular phenotype (Follicular regulatory T cells or Tfr, Foxp3^{+} Bcl6^{+}), and Tfr depletion by pretreatment with anti-CTLA4 improved tumor control. Notably, a cohort of melanoma patients undergoing sequential anti-CTLA4 and anti-PD-1 had a survival advantage over both the corresponding monotherapies or the anti-PD-1 followed by anti-CTLA4 (Eschweiler et al., 2021).

Follicular regulatory T cells are Foxp3^{+} cells that, upon antigen recognition, co-opt the differentiation pathway of Tfh and thus express the committing transcription factor Bcl6 and the chemokine receptor CXCR5, that allows relocation to germinal centers, where they modulate antibody responses (Lu and Craft, 2021). While the role of Tfr in immunization and infection models has been characterized, their significance in tumors is mostly still unknown. Nevertheless, evidence has been accumulating pointing to a central role of Tfr in modulating antitumor immunity and immunotherapy performance (**Figure 2B**). Increased Tfr frequencies are detected in peripheral blood of breast cancer patients (Song et al., 2019; Miao et al., 2021) and are shown to be a source of IL-10 that suppress antibodies production and promote Breg generation (Song et al., 2019). Whether a fraction of tumor infiltrating Tregs differentiate to Tfr in tumor associated TLS is not known, but a recent work supports this hypothesis. Eschweiler et al. reported that Tfr and activated Treg (4-1BB^{+}) isolated from human head and neck cancer share a transcriptomic signature characterized by upregulation of genes related to activation, costimulation and suppressive functions (*TNFRSF4*, *TNFRSF18*, *TNFRSF1B*, *ENTPD1*). Importantly, a fraction of activated Tregs shared TCR with Tfr, indicating a developmental relationship between the two subsets. A similar pattern was identified in murine melanoma derived Treg/Tfr, suggesting that Tfr may actually derive from tumor infiltrating Tregs that in TLS are activated by tumor-specific antigens (Eschweiler et al., 2021). Tfr represent an attractive therapeutic target given their interactions with immune ICIs and their increased suppressive functions over Bcl6^{-} Tregs (Sage et al., 2014a; Sage et al., 2014b; Eschweiler et al., 2021).

Since the anti-tumor immune response occurs locally within the tumor mass and is not restricted to the activation of effector T cells in draining secondary lymphoid organs, the cellular composition and the metabolic events occurring at the TME contribute to TLS formation, thus impacting on tumor suppression and therapeutic response. The B-cell mediated antigen presentation to CD4^{+} T cells occurring in the TLS is finely regulated by different external stimuli and by the TME conditions. In this context, the TLS-B cell interaction is crucial to modulate the early phases of T cell activation, their co-stimulatory properties and/or cell exhaustion, driving their lineage decision. More in detail, it has been described that TLS-B density positively correlates with a specific CD4^{+} T cell signature characterized by the hyper-expression of pro-inflammatory genes including POU2AF1, which encodes for the transcriptional coactivator OCA-B (also called OBF-1) that directly stimulates the IFN-γ and IL-2 promoter activities and is required for the *in vivo* generation of CD4^{+} memory T cells (Brunner et al., 2007; Shakya et al., 2015). Recently, Germain and colleagues observed that high TLS-B density in NSCLC subjects negatively correlates with the exhausted CD4^{+} T cell compartment, expressing several Treg-cell associated genes and molecules, including CD5, CD25, GITR, and Tim-3. Conversely, they found a higher percentage of CD4^{+}Foxp3^{+} Tregs in NSCLC subjects with low TLS-B density compared with those expressing high TLS-B density. This shows that high TLS-B cell density correlates positively with naïve, central memory and effector CD4^{+} T cell number but negatively with exhausted T cells and Treg cells (Germain et al., 2021).

The metabolism of the TME represents a key point in the polarization of the tumor-specific immune response. There exists evidence that low-glucose and high-lactate environment within the TME constraints T cell effector function, while promoting Treg generation and immunosuppression (Wang et al., 2018). Treg cells in tumors rely on a combination of glycolysis and fatty acid synthesis and oxidation, which allows their survival and proliferation in the hostile tumor environment (Pacella et al., 2018). A recent study has further elucidated that high-glucose conditions impair the function and stability of Tregs. Interestingly, Tregs have evolved to benefit from the symbiosis with tumors by utilizing the glycolytic by-product lactic acid (LA); indeed, they incorporate lactate-derived carbon into phosphoenolpyruvate (PEP) to provide upstream glycolytic intermediates essential for proliferation. This offers the opportunity to decrease their need for glucose, thus preserving Foxp3 induction and suppressive function (Watson et al., 2021). In addition, very recently the link between LA and PD-1 expression in TILs has been clarified; more in detail, LA upregulated PD-1 expression by eTregs through MCT1, which is controlled by Foxp3 under a low-glucose and high-LA TME. Conversely, the effect of high LA concentration on PD-1 expression by CD8^{+} T cells shows an inverse trend. This means that the efficacy of PD-1 blockade against tumors is improved by targeting the LA metabolism of Tregs (Kumagai et al., 2022). Zappasodi and colleagues recently showed that CTLA-4 blockade promotes immune cell infiltration and metabolic fitness especially in glycolysis-low tumors.

Accordingly, inhibition of tumor glycolysis ameliorates the ability of CTLA-4 blockade to induce loss of Treg stability associated with the development of anti-tumor immunity (Zappasodi et al., 2021). Moreover, increased lipid metabolism in intratumoral Tregs, which is known to boost their suppressive function, is a shared event in human and mouse cancer, occurring through the CD36 up-regulation. CD36 supports mitochondrial fitness and biogenesis *via* a peroxisome proliferator-activated receptor-β (PPAR-β)-dependent mechanism by modulating NAD^+ levels that allow Tregs to adapt to a lactic acid-enriched TME (Wang et al., 2020). Recently, it has been reported that inhibition of fatty acid binding proteins (FABP)5, one of the lipid chaperones required to facilitate uptake and intracellular lipid trafficking, causes mitochondrial damage, mtDNA release and consequent activation of the cGAS-STING-dependent type I IFN signaling; this promotes greater suppressive capacity, a phenotype also evident in tumor Tregs (Field et al., 2020).

Recent studies have revealed the importance of Blimp1 in the regulation of effector Treg (eTreg) and Tfr cell stability and suppressive function. Tfr cells express Foxp3 and belong to eTregs, and share many features with Tfh cells, as high expression levels of PD-1 and CXCR5, which allow them to traffic to B-cell follicles in response to CXCL13 (Garg et al., 2019; Shen et al., 2019). To date, few reports reported that Tfr cells are increased in cancer patients (Cha et al., 2018; Li et al., 2019), but their mechanism of action in the tumor is still unclear. However, the formation of TLS and the increased proportion of Tfh and B cells within the tumors, as observed in mice with Blimp1-specific deletion in Treg cells, are associated with favorable outcomes and with an increased response to immunotherapy in several tumors (Dixon et al., 2021).

DISCUSSION

The development of specific immune responses is a common characteristic of tumor hosts, triggered by the inflammation resulting from recognition of antigenically aberrant transformed cells. As to whether tumor specific immune responses can be triggered locally has been long a matter of debate and the discovery of tumor associated TLS and of their composition represents a central point of discussion. Animal models of secondary lymphoid organs deficiency, such as the splenectomized LTα −/− mice, have shown that the presence of tumor associated TLS is sufficient to induce effective antitumor immune responses (Schrama et al., 2008). Some characteristics of tumor associated TLS, such as the presence of mature dendritic cells, T cells and actively proliferating B cells (Ki67+) in close proximity, can be indicative of ongoing antigen specific activation. Despite very few studies have specifically associated TLS presence with tumor specific immunity (Germain et al., 2014; Kroeger et al., 2016; Overacre-Delgoffe et al., 2021), the strong prognostic value of these intratumoral lymphoid structures might indeed be related to their promoting effect on antitumor responses. Also, the predictive value of response to ICI-based immunotherapy further sustains a link between TLS and tumor specific immunity. Antibodies directed against PD-1, that achieved the best clinical performance amongst ICIs, are thought to alleviate the suppressive signals impinging on tumor specific effector T cells in the tumor microenvironment and thus reactivate their cytotoxic functions. Also regulatory T cells can express high levels of PD-1 and other immune checkpoint molecules in the tumor microenvironment, and this may undermine the efficacy of ICIs as observed in gastric cancer (Kamada et al., 2019). It is therefore important to include TLS detection and characterization in tumor specimen analysis to improve the clinical management of the disease.

Regulatory T cells are found in tumors both scattered within the mass and associated with TLS. Studies on TLS-associated Tregs overall fit with their detrimental role in cancer and provide insight on their tumor promoting activities. Treg with a follicular phenotype ($Foxp3^+$ $Bcl6^+$ $CXCR5^+$) have been the focus of recent studies, that highlighted a more powerful suppressive potential as compared to non-follicular Tregs (Sage et al., 2014a; Sage et al., 2014b; Eschweiler et al., 2021). In this view, follicular Tregs hold therapeutic potential to improve current therapies. There is indeed evidence that anti-CTLA-4 blocking antibodies can deplete Tfr, because of their increased expression of CTLA-4, and this improves the efficacy of subsequent administration of anti-PD-1 (Eschweiler et al., 2021). It has also been shown that tumor infiltrating Tfr share TCRs with activated Tregs and thus they derive from TAA-specific Tregs that migrate into tumors and get activated in TLS.

Tumor associated tertiary lymphoid structures represent an incredibly informative tool in tumor management and their characterization should be included in routine tumor pre- and postoperative analysis in order to provide the most accurate grade of disease management.

AUTHOR CONTRIBUTIONS

AR, BB, AL, VD, and SC wrote the main body. AR wrote the introduction and the discussion and prepared the figures and the table. AR, VD, SP, CT, and SJ organized the review structure and revised the manuscript.

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REFERENCES

Acton, S. E., Farrugia, A. J., Astarita, J. L., Mourão-Sá, D., Jenkins, R. P., Nye, E., et al. (2014). Dendritic Cells Control Fibroblastic Reticular Network Tension and Lymph Node Expansion. *Nature* 514, 498–502. doi:10.1038/nature13814

Ahmadvand, S., Faghih, Z., Montazer, M., Safaei, A., Mokhtari, M., Jafari, P., et al. (2019). Importance of CD45RO+ Tumor-Infiltrating Lymphocytes in Post-operative Survival of Breast Cancer Patients. *Cell Oncol.* 42, 343–356. doi:10.1007/s13402-019-00430-6

Aloisi, F., and Pujol-Borrell, R. (2006). Lymphoid Neogenesis in Chronic Inflammatory Diseases. *Nat. Rev. Immunol.* 6, 205–217. doi:10.1038/nri1786

Ansel, K. M., and Cyster, J. G. (2001). Chemokines in Lymphopoiesis and Lymphoid Organ Development. *Curr. Opin. Immunol.* 13, 172–179. doi:10.1016/s0952-7915(00)00201-6

Aoyama, S., Nakagawa, R., Mulé, J. J., and Mailloux, A. W. (2021). Inducible Tertiary Lymphoid Structures: Promise and Challenges for Translating a New Class of Immunotherapy. *Front. Immunol.* 12, 675538. doi:10.3389/fimmu.2021.675538

Asam, S., Nayar, S., Gardner, D., and Barone, F. (2021). Stromal Cells in Tertiary Lymphoid Structures: Architects of Autoimmunity. *Immunol. Rev.* 302, 184–195. doi:10.1111/imr.12987

Baratin, M., Simon, L., Jorquera, A., Ghigo, C., Dembele, D., Nowak, J., et al. (2017). T Cell Zone Resident Macrophages Silently Dispose of Apoptotic Cells in the Lymph Node. *Immunity* 47, 349–362. doi:10.1016/j.immuni.2017.07.019

Barber, D. L., Wherry, E. J., Masopust, D., Zhu, B., Allison, J. P., Sharpe, A. H., et al. (2006). Restoring Function in Exhausted CD8 T Cells during Chronic Viral Infection. *Nature* 439, 682–687. doi:10.1038/nature04444

Barone, F., Nayar, S., and Buckley, C. D. (2012). The Role of Non-hematopoietic Stromal Cells in the Persistence of Inflammation. *Front. Immunol.* 3, 416. doi:10.3389/fimmu.2012.00416

Barone, F., Gardner, D. H., Nayar, S., Steinthal, N., Buckley, C. D., and Luther, S. A. (2016). Stromal Fibroblasts in Tertiary Lymphoid Structures: A Novel Target in Chronic Inflammation. *Front. Immunol.* 7, 477. doi:10.3389/fimmu.2016.00477

Bento, D. C., Jones, E., Junaid, S., Tull, J., Williams, G. T., Godkin, A., et al. (2015). High Endothelial Venules Are Rare in Colorectal Cancers but Accumulate in Extra-tumoral Areas with Disease Progression. *Oncoimmunology* 4, e974374. doi:10.4161/2162402x.2014.974374

Bergomas, F., Grizzi, F., Doni, A., Pesce, S., Laghi, L., Allavena, P., et al. (2011). Tertiary Intratumor Lymphoid Tissue in Colo-Rectal Cancer. *Cancers* 4, 1–10. doi:10.3390/cancers4010001

Bertucci, F., Boudin, L., Finetti, P., Van Berckelaer, C., Van Dam, P., Dirix, L., et al. (2021). Immune Landscape of Inflammatory Breast Cancer Suggests Vulnerability to Immune Checkpoint Inhibitors. *Oncoimmunology* 10, 1929724. doi:10.1080/2162402x.2021.1929724

Bindea, G., Mlecnik, B., Tosolini, M., Kirilovsky, A., Waldner, M., Obenauf, A. C., et al. (2013). Spatiotemporal Dynamics of Intratumoral Immune Cells Reveal the Immune Landscape in Human Cancer. *Immunity* 39, 782–795. doi:10.1016/j.immuni.2013.10.003

Brunner, C., Sindrilaru, A., Girkontaite, I., Fischer, K.-D., Sunderkötter, C., and Wirth, T. (2007). BOB.1/OBF.1 Controls the Balance of TH1 and TH2 Immune Responses. *EMBO J.* 26, 3191–3202. doi:10.1038/sj.emboj.7601742

Bruno, T. C., Ebner, P. J., Moore, B. L., Squalls, O. G., Waugh, K. A., Eruslanov, E. B., et al. (2017). Antigen-Presenting Intratumoral B Cells Affect CD4+ TIL Phenotypes in Non-small Cell Lung Cancer Patients. *Cancer Immunol. Res.* 5, 898–907. doi:10.1158/2326-6066.cir-17-0075

Buckley, C. D., Barone, F., Nayar, S., Bénézech, C., and Caamaño, J. (2015). Stromal Cells in Chronic Inflammation and Tertiary Lymphoid Organ Formation. *Annu. Rev. Immunol.* 33, 715–745. doi:10.1146/annurev-immunol-032713-120252

Campbell, J. R., Mcdonald, B. R., Mesko, P. B., Siemers, N. O., Singh, P. B., Selby, M., et al. (2021). Fc-Optimized Anti-CCR8 Antibody Depletes Regulatory T Cells in Human Tumor Models. *Cancer Res.* 81, 2983–2994. doi:10.1158/0008-5472.can-20-3585

Castino, G. F., Cortese, N., Capretti, G., Serio, S., Di Caro, G., Mineri, R., et al. (2016). Spatial Distribution of B Cells Predicts Prognosis in Human Pancreatic Adenocarcinoma. *Oncoimmunology* 5, e1085147. doi:10.1080/2162402x.2015.1085147

Cha, Z., Gu, H., Zang, Y., Wang, Z., Li, J., Huang, W., et al. (2018). The Prevalence and Function of CD4 + CXCR5 + Foxp3 + Follicular Regulatory T Cells in Diffuse Large B Cell Lymphoma. *Int. Immunopharmacol.* 61, 132–139. doi:10.1016/j.intimp.2018.05.025

Chraa, D., Naim, A., Olive, D., and Badou, A. (2019). T Lymphocyte Subsets in Cancer Immunity: Friends or Foes. *J. Leukoc. Biol.* 105, 243–255. doi:10.1002/jlb.mr0318-097r

Colbeck, E. J., Ager, A., Gallimore, A., and Jones, G. W. (2017a). Tertiary Lymphoid Structures in Cancer: Drivers of Antitumor Immunity, Immunosuppression, or Bystander Sentinels in Disease? *Front. Immunol.* 8, 1830. doi:10.3389/fimmu.2017.01830

Colbeck, E. J., Jones, E., Hindley, J. P., Smart, K., Schulz, R., Browne, M., et al. (2017b). Treg Depletion Licenses T Cell-Driven HEV Neogenesis and Promotes Tumor Destruction. *Cancer Immunol. Res.* 5, 1005–1015. doi:10.1158/2326-6066.cir-17-0131

Cupedo, T., Jansen, W., Kraal, G., and Mebius, R. E. (2004). Induction of Secondary and Tertiary Lymphoid Structures in the Skin. *Immunity* 21, 655–667. doi:10.1016/j.immuni.2004.09.006

Cupedo, T., and Mebius, R. E. (2005). Cellular Interactions in Lymph Node Development. *J. Immunol.* 174, 21–25. doi:10.4049/jimmunol.174.1.21

Di Caro, G., Bergomas, F., Grizzi, F., Doni, A., Bianchi, P., Malesci, A., et al. (2014). Occurrence of Tertiary Lymphoid Tissue Is Associated with T-Cell Infiltration and Predicts Better Prognosis in Early-Stage Colorectal Cancers. *Clin. Cancer Res.* 20, 2147–2158. doi:10.1158/1078-0432.ccr-13-2590

Dieu-Nosjean, M.-C., Antoine, M., Danel, C., Heudes, D., Wislez, M., Poulot, V., et al. (2008). Long-term Survival for Patients with Non-small-cell Lung Cancer with Intratumoral Lymphoid Structures. *Jco* 26, 4410–4417. doi:10.1200/jco.2007.15.0284

Dieu-Nosjean, M.-C., Giraldo, N. A., Kaplon, H., Germain, C., Fridman, W. H., and Sautès-Fridman, C. (2016). Tertiary Lymphoid Structures, Drivers of the Anti-tumor Responses in Human Cancers. *Immunol. Rev.* 271, 260–275. doi:10.1111/imr.12405

Dieu-Nosjean, M.-C., Goc, J., Giraldo, N. A., Sautès-Fridman, C., and Fridman, W. H. (2014). Tertiary Lymphoid Structures in Cancer and beyond. *Trends Immunol.* 35, 571–580. doi:10.1016/j.it.2014.09.006

Dixon, M. L., Luo, L., Ghosh, S., Grimes, J. M., Leavenworth, J. D., and Leavenworth, J. W. (2021). Remodeling of the Tumor Microenvironment via Disrupting Blimp1+ Effector Treg Activity Augments Response to Anti-PD-1 Blockade. *Mol. Cancer* 20, 150. doi:10.1186/s12943-021-01450-3

Ehlers, F. A. I., Beelen, N. A., Van Gelder, M., Evers, T. M. J., Smidt, M. L., Kooreman, L. F. S., et al. (2021). ADCC-inducing Antibody Trastuzumab and Selection of KIR-HLA Ligand Mismatched Donors Enhance the NK Cell Anti-breast Cancer Response. *Cancers (Basel)* 13. doi:10.3390/cancers13133232

Endres, R., Alimzhanov, M. B., Plitz, T., Fütterer, A., Kosco-Vilbois, M. H., Nedospasov, S. A., et al. (1999). Mature Follicular Dendritic Cell Networks Depend on Expression of Lymphotoxin β Receptor by Radioresistant Stromal Cells and of Lymphotoxin β and Tumor Necrosis Factor by B Cells. *J. Exp. Med.* 189, 159–168. doi:10.1084/jem.189.1.159

Engelhard, V. H., Rodriguez, A. B., Mauldin, I. S., Woods, A. N., Peske, J. D., and Slingluff, C. L., Jr. (2018). Immune Cell Infiltration and Tertiary Lymphoid Structures as Determinants of Antitumor Immunity. *J. I.* 200, 432–442. doi:10.4049/jimmunol.1701269

Eschweiler, S., Clarke, J., Ramírez-Suástegui, C., Panwar, B., Madrigal, A., Chee, S. J., et al. (2021). Intratumoral Follicular Regulatory T Cells Curtail Anti-PD-1 Treatment Efficacy. *Nat. Immunol.* 22, 1052–1063. doi:10.1038/s41590-021-00958-6

Field, C. S., Baixauli, F., Kyle, R. L., Puleston, D. J., Cameron, A. M., Sanin, D. E., et al. (2020). Mitochondrial Integrity Regulated by Lipid Metabolism Is a Cell-Intrinsic Checkpoint for Treg Suppressive Function. *Cell Metab.* 31, 422–437. doi:10.1016/j.cmet.2019.11.021

Figenschau, S. L., Fismen, S., Fenton, K. A., Fenton, C., and Mortensen, E. S. (2015). Tertiary Lymphoid Structures Are Associated with Higher Tumor Grade in Primary Operable Breast Cancer Patients. *BMC Cancer* 15, 101. doi:10.1186/s12885-015-1116-1

Filer, A., Raza, K., Salmon, M., and Buckley, C. D. (2008). The Role of Chemokines in Leucocyte-Stromal Interactions in Rheumatoid Arthritis. *Front. Biosci.* 13, 2674–2685. doi:10.2741/2874

Finkin, S., Yuan, D., Stein, I., Taniguchi, K., Weber, A., Unger, K., et al. (2015). Ectopic Lymphoid Structures Function as Microniches for Tumor Progenitor Cells in Hepatocellular Carcinoma. *Nat. Immunol.* 16, 1235–1244. doi:10.1038/ni.3290

Flavell, S. J., Hou, T. Z., Lax, S., Filer, A. D., Salmon, M., and Buckley, C. D. (2008). Fibroblasts as Novel Therapeutic Targets in Chronic Inflammation. *Br. J. Pharmacol.* 153 (Suppl. 1), S241–S246. doi:10.1038/sj.bjp.0707487

Förster, R., Schubel, A., Breitfeld, D., Kremmer, E., Renner-Müller, I., Wolf, E., et al. (1999). CCR7 Coordinates the Primary Immune Response by Establishing Functional Microenvironments in Secondary Lymphoid Organs. *Cell* 99, 23–33. doi:10.1016/s0092-8674(00)80059-8

Fridman, W. H., Zitvogel, L., Sautès–Fridman, C., and Kroemer, G. (2017). The Immune Contexture in Cancer Prognosis and Treatment. *Nat. Rev. Clin. Oncol.* 14, 717–734. doi:10.1038/nrclinonc.2017.101

Galon, J., Costes, A., Sanchez-Cabo, F., Kirilovsky, A., Mlecnik, B., Lagorce-Pages, C., et al. (2006). Type, Density, and Location of Immune Cells within Human Colorectal Tumors Predict Clinical Outcome. *Science* 313, 1960–1964. doi:10.1126/science.1129139

Garaud, S., Buisseret, L., Solinas, C., Gu-Trantien, C., De Wind, A., Van Den Eynden, G., et al. (2019). Tumor Infiltrating B-Cells Signal Functional Humoral Immune Responses in Breast Cancer. *JCI Insight* 5. doi:10.1172/jci.insight.129641

García-Hernández, M. d. l. L., Uribe-Uribe, N. O., Espinosa-González, R., Kast, W. M., Khader, S. A., and Rangel-Moreno, J. (2017). A Unique Cellular and Molecular Microenvironment Is Present in Tertiary Lymphoid Organs of Patients with Spontaneous Prostate Cancer Regression. *Front. Immunol.* 8, 563. doi:10.3389/fimmu.2017.00563

Garg, G., Muschaweckh, A., Moreno, H., Vasanthakumar, A., Floess, S., Lepennetier, G., et al. (2019). Blimp1 Prevents Methylation of Foxp3 and Loss of Regulatory T Cell Identity at Sites of Inflammation. *Cell Rep.* 26, 1854–1868. e1855. doi:10.1016/j.celrep.2019.01.070

Germain, C., Devi-Marulkar, P., Knockaert, S., Biton, J., Kaplon, H., Letaïef, L., et al. (2021). Tertiary Lymphoid Structure-B Cells Narrow Regulatory T Cells Impact in Lung Cancer Patients. *Front. Immunol.* 12, 626776. doi:10.3389/fimmu.2021.626776

Germain, C., Gnjatic, S., Tamzalit, F., Knockaert, S., Remark, R., Goc, J., et al. (2014). Presence of B Cells in Tertiary Lymphoid Structures Is Associated with a Protective Immunity in Patients with Lung Cancer. *Am. J. Respir. Crit. Care Med.* 189, 832–844. doi:10.1164/rccm.201309-1611oc

Geurtsvankessel, C. H., Willart, M. A. M., Bergen, I. M., Van Rijt, L. S., Muskens, F., Elewaut, D., et al. (2009). Dendritic Cells Are Crucial for Maintenance of Tertiary Lymphoid Structures in the Lung of Influenza Virus-Infected Mice. *J. Exp. Med.* 206, 2339–2349. doi:10.1084/jem.20090410

Gobert, M., Treilleux, I., Bendriss-Vermare, N., Bachelot, T., Goddard-Leon, S., Arfi, V., et al. (2009). Regulatory T Cells Recruited through CCL22/CCR4 Are Selectively Activated in Lymphoid Infiltrates Surrounding Primary Breast Tumors and Lead to an Adverse Clinical Outcome. *Cancer Res.* 69, 2000–2009. doi:10.1158/0008-5472.can-08-2360

Goc, J., Germain, C., Vo-Bourgais, T. K. D., Lupo, A., Klein, C., Knockaert, S., et al. (2014). Dendritic Cells in Tumor-Associated Tertiary Lymphoid Structures Signal a Th1 Cytotoxic Immune Contexture and License the Positive Prognostic Value of Infiltrating CD8+ T Cells. *Cancer Res.* 74, 705–715. doi:10.1158/0008-5472.can-13-1342

Gräbner, R., Lötzer, K., Döpping, S., Hildner, M., Radke, D., Beer, M., et al. (2009). Lymphotoxin β Receptor Signaling Promotes Tertiary Lymphoid Organogenesis in the Aorta Adventitia of Aged ApoE−/− Mice. *J. Exp. Med.* 206, 233–248. doi:10.1084/jem.20080752

Gu-Trantien, C., Loi, S., Garaud, S., Equeter, C., Libin, M., De Wind, A., et al. (2013). CD4+ Follicular Helper T Cell Infiltration Predicts Breast Cancer Survival. *J. Clin. Invest.* 123, 2873–2892. doi:10.1172/jci67428

Guedj, K., Khallou-Laschet, J., Clement, M., Morvan, M., Gaston, A.-T., Fornasa, G., et al. (2014). M1 Macrophages Act as LTβR-independent Lymphoid Tissue Inducer Cells during Atherosclerosis-Related Lymphoid Neogenesis. *Cardiovasc Res.* 101, 434–443. doi:10.1093/cvr/cvt263

Halle, S., Dujardin, H. C., Bakocevic, N., Fleige, H., Danzer, H., Willenzon, S., et al. (2009). Induced Bronchus-Associated Lymphoid Tissue Serves as a General Priming Site for T Cells and Is Maintained by Dendritic Cells. *J. Exp. Med.* 206, 2593–2601. doi:10.1084/jem.20091472

Heinhuis, K. M., Ros, W., Kok, M., Steeghs, N., Beijnen, J. H., and Schellens, J. H. M. (2019). Enhancing Antitumor Response by Combining Immune Checkpoint Inhibitors with Chemotherapy in Solid Tumors. *Ann. Oncol.* 30, 219–235. doi:10.1093/annonc/mdy551

Helmink, B. A., Reddy, S. M., Gao, J., Zhang, S., Basar, R., Thakur, R., et al. (2020). B Cells and Tertiary Lymphoid Structures Promote Immunotherapy Response. *Nature* 577, 549–555. doi:10.1038/s41586-019-1922-8

Hennequin, A., Derangère, V., Boidot, R., Apetoh, L., Vincent, J., Orry, D., et al. (2016). Tumor Infiltration by Tbet+ Effector T Cells and CD20+ B Cells Is Associated with Survival in Gastric Cancer Patients. *Oncoimmunology* 5, e1054598. doi:10.1080/2162402x.2015.1054598

Hindley, J. P., Jones, E., Smart, K., Bridgeman, H., Lauder, S. N., Ondondo, B., et al. (2012). T-cell Trafficking Facilitated by High Endothelial Venules Is Required for Tumor Control after Regulatory T-Cell Depletion. *Cancer Res.* 72, 5473–5482. doi:10.1158/0008-5472.can-12-1912

Hiraoka, N., Ino, Y., Yamazaki-Itoh, R., Kanai, Y., Kosuge, T., and Shimada, K. (2015). Intratumoral Tertiary Lymphoid Organ Is a Favourable Prognosticator in Patients with Pancreatic Cancer. *Br. J. Cancer* 112, 1782–1790. doi:10.1038/bjc.2015.145

Hong, S., Zhang, Z., Liu, H., Tian, M., Zhu, X., Zhang, Z., et al. (2018). B Cells Are the Dominant Antigen-Presenting Cells that Activate Naive CD4+ T Cells upon Immunization with a Virus-Derived Nanoparticle Antigen. *Immunity* 49, 695–708. doi:10.1016/j.immuni.2018.08.012

Hua, Z., and Hou, B. (2020). The Role of B Cell Antigen Presentation in the Initiation of CD4+ T Cell Response. *Immunol. Rev.* 296, 24–35. doi:10.1111/imr.12859

Hughes, C. E., Benson, R. A., Bedaj, M., and Maffia, P. (2016). Antigen-Presenting Cells and Antigen Presentation in Tertiary Lymphoid Organs. *Front. Immunol.* 7, 481. doi:10.3389/fimmu.2016.00481

Johansson-Percival, A., and Ganss, R. (2021). Therapeutic Induction of Tertiary Lymphoid Structures in Cancer through Stromal Remodeling. *Front. Immunol.* 12, 674375. doi:10.3389/fimmu.2021.674375

Johansson-Percival, A., He, B., Li, Z.-J., Kjellén, A., Russell, K., Li, J., et al. (2017). De Novo induction of Intratumoral Lymphoid Structures and Vessel Normalization Enhances Immunotherapy in Resistant Tumors. *Nat. Immunol.* 18, 1207–1217. doi:10.1038/ni.3836

Johansson-Percival, A., Li, Z.-J., Lakhiani, D. D., He, B., Wang, X., Hamzah, J., et al. (2015). Intratumoral LIGHT Restores Pericyte Contractile Properties and Vessel Integrity. *Cell Rep.* 13, 2687–2698. doi:10.1016/j.celrep.2015.12.004

Jones, G. W., Hill, D. G., and Jones, S. A. (2016). Understanding Immune Cells in Tertiary Lymphoid Organ Development: It Is All Starting to Come Together. *Front. Immunol.* 7, 401. doi:10.3389/fimmu.2016.00401

Joshi, N. S., Akama-Garren, E. H., Lu, Y., Lee, D.-Y., Chang, G. P., Li, A., et al. (2015). Regulatory T Cells in Tumor-Associated Tertiary Lymphoid Structures Suppress Anti-tumor T Cell Responses. *Immunity* 43, 579–590. doi:10.1016/j.immuni.2015.08.006

Kamada, T., Togashi, Y., Tay, C., Ha, D., Sasaki, A., Nakamura, Y., et al. (2019). PD-1 + Regulatory T Cells Amplified by PD-1 Blockade Promote Hyperprogression of Cancer. *Proc. Natl. Acad. Sci. U.S.A.* 116, 9999–10008. doi:10.1073/pnas.1822001116

Kang, W., Feng, Z., Luo, J., He, Z., Liu, J., Wu, J., et al. (2021). Tertiary Lymphoid Structures in Cancer: The Double-Edged Sword Role in Antitumor Immunity and Potential Therapeutic Induction Strategies. *Front. Immunol.* 12, 689270. doi:10.3389/fimmu.2021.689270

Kaplan, D. H., Shankaran, V., Dighe, A. S., Stockert, E., Aguet, M., Old, L. J., et al. (1998). Demonstration of an Interferon γ-dependent Tumor Surveillance System in Immunocompetent Mice. *Proc. Natl. Acad. Sci. U.S.A.* 95, 7556–7561. doi:10.1073/pnas.95.13.7556

Keir, M. E., Butte, M. J., Freeman, G. J., and Sharpe, A. H. (2008). PD-1 and its Ligands in Tolerance and Immunity. *Annu. Rev. Immunol.* 26, 677–704. doi:10.1146/annurev.immunol.26.021607.090331

Koscsó, B., Kurapati, S., Rodrigues, R. R., Nedjic, J., Gowda, K., Shin, C., et al. (2020). Gut-resident CX3CR1hi Macrophages Induce Tertiary Lymphoid Structures and IgA Response *In Situ*. *Sci. Immunol.* 5. doi:10.1126/sciimmunol.aax0062

Kroeger, D. R., Milne, K., and Nelson, B. H. (2016). Tumor-Infiltrating Plasma Cells Are Associated with Tertiary Lymphoid Structures, Cytolytic T-Cell

Responses, and Superior Prognosis in Ovarian Cancer. *Clin. Cancer Res.* 22, 3005–3015. doi:10.1158/1078-0432.ccr-15-2762

Kumagai, S., Koyama, S., Itahashi, K., Tanegashima, T., Lin, Y.-t., Togashi, Y., et al. (2022). Lactic Acid Promotes PD-1 Expression in Regulatory T Cells in Highly Glycolytic Tumor Microenvironments. *Cancer Cell* 40, 201–218. doi:10.1016/j.ccell.2022.01.001

Kurose, K., Ohue, Y., Wada, H., Iida, S., Ishida, T., Kojima, T., et al. (2015). Phase Ia Study of FoxP3+ CD4 Treg Depletion by Infusion of a Humanized Anti-CCR4 Antibody, KW-0761, in Cancer Patients. *Clin. Cancer Res.* 21, 4327–4336. doi:10.1158/1078-0432.ccr-15-0357

Ladányi, A., Kiss, J., Somlai, B., Gilde, K., Fejős, Z., Mohos, A., et al. (2007). Density of DC-LAMP+ Mature Dendritic Cells in Combination with Activated T Lymphocytes Infiltrating Primary Cutaneous Melanoma Is a Strong Independent Prognostic Factor. *Cancer Immunol. Immunother.* 56, 1459–1469. doi:10.1007/s00262-007-0286-3

Le Hir, M., Bluethmann, H., Kosco-Vilbois, M. H., Müller, M., Di Padova, F., Moore, M., et al. (1996). Differentiation of Follicular Dendritic Cells and Full Antibody Responses Require Tumor Necrosis Factor Receptor-1 Signaling. *J. Exp. Med.* 183, 2367–2372. doi:10.1084/jem.183.5.2367

Li, H., Wang, J., Liu, H., Lan, T., Xu, L., Wang, G., et al. (2020). Existence of Intratumoral Tertiary Lymphoid Structures Is Associated with Immune Cells Infiltration and Predicts Better Prognosis in Early-Stage Hepatocellular Carcinoma. *Aging* 12, 3451–3472. doi:10.18632/aging.102821

Li, L., Ma, Y., and Xu, Y. (2019). Follicular Regulatory T Cells Infiltrated the Ovarian Carcinoma and Resulted in CD8 T Cell Dysfunction Dependent on IL-10 Pathway. *Int. Immunopharmacol.* 68, 81–87. doi:10.1016/j.intimp.2018.12.051

Locy, H., De Mey, S., De Mey, W., De Ridder, M., Thielemans, K., and Maenhout, S. K. (2018). Immunomodulation of the Tumor Microenvironment: Turn Foe into Friend. *Front. Immunol.* 9, 2909. doi:10.3389/fimmu.2018.02909

Lok, L. S. C., and Clatworthy, M. R. (2021). Neutrophils in Secondary Lymphoid Organs. *Immunology* 164, 677–688. doi:10.1111/imm.13406

Lok, L. S. C., Dennison, T. W., Mahbubani, K. M., Saeb-Parsy, K., Chilvers, E. R., and Clatworthy, M. R. (2019). Phenotypically Distinct Neutrophils Patrol Uninfected Human and Mouse Lymph Nodes. *Proc. Natl. Acad. Sci. U.S.A.* 116, 19083–19089. doi:10.1073/pnas.1905054116

Lorenz, R. G., Chaplin, D. D., Mcdonald, K. G., Mcdonough, J. S., and Newberry, R. D. (2003). Isolated Lymphoid Follicle Formation Is Inducible and Dependent upon Lymphotoxin-Sufficient B Lymphocytes, Lymphotoxin β Receptor, and TNF Receptor I Function. *J. Immunol.* 170, 5475–5482. doi:10.4049/jimmunol.170.11.5475

Lu, Y., and Craft, J. (2021). T Follicular Regulatory Cells: Choreographers of Productive Germinal Center Responses. *Front. Immunol.* 12, 679909. doi:10.3389/fimmu.2021.679909

Ludewig, B., Odermatt, B., Landmann, S., Hengartner, H., and Zinkernagel, R. M. (1998). Dendritic Cells Induce Autoimmune Diabetes and Maintain Disease via De Novo Formation of Local Lymphoid Tissue. *J. Exp. Med.* 188, 1493–1501. doi:10.1084/jem.188.8.1493

Luen, S. J., Salgado, R., Fox, S., Savas, P., Eng-Wong, J., Clark, E., et al. (2017). Tumour-infiltrating Lymphocytes in Advanced HER2-Positive Breast Cancer Treated with Pertuzumab or Placebo in Addition to Trastuzumab and Docetaxel: a Retrospective Analysis of the CLEOPATRA Study. *Lancet Oncol.* 18, 52–62. doi:10.1016/s1470-2045(16)30631-3

Manzo, A., Bombardieri, M., Humby, F., and Pitzalis, C. (2010). Secondary and Ectopic Lymphoid Tissue Responses in Rheumatoid Arthritis: from Inflammation to Autoimmunity and Tissue Damage/remodeling. *Immunol. Rev.* 233, 267–285. doi:10.1111/j.0105-2896.2009.00861.x

Martinet, L., Garrido, I., Filleron, T., Le Guellec, S., Bellard, E., Fournie, J.-J., et al. (2011). Human Solid Tumors Contain High Endothelial Venules: Association with T- and B-Lymphocyte Infiltration and Favorable Prognosis in Breast Cancer. *Cancer Res.* 71, 5678–5687. doi:10.1158/0008-5472.can-11-0431

Martinet, L., Le Guellec, S., Filleron, T., Lamant, L., Meyer, N., Rochaix, P., et al. (2012). High Endothelial Venules (HEVs) in Human Melanoma Lesions. *Oncoimmunology* 1, 829–839. doi:10.4161/onci.20492

Matsumoto, M., Mariathasan, S., Nahm, M. H., Baranyay, F., Peschon, J. J., and Chaplin, D. D. (1996). Role of Lymphotoxin and the Type I TNF Receptor in the Formation of Germinal Centers. *Science* 271, 1289–1291. doi:10.1126/science.271.5253.1289

Mcmullen, T. P. W., Lai, R., Dabbagh, L., Wallace, T. M., and De Gara, C. J. (2010). Survival in Rectal Cancer Is Predicted by T Cell Infiltration of Tumour-Associated Lymphoid Nodules. *Clin. Exp. Immunol.* 161, 81–88. doi:10.1111/j.1365-2249.2010.04147.x

Miao, X., Guo, Q., Pan, Z., Xu, X., Shao, X., and Wang, X. (2021). The Characteristics and Novel Clinical Implications of CD4+CXCR5+Foxp3+ Follicular Regulatory T Cells in Breast Cancer. *Ann. Transl. Med.* 9, 1332. doi:10.21037/atm-21-3848

Milne, K., Köbel, M., Kalloger, S. E., Barnes, R. O., Gao, D., Gilks, C. B., et al. (2009). Systematic Analysis of Immune Infiltrates in High-Grade Serous Ovarian Cancer Reveals CD20, FoxP3 and TIA-1 as Positive Prognostic Factors. *PLoS One* 4, e6412. doi:10.1371/journal.pone.0006412

Mittal, D., Gubin, M. M., Schreiber, R. D., and Smyth, M. J. (2014). New Insights into Cancer Immunoediting and its Three Component Phases-Elimination, Equilibrium and Escape. *Curr. Opin. Immunol.* 27, 16–25. doi:10.1016/j.coi.2014.01.004

Montfort, A., Pearce, O., Maniati, E., Vincent, B. G., Bixby, L., Böhm, S., et al. (2017). A Strong B-Cell Response Is Part of the Immune Landscape in Human High-Grade Serous Ovarian Metastases. *Clin. Cancer Res.* 23, 250–262. doi:10.1158/1078-0432.ccr-16-0081

Moyron-Quiroz, J. E., Rangel-Moreno, J., Hartson, L., Kusser, K., Tighe, M. P., Klonowski, K. D., et al. (2006). Persistence and Responsiveness of Immunologic Memory in the Absence of Secondary Lymphoid Organs. *Immunity* 25, 643–654. doi:10.1016/j.immuni.2006.08.022

Moyron-Quiroz, J. E., Rangel-Moreno, J., Kusser, K., Hartson, L., Sprague, F., Goodrich, S., et al. (2004). Role of Inducible Bronchus Associated Lymphoid Tissue (iBALT) in Respiratory Immunity. *Nat. Med.* 10, 927–934. doi:10.1038/nm1091

Muniz, L. R., Pacer, M. E., Lira, S. A., and Furtado, G. C. (2011). A Critical Role for Dendritic Cells in the Formation of Lymphatic Vessels within Tertiary Lymphoid Structures. *J. I.* 187, 828–834. doi:10.4049/jimmunol.1004233

Munoz-Erazo, L., Rhodes, J. L., Marion, V. C., and Kemp, R. A. (2020). Tertiary Lymphoid Structures in Cancer - Considerations for Patient Prognosis. *Cell Mol. Immunol.* 17, 570–575. doi:10.1038/s41423-020-0457-0

Muramatsu, M., Kinoshita, K., Fagarasan, S., Yamada, S., Shinkai, Y., and Honjo, T. (2000). Class Switch Recombination and Hypermutation Require Activation-Induced Cytidine Deaminase (AID), a Potential RNA Editing Enzyme. *Cell* 102, 553–563. doi:10.1016/s0092-8674(00)00078-7

N, J., J, T., Sl, N., and Gt, B. (2021). Tertiary Lymphoid Structures and B Lymphocytes in Cancer Prognosis and Response to Immunotherapies. *Oncoimmunology* 10, 1900508. doi:10.1080/2162402x.2021.1900508

Neyt, K., Perros, F., Geurtsvankessel, C. H., Hammad, H., and Lambrecht, B. N. (2012). Tertiary Lymphoid Organs in Infection and Autoimmunity. *Trends Immunol.* 33, 297–305. doi:10.1016/j.it.2012.04.006

Overacre-Delgoffe, A. E., Bumgarner, H. J., Cillo, A. R., Burr, A. H. P., Tometich, J. T., Bhattacharjee, A., et al. (2021). Microbiota-specific T Follicular Helper Cells Drive Tertiary Lymphoid Structures and Anti-tumor Immunity against Colorectal Cancer. *Immunity* 54, 2812–2824. e2814. doi:10.1016/j.immuni.2021.11.003

Pacella, I., Procaccini, C., Focaccetti, C., Miacci, S., Timperi, E., Faicchia, D., et al. (2018). Fatty Acid Metabolism Complements Glycolysis in the Selective Regulatory T Cell Expansion during Tumor Growth. *Proc. Natl. Acad. Sci. U. S. A.* 115, E6546–E6555. doi:10.1073/pnas.1720113115

Pagès, F., Berger, A., Camus, M., Sanchez-Cabo, F., Costes, A., Molidor, R., et al. (2005). Effector Memory T Cells, Early Metastasis, and Survival in Colorectal Cancer. *N. Engl. J. Med.* 353, 2654–2666. doi:10.1056/nejmoa051424

Paijens, S. T., Vledder, A., De Bruyn, M., and Nijman, H. W. (2021). Tumor-infiltrating Lymphocytes in the Immunotherapy Era. *Cell Mol. Immunol.* 18, 842–859. doi:10.1038/s41423-020-00565-9

Pardoll, D. M. (2012). The Blockade of Immune Checkpoints in Cancer Immunotherapy. *Nat. Rev. Cancer* 12, 252–264. doi:10.1038/nrc3239

Park, S., Jiang, Z., Mortenson, E. D., Deng, L., Radkevich-Brown, O., Yang, X., et al. (2010). The Therapeutic Effect of Anti-HER2/neu Antibody Depends on Both Innate and Adaptive Immunity. *Cancer Cell* 18, 160–170. doi:10.1016/j.ccr.2010.06.014

Perez-Shibayama, C., Gil-Cruz, C., and Ludewig, B. (2019). Fibroblastic Reticular Cells at the Nexus of Innate and Adaptive Immune Responses. *Immunol. Rev.* 289, 31–41. doi:10.1111/imr.12748

Peske, J. D., Woods, A. B., and Engelhard, V. H. (2015). Control of CD8 T-Cell Infiltration into Tumors by Vasculature and Microenvironment. *Adv. Cancer Res.* 128, 263–307. doi:10.1016/bs.acr.2015.05.001

Petitprez, F., de Reyniès, A., Keung, E. Z., Chen, T. W.-W., Sun, C.-M., Calderaro, J., et al. (2020). B Cells Are Associated with Survival and Immunotherapy Response in Sarcoma. *Nature* 577, 556–560. doi:10.1038/s41586-019-1906-8

Piconese, S., Timperi, E., Pacella, I., Schinzari, V., Tripodo, C., Rossi, M., et al. (2014). Human OX40 Tunes the Function of Regulatory T Cells in Tumor and Nontumor Areas of Hepatitis C Virus-Infected Liver Tissue. *Hepatology* 60, 1494–1507. doi:10.1002/hep.27188

Polesso, F., Weinberg, A. D., and Moran, A. E. (2019). Late-Stage Tumor Regression after PD-L1 Blockade Plus a Concurrent OX40 Agonist. *Cancer Immunol. Res.* 7, 269–281. doi:10.1158/2326-6066.cir-18-0222

Posch, F., Silina, K., Leibl, S., Mündlein, A., Moch, H., Siebenhüner, A., et al. (2018). Maturation of Tertiary Lymphoid Structures and Recurrence of Stage II and III Colorectal Cancer. *Oncoimmunology* 7, e1378844. doi:10.1080/2162402x.2017.1378844

Puga, I., Cols, M., Barra, C. M., He, B., Cassis, L., Gentile, M., et al. (2011). B Cell-Helper Neutrophils Stimulate the Diversification and Production of Immunoglobulin in the Marginal Zone of the Spleen. *Nat. Immunol.* 13, 170–180. doi:10.1038/ni.2194

Remark, R., Alifano, M., Cremer, I., Lupo, A., Dieu-Nosjean, M.-C., Riquet, M., et al. (2013). Characteristics and Clinical Impacts of the Immune Environments in Colorectal and Renal Cell Carcinoma Lung Metastases: Influence of Tumor Origin. *Clin. Cancer Res.* 19, 4079–4091. doi:10.1158/1078-0432.ccr-12-3847

Rosato, P. C., Wijeyesinghe, S., Stolley, J. M., Nelson, C. E., Davis, R. L., Manlove, L. S., et al. (2019). Virus-specific Memory T Cells Populate Tumors and Can Be Repurposed for Tumor Immunotherapy. *Nat. Commun.* 10, 567. doi:10.1038/s41467-019-08534-1

Rosenthal, R., Cadieux, E. L., Cadieux, E. L., Salgado, R., Bakir, M. A., Moore, D. A., et al. (2019). Neoantigen-directed Immune Escape in Lung Cancer Evolution. *Nature* 567, 479–485. doi:10.1038/s41586-019-1032-7

Rosser, E. C., and Mauri, C. (2015). Regulatory B Cells: Origin, Phenotype, and Function. *Immunity* 42, 607–612. doi:10.1016/j.immuni.2015.04.005

Rubin, E., and Kan, Y. (1985). A SIMPLE SENSITIVE PRENATAL TEST FOR HYDROPS FETALIS CAUSED BY α-THALASSAEMIA. *Lancet* 325, 75–77. doi:10.1016/s0140-6736(85)91967-1

Sage, P. T., Alvarez, D., Godec, J., Von Andrian, U. H., and Sharpe, A. H. (2014a). Circulating T Follicular Regulatory and Helper Cells Have Memory-like Properties. *J. Clin. Invest.* 124, 5191–5204. doi:10.1172/jci76861

Sage, P. T., Paterson, A. M., Lovitch, S. B., and Sharpe, A. H. (2014b). The Coinhibitory Receptor CTLA-4 Controls B Cell Responses by Modulating T Follicular Helper, T Follicular Regulatory, and T Regulatory Cells. *Immunity* 41, 1026–1039. doi:10.1016/j.immuni.2014.12.005

Sato, Y., Shimoda, M., Sota, Y., Miyake, T., Tanei, T., Kagara, N., et al. (2021). Enhanced Humoral Immunity in Breast Cancer Patients with High Serum Concentration of anti-HER2 Autoantibody. *Cancer Med.* 10, 1418–1430. doi:10.1002/cam4.3742

Sautès-Fridman, C., Lawand, M., Giraldo, N. A., Kaplon, H., Germain, C., Fridman, W. H., et al. (2016). Tertiary Lymphoid Structures in Cancers: Prognostic Value, Regulation, and Manipulation for Therapeutic Intervention. *Front. Immunol.* 7, 407. doi:10.3389/fimmu.2016.00407

Sautès-Fridman, C., Petitprez, F., Calderaro, J., and Fridman, W. H. (2019). Tertiary Lymphoid Structures in the Era of Cancer Immunotherapy. *Nat. Rev. Cancer* 19, 307–325. doi:10.1038/s41568-019-0144-6

Schlößer, H. A., Thelen, M., Lechner, A., Wennhold, K., Garcia-Marquez, M. A., Rothschild, S. I., et al. (2019). B Cells in Esophago-Gastric Adenocarcinoma Are Highly Differentiated, Organize in Tertiary Lymphoid Structures and Produce Tumor-specific Antibodies. *Oncoimmunology* 8, e1512458. doi:10.1080/2162402X.2018.1512458

Schrama, D., Voigt, H., Eggert, A. O., Xiang, R., Zhou, H., Schumacher, T. N. M., et al. (2008). Immunological Tumor Destruction in a Murine Melanoma Model by Targeted LTα Independent of Secondary Lymphoid Tissue. *Cancer Immunol. Immunother.* 57, 85–95. doi:10.1007/s00262-007-0352-x

Schröder, A. E., Greiner, A., Seyfert, C., and Berek, C. (1996). Differentiation of B Cells in the Nonlymphoid Tissue of the Synovial Membrane of Patients with Rheumatoid Arthritis. *Proc. Natl. Acad. Sci. U.S.A.* 93, 221–225. doi:10.1073/pnas.93.1.221

Schumacher, T. N., and Thommen, D. S. (2022). Tertiary Lymphoid Structures in Cancer. *Science* 375, eabf9419. doi:10.1126/science.abf9419

Schumacher, T. N., and Schreiber, R. D. (2015). Neoantigens in Cancer Immunotherapy. *Science* 348, 69–74. doi:10.1126/science.aaa4971

Schweiger, T., Berghoff, A. S., Glogner, C., Glueck, O., Rajky, O., Traxler, D., et al. (2016). Tumor-infiltrating Lymphocyte Subsets and Tertiary Lymphoid Structures in Pulmonary Metastases from Colorectal Cancer. *Clin. Exp. Metastasis* 33, 727–739. doi:10.1007/s10585-016-9813-y

Selby, M. J., Engelhardt, J. J., Quigley, M., Henning, K. A., Chen, T., Srinivasan, M., et al. (2013). Anti-CTLA-4 Antibodies of IgG2a Isotype Enhance Antitumor Activity through Reduction of Intratumoral Regulatory T Cells. *Cancer Immunol. Res.* 1, 32–42. doi:10.1158/2326-6066.cir-13-0013

Seow, D. Y. B., Yeong, J. P. S., Lim, J. X., Chia, N., Lim, J. C. T., Ong, C. C. H., et al. (2020). Tertiary Lymphoid Structures and Associated Plasma Cells Play an Important Role in the Biology of Triple-Negative Breast Cancers. *Breast Cancer Res. Treat.* 180, 369–377. doi:10.1007/s10549-020-05548-y

Shakya, A., Goren, A., Shalek, A., German, C. N., Snook, J., Kuchroo, V. K., et al. (2015). Oct1 and OCA-B Are Selectively Required for CD4 Memory T Cell Function. *J. Exp. Med.* 212, 2115–2131. doi:10.1084/jem.20150363

Shankaran, V., Ikeda, H., Bruce, A. T., White, J. M., Swanson, P. E., Old, L. J., et al. (2001). IFNγ and Lymphocytes Prevent Primary Tumour Development and Shape Tumour Immunogenicity. *Nature* 410, 1107–1111. doi:10.1038/35074122

Sharma, A., Subudhi, S. K., Blando, J., Vence, L., Wargo, J., Allison, J. P., et al. (2019). Anti-CTLA-4 Immunotherapy Does Not Deplete FOXP3+ Regulatory T Cells (Tregs) in Human Cancers-Response. *Clin. Cancer Res.* 25, 3469–3470. doi:10.1158/1078-0432.ccr-19-0402

Shen, E., Rabe, H., Luo, L., Wang, L., Wang, Q., Yin, J., et al. (2019). Control of Germinal Center Localization and Lineage Stability of Follicular Regulatory T Cells by the Blimp1 Transcription Factor. *Cell Rep.* 29, 1848–1861. e1846. doi:10.1016/j.celrep.2019.10.012

Siliņa, K., Soltermann, A., Attar, F. M., Casanova, R., Uckeley, Z. M., Thut, H., et al. (2018). Germinal Centers Determine the Prognostic Relevance of Tertiary Lymphoid Structures and Are Impaired by Corticosteroids in Lung Squamous Cell Carcinoma. *Cancer Res.* 78, 1308–1320. doi:10.1158/0008-5472.CAN-17-1987

Simoni, Y., Becht, E., Fehlings, M., Loh, C. Y., Koo, S.-L., Teng, K. W. W., et al. (2018). Bystander CD8+ T Cells Are Abundant and Phenotypically Distinct in Human Tumour Infiltrates. *Nature* 557, 575–579. doi:10.1038/s41586-018-0130-2

Simpson, T. R., Li, F., Montalvo-Ortiz, W., Sepulveda, M. A., Bergerhoff, K., Arce, F., et al. (2013). Fc-dependent Depletion of Tumor-Infiltrating Regulatory T Cells Co-defines the Efficacy of Anti-CTLA-4 Therapy against Melanoma. *J. Exp. Med.* 210, 1695–1710. doi:10.1084/jem.20130579

Singhal, S., Bhojnagarwala, P. S., O'brien, S., Moon, E. K., Garfall, A. L., Rao, A. S., et al. (2016). Origin and Role of a Subset of Tumor-Associated Neutrophils with Antigen-Presenting Cell Features in Early-Stage Human Lung Cancer. *Cancer Cell* 30, 120–135. doi:10.1016/j.ccell.2016.06.001

Smyth, M. J., Thia, K. Y. T., Street, S. E. A., Macgregor, D., Godfrey, D. I., and Trapani, J. A. (2000). Perforin-mediated Cytotoxicity Is Critical for Surveillance of Spontaneous Lymphoma. *J. Exp. Med.* 192, 755–760. doi:10.1084/jem.192.5.755

Sofopoulos, M., Fortis, S. P., Vaxevanis, C. K., Sotiriadou, N. N., Arnogiannaki, N., Ardavanis, A., et al. (2019). The Prognostic Significance of Peritumoral Tertiary Lymphoid Structures in Breast Cancer. *Cancer Immunol. Immunother.* 68, 1733–1745. doi:10.1007/s00262-019-02407-8

Song, H., Liu, A., Liu, G., Wu, F., and Li, Z. (2019). T Follicular Regulatory Cells Suppress Tfh-Mediated B Cell Help and Synergistically Increase IL-10-producing B Cells in Breast Carcinoma. *Immunol. Res.* 67, 416–423. doi:10.1007/s12026-019-09090-y

Suematsu, S., and Watanabe, T. (2004). Generation of a Synthetic Lymphoid Tissue-like Organoid in Mice. *Nat. Biotechnol.* 22, 1539–1545. doi:10.1038/nbt1039

Thaunat, O., Patey, N., Caligiuri, G., Gautreau, C., Mamani-Matsuda, M., Mekki, Y., et al. (2010). Chronic Rejection Triggers the Development of an Aggressive Intragraft Immune Response through Recapitulation of Lymphoid Organogenesis. *J. I.* 185, 717–728. doi:10.4049/jimmunol.0903589

Thompson, E. D., Enriquez, H. L., Fu, Y.-X., and Engelhard, V. H. (2010). Tumor Masses Support Naive T Cell Infiltration, Activation, and Differentiation into Effectors. *J. Exp. Med.* 207, 1791–1804. doi:10.1084/jem.20092454

Topalian, S. L., Drake, C. G., and Pardoll, D. M. (2015). Immune Checkpoint Blockade: a Common Denominator Approach to Cancer Therapy. *Cancer Cell* 27, 450–461. doi:10.1016/j.ccell.2015.03.001

Trajkovski, G., Ognjenovic, L., Karadzov, Z., Jota, G., Hadzi-Manchev, D., Kostovski, O., et al. (2018). Tertiary Lymphoid Structures in Colorectal Cancers and Their Prognostic Value. *Open Access Maced. J. Med. Sci.* 6, 1824–1828. doi:10.3889/oamjms.2018.341

Tsao, L. C., Crosby, E. J., Trotter, T. N., Agarwal, P., Hwang, B. J., Acharya, C., et al. (2019). CD47 Blockade Augmentation of Trastuzumab Antitumor Efficacy Dependent on Antibody-dependent Cellular Phagocytosis. *JCI Insight* 4. doi:10.1172/jci.insight.131882

Upton, R., Banuelos, A., Feng, D., Biswas, T., Kao, K., Mckenna, K., et al. (2021). Combining CD47 Blockade with Trastuzumab Eliminates HER2-Positive Breast Cancer Cells and Overcomes Trastuzumab Tolerance. *Proc. Natl. Acad. Sci. U. S. A.* 118. doi:10.1073/pnas.2026849118

Wang, H., Franco, F., Tsui, Y.-C., Xie, X., Trefny, M. P., Zappasodi, R., et al. (2020). CD36-mediated Metabolic Adaptation Supports Regulatory T Cell Survival and Function in Tumors. *Nat. Immunol.* 21, 298–308. doi:10.1038/s41590-019-0589-5

Wang, Y.-a., Li, X.-L., Mo, Y.-Z., Fan, C.-M., Tang, L., Xiong, F., et al. (2018). Effects of Tumor Metabolic Microenvironment on Regulatory T Cells. *Mol. Cancer* 17, 168. doi:10.1186/s12943-018-0913-y

Watson, M. J., Vignali, P. D. A., Mullett, S. J., Overacre-Delgoffe, A. E., Peralta, R. M., Grebinoski, S., et al. (2021). Metabolic Support of Tumour-Infiltrating Regulatory T Cells by Lactic Acid. *Nature* 591, 645–651. doi:10.1038/s41586-020-03045-2

Weinstein, A. M., Chen, L., Brzana, E. A., Patil, P. R., Taylor, J. L., Fabian, K. L., et al. (2017). Tbet and IL-36γ Cooperate in Therapeutic DC-mediated Promotion of Ectopic Lymphoid Organogenesis in the Tumor Microenvironment. *Oncoimmunology* 6, e1322238. doi:10.1080/2162402X.2017.1322238

Weinstein, A. M., and Storkus, W. J. (2015). Therapeutic Lymphoid Organogenesis in the Tumor Microenvironment. *Adv. Cancer Res.* 128, 197–233. doi:10.1016/bs.acr.2015.04.003

Wirsing, A. M., Ervik, I. K., Seppola, M., Uhlin-Hansen, L., Steigen, S. E., and Hadler-Olsen, E. (2018). Presence of High-Endothelial Venules Correlates with a Favorable Immune Microenvironment in Oral Squamous Cell Carcinoma. *Mod. Pathol.* 31, 910–922. doi:10.1038/s41379-018-0019-5

Wirsing, A. M., Rikardsen, O. G., Steigen, S. E., Uhlin-Hansen, L., and Hadler-Olsen, E. (2014). Characterisation and Prognostic Value of Tertiary Lymphoid Structures in Oral Squamous Cell Carcinoma. *BMC Clin. Pathol.* 14, 38. doi:10.1186/1472-6890-14-38

Yamaguchi, K., Ito, M., Ohmura, H., Hanamura, F., Nakano, M., Tsuchihashi, K., et al. (2020). Helper T Cell-Dominant Tertiary Lymphoid Structures Are Associated with Disease Relapse of Advanced Colorectal Cancer. *Oncoimmunology* 9, 1724763. doi:10.1080/2162402x.2020.1724763

Zappasodi, R., Serganova, I., Cohen, I. J., Maeda, M., Shindo, M., Senbabaoglu, Y., et al. (2021). CTLA-4 Blockade Drives Loss of Treg Stability in Glycolysis-Low Tumours. *Nature* 591, 652–658. doi:10.1038/s41586-021-03326-4

Zhang, N.-N., Qu, F.-J., Liu, H., Li, Z.-J., Zhang, Y.-C., Han, X., et al. (2021). Prognostic Impact of Tertiary Lymphoid Structures in Breast Cancer Prognosis: a Systematic Review and Meta-Analysis. *Cancer Cell Int.* 21, 536. doi:10.1186/s12935-021-02242-x

Zhu, W., Germain, C., Liu, Z., Sebastian, Y., Devi, P., Knockaert, S., et al. (2015). A High Density of Tertiary Lymphoid Structure B Cells in Lung Tumors Is Associated with Increased CD4+T Cell Receptor Repertoire Clonality. *Oncoimmunology* 4, e1051922. doi:10.1080/2162402x.2015.1051922

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Fine-tuning of regulatory T cells is indispensable for the metabolic steatosis-related hepatocellular carcinoma: A review

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The majority of chronic hepatic diseases are caused by nutritional imbalance. These nutritional inequities include excessive intake of alcohol and fat, which causes alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD), respectively. The pathogenesis of hepatic diseases is mainly dependent on oxidative stress, autophagy, DNA damage, and gut microbiota and their metabolites. These factors influence the normal physiology of the liver and impact the hepatic microenvironment. The hepatic microenvironment contains several immune cells and inflammatory cytokines which interact with each other and contribute to the progression of chronic hepatic diseases. Among these immune cells, Foxp3$^+$ CD4$^+$ regulatory T cells (Tregs) are the crucial subset of CD4$^+$ T cells that create an immunosuppressive environment. This review emphasizes the function of Tregs in the pathogenesis of ALD and NAFLD and their role in the progression of NAFLD-associated hepatocellular carcinoma (HCC). Briefly, Tregs establish an immunosuppressive landscape in the liver by interacting with the innate immune cells and gut microbiota and their metabolites. Meanwhile, with the advancement of steatosis, these Tregs inhibit the proliferation, activation and functions of other cytotoxic T cells and support the progression of simple steatosis to HCC. Briefly, it can be suggested that targeting Tregs can act as a favourable prognostic indicator by modulating steatosis and insulin resistance during the pathogenesis of hepatic steatosis and NAFLD-associated HCC.

KEYWORDS

regulatory (Treg) cell, forkhead box P3 (FOXP3), hepatocellular carcinoma, metabolic liver diseases, nonalcoholic fatty liver disease -NAFLD, alcoholic liver disease -ALD, NAFLD-associated HCC

Introduction

Human beings acquire a sophisticated immune system that actively exists with complex biological mechanisms to defend the host by attacking and destroying the foreign substances (antigen) and transformed or infected cells. Simultaneously, numerous immune regulatory processes are projected to dodge the auto-immune mechanisms resulting against the body's own tissues. The immune system retains a distinct $CD4^+$ cells population, regulatory T cells (Tregs), known to serve essential modulatory roles in immune homeostasis by maintaining peripheral tolerance and controlling the pathological and physiological immune response (Vignali et al., 2008; Lu et al., 2017). These Treg cells assist in limiting the inflammatory responses and abolish autoreactive T cells (Peterson, 2012; Scheinecker et al., 2020).

In 1970, it was first proposed that the presence of thymic-derived suppressor T cells, other than helper T (Th) cells, plays a role in self-tolerance by restricting the effector immune reactions (Gershon and Kondo, 1970). These suppressor T cells were later found responsible for the over-production of immunosuppressive cytokines, such as transforming growth factor β (TGF-β) and interleukin 10 (IL-10), which take part in the immune suppression (O'Garra and Murphy, 1994; Cottrez et al., 2000). Afterward, Sakaguchi et al. (1995), identified the IL-2 receptor α-chain (CD25 molecule) on the surface of these suppressive T cells and named Treg cells. It has been estimated that 5% to 50% of $CD4^+$ T cells in the human peripheral blood are comprised of naturally arising $CD25^+$ Treg cells that suppress immune activity (Sakaguchi et al., 1995; Gregg et al., 2005). Moreover, $CD25^+CD4^+$ Tregs were determined to express forkhead box P3 (FOXP3), a transcriptional factor which regulated the development and proper functioning of Treg cells (Hori et al., 2003). Therefore, these suppressor T cells can be characterized as $CD4^+CD25^+FOXP3^+$ Treg cells in murine and humans (Ziegler, 2006).

Multiple mechanisms have been defined for the proper functioning of Treg cells. These include the secretion of cytokines and soluble factors, cell-to-cell contact, and changes in the extracellular milieu to target a diverse population of immune cell populations, such as antigen-presenting cells (APCs), $CD8^+$ T killers and cell counterparts of conventional $CD4^+$ T cells (Vignali et al., 2008). Emerging evidence suggests the presence of these Treg cells population residing or infiltrating in numerous peripheral organs where they mediate tissue-specific functions. For instance, peroxisome proliferator-activated receptor-γ (PPAR-γ) expressing visceral adipose tissue-specific Treg population execute highly distinct functions, including the regulation of numerous genes known to have crucial functions in lipid and glucose metabolism (Cipolletta et al., 2012). Similarly, in muscle and lung, the presence of amphiregulin, a ligand of epidermal growth factor receptor, expressing Treg cells population can directly expedite the tissue repairing process (Burzyn et al., 2013; Arpaia et al., 2015). $Foxp3^+$ Tregs play various roles in liver homeostasis and pathogenesis by interacting with other hepatic immune cells and hepatocytes. Hepatocytes have exhibited enclysis ability to engulf $CD4^+$ T cells, predominantly Tregs, during hepatic inflammation to regulate T cell population (Davies et al., 2019).

Although the non-lymphoid tissue-specific Tregs molecular signature and functions have been interrogated in numerous investigations, our knowledge of the functions and fundamental biology of these liver-specific Treg cells and how they vary from other non-lymphoid Treg cells and immune cells is yet overlooked. Here, we reviewed the currently available studies about Treg cells that infiltrate the liver, emphasizing the mechanisms in the progression of chronic liver diseases from simple steatosis to hepatocellular carcinoma (HCC).

Treg cells in hepatic microenvironment

One of the largest internal tissues, the liver, connects with the gastrointestinal tract through the portal vein, which delivers multiple pathogenic and non-pathogenic antigens derived from the gastrointestinal tract (Jenne and Kubes, 2013; Kubes and Jenne, 2018). Therefore, besides enduring non-pathogenic organisms, it plays frontline immunological functions by establishing and escalating specific immune responses (Jenne and Kubes, 2013). As a vast organ, liver retains a predominant population of cells, including T lymphocytes, which are immunologically active and maintain essential immunological functions (Parker and Picut, 2012). The phenotypic characteristics and function of Treg cells differ between intrahepatic and circulatory compartments. This difference is due to the residence of Tregs in the hepatic microenvironment, which are deprived of sufficient oxygen while supplemented with inflammatory cytokines, hormones and metabolic products (Jeffery et al., 2016).

As the gut and liver are connected *via* the portal vein, several environmental factors, including dietary nutrients and metabolites, impact the functionality of Treg cells (Zeng and Chi, 2015). It is evident that gut microbiota and their metabolites strongly affect immune responses (Wu and Wu, 2012). Several microbial metabolites and bile acids have been linked with the expansion and stability of Tregs (Hang et al., 2019; Campbell et al., 2020; Song et al., 2020; Wiechers et al., 2021). Increasing evidence suggests that intake of short-chain fatty acids (SCFAs), metabolites produced by numerous symbionts, improves the proportion of Treg cells in mice treated with antibiotics (Arpaia et al., 2013). Similarly, oral gavage of microbes or SCFAs also activates Treg cells in response to several diseases (Ben Ya'acov et al., 2015; Smith et al., 2013). These gut microbiota and their metabolites also influence the normal physiology and immune response in the

liver (Visekruna and Luu, 2021). It can be suggested that microbiota and their metabolites can also play a vital role in stabilizing the Treg cells.

Bile acids, cholesterol metabolites, are immensely produced in the liver through a multiple-step complex process that involves peroxisomal, mitochondrial, and cytosolic enzymes (Russell, 2003). These bile acids are best known to regulate metabolic processes, cellular processes, and the immune system (Chiang and Ferrell, 2019; Fiorucci et al., 2021). Bile acids exert their function by activating numerous receptors, including RAR-related orphan receptor γt (RORγt), liver X receptors α/β (LXRα/β), farnesoid X receptor (FXR), vitamin D receptor (VDR), constitutive androstane receptor (CAR), pregnane X receptor (PXR), and membrane-bound G protein-coupled receptors Takeda G protein-coupled receptor 5 (TGR5) (Keitel et al., 2019; Shin and Wang, 2019; Cai et al., 2021). Among these receptors, FXR and TGR5 have been acknowledged as legitimate targets for treating metabolic-associated NAFLD (Arab et al., 2017). Activation of these receptors also influences and shapes the innate immune response, thus playing critical roles in the progression and development of NAFLD-related HCC (Schubert et al., 2017). Increasing evidence indicates that bile acids and their receptors also influence the adaptive immune system (Campbell et al., 2020; Song et al., 2020); however, the role of these receptors in adaptive immune response, especially in liver resident Tregs, is not studied well. A recent study identified isoallo-LCA and 3-oxo-LCA as metabolites of lithocholic acid (LCA). Among these, 3-oxo-LCA directly binds to the RORγt and suppresses the differentiation of Th17 cells. Meanwhile, isoallo-LCA stimulates mitochondrial ROS production and promotes Treg differentiation by increasing FOXP3 expression (Hang et al., 2019). Interestingly, obeticholic acid (OCA), FXR agonist, was approved by FDA for the treatment of primary sclerosing (Ali and Lindor, 2016). On the other hand, OCA ameliorates fibrosis and NASH; thus, it exerts beneficial effects by reducing hepatic cirrhosis (Shah and Kowdley, 2020). However, the effect of OCA and other bile acid molecules on the population and function of $FOXP3^{+}CD4^{+}$ cells is not studied.

It has been documented that fat-soluble vitamins are highly prevalent in the liver (Stacchiotti et al., 2021). A metabolite of vitamin A, all-trans retinoic acid (RA), plays a role in the growth, differentiation and proper functioning of immune cells, including Treg cells (Liu et al., 2015). In the liver, RA is produced by stellate cells, which increases the Foxp3 in $CD4^{+}$ T cells and takes part in the development (Dunham et al., 2013), functioning and stability of the Treg cells during the inflammatory microenvironment (Zhou et al., 2010; Lu et al., 2014). TGF-β is considered one of the essential immunosuppressive cytokines that activate the population of $CD4^{+}$ $CD25^{+}$ $FoxP3^{+}$ Treg cells (Kanamori et al., 2016). RA can directly influence and promote the TGF-β-dependent differentiation of naive T cells into the $FoxP3^{+}$ Treg cells (Mucida et al., 2009; Martínez-Blanco et al., 2021).

Besides the TGF-β in an inflammatory hepatic microenvironment, several other pro-inflammatory cytokines, including IL-1, IL-12, IL-6, IL-8, and TNFα, are also present. However, it was reported that IL-2 is deficient, which is necessary for the overall survival of Treg cells (Chen et al., 2016a). Activation of APCs is known to secrete these pro-inflammatory cytokines (Blanco et al., 2008). These intrahepatic resident Treg cells also rely on the APCs for their differentiation, proper functioning, and survival. Tregs interact with APCs by binding with CD80/86 through CD28/CTLA-4, engaging with MHC Class II through TCR in the presence of antigen, and responding to APC secretory cytokines through cytokine receptors (Goddard et al., 2004; Lai et al., 2007). However, TGF-β, in combination with higher IL-6, generates IL-17 producing Th17 cells from naïve T cells, which further suppresses the induction of Treg cells (Bettelli et al., 2006). In addition, hepatocytes also play an important role in the differentiation of $Foxp3^{+}$ Tregs upon TCR stimulation from the $CD4^{+}$ T cells *via* Notch-signaling (Burghardt et al., 2014). Resident Treg cells, even present in slightly lower regulatory potential, acquire an intact functional ability and affirm short-term lineage during culturing conditions that imitate and maintain the intrahepatic microenvironment (Chen et al., 2016a).

Immune metabolic pathways have been well studied for the activation, differentiation, survival, and proliferation of the immune cells, including metabolically active Treg cells (O'Neill et al., 2016; Wang et al., 2019). The expression of Foxp3, a pivotal transcription factor of Treg, is known to involve in metabolic pathways, such as glycolysis and fatty-acid oxidation (Fontenot et al., 2003; Gerriets et al., 2016; Angelin et al., 2017). During inflammatory circumstances, the ratios of metabolic supply-and-demand dramatically alter. Inflammatory lesions induce the level of hypoxia-inducible factor-1α (HIF-1α) in the tissues deficient in enough oxygen. HIF-1α plays a vital role in enhancing the population and suppressive roles of thymic Treg (Ben-Shoshan et al., 2008; Clambey et al., 2012) by directly binding the promoter region of FOXP3 (Clambey et al., 2012). However, during an oxygen-deficient environment, the ablation of mTOR signaling is found to be involved in the elimination of the HIF-1α functions (Land and Tee, 2007). Activating transcription factor 3 (ATF3), a member of ATF/CREB transcriptional family, is induced at early inflammatory responses by cytokines, i.e., IFN-α, IFN-β, IFN-γ and IL-4 (Farber, 1992; Drysdale et al., 1996). The absence of ATF3 escalated the mTOR-dependent induction of the HIF-1α, which minimized the $Foxp3^{+}$ Treg cells (Zhu et al., 2018). Therefore, the hypoxic anti-inflammatory process induces HIF-1α that improves the population and functions of Treg cells by strengthening their effectiveness and reducing the proliferation of effector cells (Ben-Shoshan et al., 2008).

Conclusively, it is evident that hepatic immune responses and Treg population and functionality are greatly dependent on the

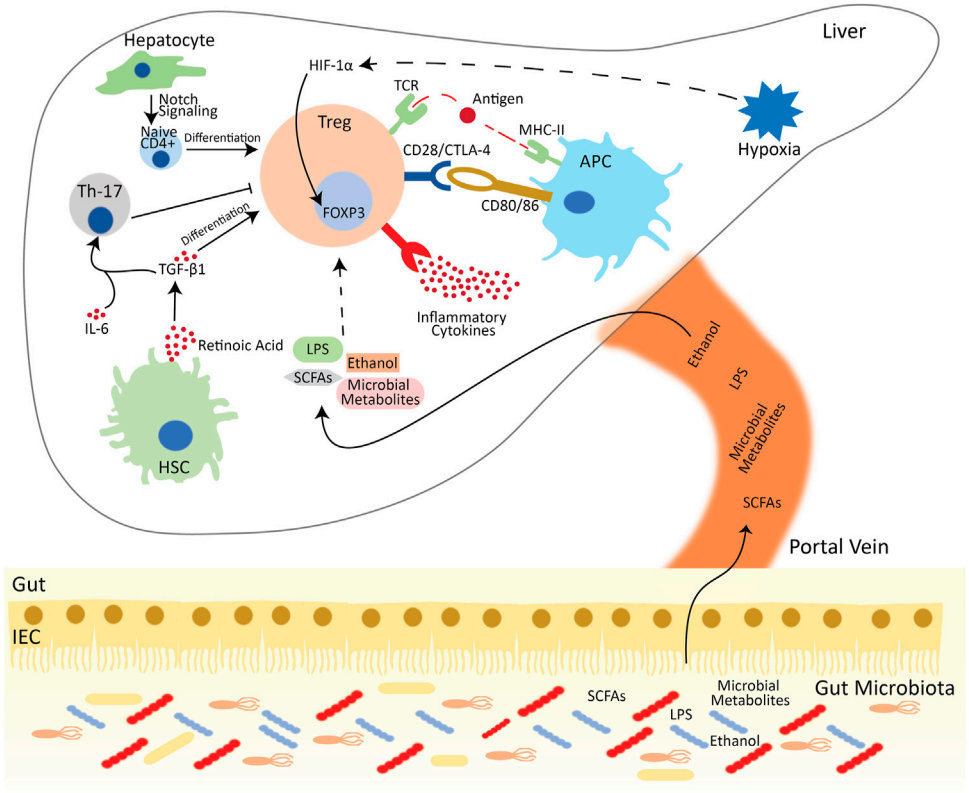

FIGURE 1
Treg mediated immune suppression in the hepatic microenvironment. Tregs are activated by interacting with the MHC-II on the surface of APCs in the presence of antigen. Tregs also suppress conventional T cells by interacting with pro-inflammatory cytokines and pairing with CD80/86 *via* CTL-4/CD28 and deprive the co-stimulatory signal to responder T cells. Besides the APC, Tregs also interact with other hepatic cells. Hepatocytes differentiate the naïve $CD4^+$ cells into $FOXP3^+$ Tregs *via* notch signaling. HSCs release retinoic acid, which activates the TGF-β signaling and aids Tregs differentiation. Meanwhile, in the presence of IL-6, TGF-β activates Th-17 cells, which reduces the activation and development of Tregs. Additionally, in oxygen-deprived environment, HIF-1α directly interacts with the FOXP3 and enhance the population of Tregs. Besides the intrahepatic regulation of Treg development and functions, gut microbiota and their metabolites also influence the Treg functions. Abbreviations: APC, Antigen presenting cell; Treg, regulatory T cell; Th-17, T helper 17 cell; HSC, hepatic stellate cell; IL, interleukin; CTLA4, cytotoxic T lymphocyte-associated antigen 4; DC, dendritic cell; CD, cluster of differentiation; TGF-β1, transforming growth factor-beta 1; MHC, major histocompatibility complex; IEC, intestinal epithelial cells; LPS, lipopolysaccharide; SCFA, Short-chain fatty acids.

hepatic microenvironment (Figure 1). This hepatic microenvironment has an excessive hypoxic atmosphere due to higher blood supply, especially around zone 3 (Jeffery et al., 2016). Meanwhile, other environmental signallings, including environmental factors, microbial metabolites and metabolic pathways, also influence the transcriptional programming of Foxp3 and the functional plasticity of Treg cells.

Functions of Tregs in metabolic-associated chronic hepatic diseases

As mentioned earlier, the human liver acquires a large amount of blood supply, ~70%–80%, from the portal vein, which is intensified with numerous metabolites and nutrients (Balmer et al., 2014). Therefore, the liver-resident immune cells, e.g., APCs, Tregs and T effector cells (Teffs), are continuously exposed to numerous signals which significantly impact their activation and alter their functions. Besides the effects of metabolites and nutrients on Tregs, innate immune cells and pro-inflammatory cytokines also impact the Tregs (Figure 2). Reported data on the immunosuppressive functions of Tregs suggest that intrahepatic suppression/overexpression or inadequate regulation of Tregs contribute to the onset of various diseases, including chronic hepatitis B&C virus (Cabrera et al., 2004), HCC (Unitt et al., 2005), autoimmune hepatitis (Longhi et al., 2004), alcoholic liver disease (ALD) (Matos et al., 2013), non-alcoholic liver disease (NAFLD) (Rau et al., 2016), primary biliary cirrhosis (Lan et al., 2006), acute rejection after liver transplant (Demirkiran et al., 2006). Here, we will be focusing on the function of

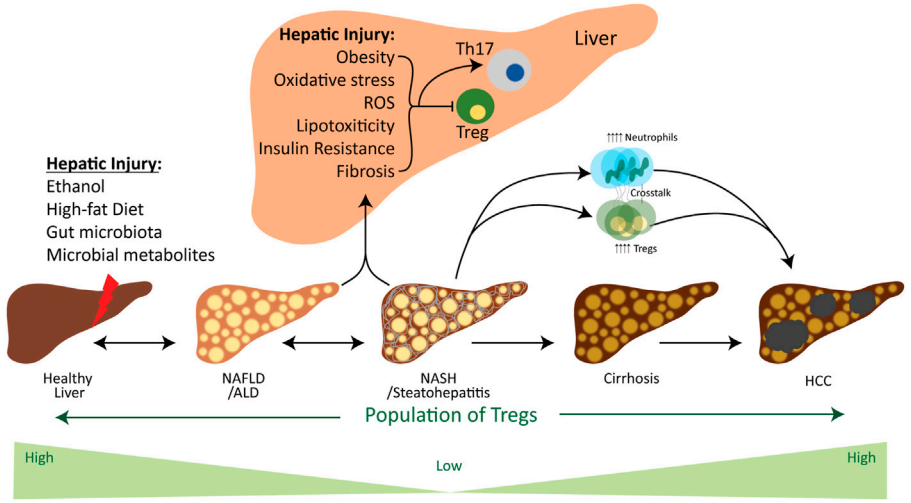

FIGURE 2
Influence of factors involved in the chronic liver disease spectrum on the Treg cells. Hepatic injury is caused by various environmental stressors, including ethanol, a high-fat diet and gut microbiota and their metabolites. These stressors initiate the simple steatosis and lead to steatohepatitis and cirrhosis in the liver, which finally progresses towards the tumor development. In the beginning, environmental stressors induce hepatic oxidative stress, ROS, lipotoxicity, insulin resistance, fibrosis and obesity, which are the pathogenic factors for ALD, NAFLD, NASH and HCC. These factors considerably contribute in downregulating the activation of Tregs and their population for the development of steatosis and fibrosis. However, the progression of NASH towards the tumor development increases the population of Tregs to establish a pro-tumor microenvironment. Meanwhile, adaptive and innate immune systems communicate with each other through Tregs and neutrophils, which aid in the progression of NAFLD-associated HCC. Abbreviations: Treg, regulatory T cell; Th-17, T helper 17 cell; NAFLD, Non-alcoholic fatty liver disease; ALD, alcoholic liver disease; NASH, non-alcoholic steatohepatitis; HCC, hepatocellular carcinoma.

Tregs in metabolic-associated chronic hepatic diseases, including ALD, NAFLD, NASH, and NAFLD-associated HCC.

Role of Treg in alcoholic liver disease

Alcohol abuse, an emerging and alarming health concern with high mortality worldwide, encompasses a broad range of injuries. These include alcoholic liver disease (ALD) with mild steatosis to steatohepatitis, fibrosis, and cirrhosis, leading to hepatocellular cancer (O'Shea et al., 2010; Breitkopf et al., 2009). It has been estimated that excessive alcohol intake is responsible for up to 4% of the yearly deaths (Singal and Anand, 2013). Ample evidence suggests that innate and adaptive immune responses are entailed in the development, pathogenesis and progression of ALD (Mille r et al., 2011; Albano, 2012; Gao et al., 2019). Alcoholic hepatic injury recruits peripheral immune-related cells, including infiltrating monocytes, neutrophils and T lymphocytes (Chedid et al., 1993; Nagy, 2015). Excessive exposure to alcohol or chronic ALD leads to the dysregulation of the balance between these immune cells and disruption of the immune activity. It contributes to the development of the unresolved inflammation features of ALD.

The role of inflammatory response activation and aberrant immune responses in worsening the ALD and its outcome has been extensively studied. It is generally believed that mild or excessive ethanol abuse may modify the immune responses and functions (Szabo et al., 2011; Azizov et al., 2020; Luck et al., 2021). Ethanol impairs the functions of APCs and monocytes, decreases the proliferation of T cells and interferes with the expression of adhesion molecules (Szabo and Mandrekar, 2009). During the progression of ALD, there is an increase in the production of pro-inflammatory chemokines/cytokines by the liver resident macrophages, i.e., kupffer cells (KC) (Slevin et al., 2020). Meanwhile, damaged hepatocytes may produce several antigens to initiate the intrahepatic immune responses, resulting in massive intrahepatic inflammatory cell recruitment, including Treg cells (Viitala et al., 2000).

With the advancement in experimental techniques, multiple T cell subtypes have been identified. It has been recognized that alcohol exposure impacts the T cells phenotypes and Treg cells population (Matos et al., 2013). Earlier studies elucidating the molecular mechanism underlying the progression and development of ALD illustrated the presence of $CD4^+$ and $CD8^+$ cells in the liver biopsies from ALD patients (Chedid et al., 1993). The population of peripheral blood $CD4^+/CD25^+$ Treg cells is not altered (non-significant increase) in chronic alcoholic patients. Still, it significantly decreases with the increased inflammatory cytokines in patients with alcoholic hepatitis (AH) (Almeida et al., 2013). A similar relative and

absolute Treg population in AH, regardless of chronic hepatopathy symptoms, concluded that decreased $CD4^+$/$CD25^+$ Treg cells in AH depend on the acute hepatic inflammation (Almeida et al., 2013).

Similarly, Treg cells are most likely to participate in the pathogenesis of viral hepatitis in individuals with alcohol abuse. It was evident by the enhanced $CD4^+$ $FOXP3^+$ and $CD25^+$ $FOXP3^+$ Treg cell subtypes which were prompted in mice immunized with DCs, isolated from ethanol-fed mice, and loaded with HCV core (Ortiz and Wands, 2014). In contrast to decreased circulatory $CD4^+$/$CD25^+$ Treg cells in alcoholic patients (Almeida et al., 2013), another study reported an increased population of circulatory $CD4^+$/$CD25^+$ Treg cells in alcoholic patients (Ribeiro et al., 2017), suggested the role of Treg cells in reducing the detrimental effects of excessive alcohol intake on the liver. Meanwhile, it has been investigated that inflammatory immune response in ALD has resulted from the increased Th17 population (Kasztelan-Szczerbińska et al., 2015), and Treg cells reduce the development and differentiation of Th17 cells by modulating the levels of anti-inflammatory IL-10 and TGF-β cytokines (Chaudhry et al., 2011; O'Garra and Vieira, 2004; Lee, 2018). Likewise, the increased distribution of Treg cells close to the portal tract in the inflamed human liver demonstrates the close association with the suppression of immune response (Oo et al., 2010). Therefore, it can be suggested that an increased Treg cells population in patients with extreme ethanol might reduce the alcoholic hepatic inflammatory responses.

As previously described, the liver is directly connected to the gut and is continuously susceptible to harmless antigens and byproducts of gut bacteria. Currently available studies have discovered that alterations in the intestinal microbiome serve an important regulatory role in initiating ALD in humans and murine (Yan et al., 2011; Mutlu et al., 2012). Likewise, excessive alcohol intake may damage microbiome balance, distort the intestinal barrier, and lead to a dysfunctional liver and other vital organs (Bishehsari et al., 2017). Alcohol-induced intestinal barrier disruption helps the endotoxins, including lipopolysaccharide (LPS), and cytokines, including IL-6 and TNFα, to translocate into the liver and interact with hepatocytes and immune cells (Bala et al., 2014; Bishehsari et al., 2017; Zheng and Wang, 2021), which play pivotal roles in ALD and hepatic inflammation. Evidence of the ethanol-dependent modifications in the expression of $CD4^+$ T Cell subsets in LPS-stimulated peripheral blood mononuclear cells suggests that ethanol impedes the Foxp3 kinetics and the production of IL-1 and TNF-α after the LPS challenge, thereby affecting the Treg/Th17 cells balance (von Haefen et al., 2011).

Ethanol intake damages the liver by reducing the population of Treg cells while increasing the Th17 cells population along with the production of IL-17 and increasing the intestinal permeability by reducing the expression of tight junction proteins (Wang et al., 2011; Chen et al., 2016b). Probiotic supplementation is well known to improve the functions of the intestinal barrier by improving the expression of tight junction proteins and exerting protective effects in response to damaging factors, including alcohol abuse (Rao and Samak, 2013). Lactobacillus rhamnosus GG (LGG) is a probiotic that strives to treat and prevent various diseases by stimulating immune responses (Segers and Lebeer, 2014). A recent study showed that LGG supernatant supplementation ameliorates ALD by improving the population of Treg cells and decreasing the Th17 population (Chen et al., 2016b).

Alcohol consumption, either acute or chronic, lowers the antigen presentation by DCs, and reduces the T-cell proliferation and activation by affecting the levels of costimulatory molecules (Eken et al., 2011). Hepatic resident APCs activate the Treg cells from naive $CD4^+$ precursors (Bamboat et al., 2009). In ALD-cirrhosis patients, diminished levels of circulatory IL-1β, IL-6, IL-12, and TNF-α inflammatory cytokines, along with fewer number of circulatory DCs have been observed (Laso et al., 2007). Excessive alcohol consumption impairs the production of cytokines IL-6, IL-12, IL-17A and IFN-γ from APCs which are basically involved in initiating the adaptive immune response (Heinz and Waltenbaugh, 2007). Generally, ethanol diminishes the expression of stimulatory molecules on the surface of DCs, impairs their ability to prime T cells, affects the activation of naive T cells, and restricts the development of allogeneic T cells. Upon CpG stimulation, hepatic DCs collected from ethanol-fed mice exhibited reduced functional maturation (Lau et al., 2006). It implies that the alcohol-induced dysfunctional DCs might serve as a protective mechanism for immunosuppression by excessive alcohol drinking (Szabo, 1999; Lau et al., 2006) and may impair the Treg population.

Meanwhile, cell-cell interaction between DCs and Treg cells depends on the MHC-II for activation of Treg cells. Individuals with chronic alcohol intake may show lower MHC II-dependent T cell response (Chang and Norman, 1999). It can be advocated that besides the exclusive effects of alcohol on the cells responsible for innate immune response, early consequences on the adaptive immune system by alcohol contribute to the AH and ALD. Similarly, dysfunctional Treg cells are well known to contribute to the development and progression of these alcoholic diseases. However, the influence of alcohol on the roles of liver resident Treg cells is not studied well. Therefore, further investigations are necessary to improve our understanding of mechanisms underlying ALD and provide aid in finding novel therapeutic targets to treat AH and ALD.

Role of Treg in non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD), affecting one-third of the population, is known for the presence of hepatic steatosis, which accelerates a series of hepatic diseases ranging

from non-alcoholic fatty liver (NAFL) to non-alcoholic steatohepatitis (NASH) and progress to cirrhosis and hepatocellular carcinoma (HCC) (Younossi et al., 2016; Younossi et al., 2018). Hypothetically, the progression of NAFLD has been illustrated in a "multi-hit" manner, which initiates the accumulation of lipids in hepatocytes. Afterward, an increase in free fatty acids secretion from adipocytes, oxidative stress, decreased adiponectin, and increased pro-inflammatory cytokines (resistin, leptin, TNFα, and IL-6) collectively prompt the development of hepatic steatosis and inflammation (Starley et al., 2010). Meanwhile, several other factors, including TGF-β1-dependent collagen deposition, macrophage activation, hepatic reactive oxygen species (ROS), metabolically active natural killer T cells (NK-T) and $CD8^+$ T cells, and imbalance between Th17 and Treg, take part in the disease progression beyond NAFL (Wolf et al., 2014; Paquissi, 2016).

Increasing evidence suggested the close association of NASH with activated innate immune response in mice (Tosello-Trampont et al., 2012) and humans (Malehmir et al., 2019). However, the role of adaptive immunity and Treg cells in NASH hasn't been studied well. A recent clinical study showed the low population of resting Tregs in the peripheral blood of NASH patients and an increase in intrahepatic Th17 cells (Rau et al., 2016), suggesting that higher Th17/rTreg is engaged in NAFL to NASH progression. Another study indicated that NAFLD-related severe hepatic inflammation in children was linked with higher intralobular $Foxp3^+$ lymphocytes, while adults exhibited decreased $Foxp3^+$ and higher $IL\text{-}17A^+$ lymphocytes in portal/periportal (P/P) tracts (Cairoli et al., 2021). Similarly, the high-fat diet (HFD)-induced NAFLD model showed an elevated population of intrahepatic Th17 cells. This increased Th17 and IL-17 are linked with the development of steatosis and the expression of pro-inflammatory cytokines (Tang et al., 2011). Subsequently, increased Th17 in liver during the chronic liver injuries exhibits a decreased proportion of Treg cells with the increased IL-6, IL-17 and IL-23 (He et al., 2017; Khanam et al., 2019).

Insulin resistance (IR), lipotoxicity and adipose inflammation are the hallmarks of NAFLD. Generally, lipotoxicity accelerates the pathogenesis of NAFLD by aggravating hepatic inflammation, adipose tissue inflammation and IR (Manco, 2017). It has been implicated that $CD4^+$ T cells, especially Tregs, play critical roles in the regulation of IR, adipose inflammation and obesity. For instance, Tregs repress the immune response, whereas Th1 and Th17 cells enhance adipose inflammation (Bluestone et al., 2009; Newton et al., 2016). Upon activation, resting naive $CD4^+$ T lymphocytes are differentiated into Tregs and Teffs to and employ immunological responses. Obesity and IR affect the Tregs cells by suppressing their differentiation or impairing their functions (Feuerer et al., 2009; Cipolletta et al., 2012; Wagner et al., 2013). It was found that maintenance of Tregs in VATs of HFD-induced obese animal model significantly reduces adipocyte size and decreases the body weight gain and visceral adipose tissue weight; thus, impairing Tregs worsens HFD-induced obesity and IR in mice (Tian et al., 2011; Cipolletta et al., 2012).

Several cellular metabolic activities, including inflammation, stress responses, and cell survival, are responsible for the oxidative stress and production of ROS within the cells (Pizzino et al., 2017). ROS production promotes hepatic inflammation, fibrogenesis and lipotoxicity in NAFLD (Delli Bovi et al., 2021). Lack of fatty acid β-oxidation along with intensive lipogenesis cause the excessive accumulation of triglycerides within the hepatocytes. During the process of NASH, increased ROS combines triglycerides and leads to IR and hepatic steatosis (Mansouri et al., 2018). Oxidative stress in the liver exerts detrimental effects on the hepatic Tregs. It was observed that oxidative stress induces Treg apoptosis and leads to their deletion within the steatotic liver (Ma et al., 2007), and consequently increases hepatic inflammation and avitivates TNF-α signaling pathway. This results in further hepatic injury, including the progression of simple steatosis to steatohepatitis, especially when liver is exposed to endotoxins, e.g., LPS, which can be delivered to liver or endogenously produced by gut microbiota (Ma et al., 2007; An et al., 2021). Moreover, adoptive transfer of Tregs have decreased HFD-induced intrahepatic TNF-α signaling and diminished the LPS-induced hepatotoxicity (Ma et al., 2007).

As mentioned earlier, activation of Tregs and their ability to perform suppressive functions are somehow dependent on the MHC-II. Liver sinusoidal endothelial cells (LSECs) and KCs are the key MHC-II expressing hepatic APC populations, which display antigen to Tregs and other $CD4^+$ T cells (Wiegard et al., 2005). These Treg cells stimulated by KC and LSEC suppress the other $CD4^+$ T cells proliferation (Wiegard et al., 2005). Similarly, hepatocytes can express MHC-II during hepatic inflammation, which helps them contribute to inflammatory immune responses by overcoming Treg suppressive functions during microbial antigenic signals (Herkel et al., 2003; Wiegard et al., 2005). The inflammatory hepatic microenvironment, i.e., TNF-α, IFN-γ and oxidative stress induced by KCs and DCs impair the survival and induce the apoptosis of $Foxp3^+$ Treg cells during the apoptosis of hepatocytes and NASH (Ma et al., 2007; Roh et al., 2018). However, another study reported that the population of hepatic Tregs enhanced during the NASH, and depletion of Tregs can significantly inhibit the development of HCC from choline-deficient, high-fat diet feeding and diethylnitrosamine injection-induced NASH model (Wang et al., 2021). These opposing findings describing the functions of Tregs in NASH could have resulted from the different NASH models, or there is a probability that Tregs exert contrasting functions during the early and late NASH.

KLF10 is a well-known responsive transcription factor of TGF-β1, which regulates the functions and differentiation of Teffs and Tregs (Cao et al., 2009). A recent study concluded that the expression of KLF10 is significantly reduced in Teffs and

Tregs isolated from peripheral blood and spleen of HFD-induced and obese mice (Wara et al., 2020). It is known that diet-induced obesity and liver diseases increase the immune cell accumulation in the liver and aggravate hepatic inflammation and lipid metabolic dysfunction (Dallio et al., 2021). $CD4^+$ T cell-specific KLF10 deficiency leads to inflammation in adipose tissue, IR, obesity and the onset of NAFLD with impaired Treg accumulation (Wara et al., 2020). However, adoptive transfer of Tregs in $CD4^+$ T cell-specific KLF10 deficient mice impede obesity, IR, adipose tissue inflammation and fatty liver phenotype (Wara et al., 2020). Overall, $Foxp3^+$ Tregs exert protective roles during the progression of NAFLD from simple steatosis to steatohepatitis. Therefore, it can be concluded that despite the activation and marginal clonal expansion of hepatic T cells in NASH, the increased population of Treg counterbalance these effects. Meanwhile, the adoptive transfer of Treg in NASH aggravates the disease severity (Dywicki et al., 2022). Overall, the current understandings of the protective function of Tregs are still limited and need additional investigations to provide aid in treating NAFLD and NASH through Treg targeted therapy.

Role of Treg in NAFLD-associated HCC

HCC is deemed a major histological type of primary liver cancer which accounts for approximately 75% of all hepatic cancers (McGlynn et al., 2015). Leading evidence suggests that a high incidence of NAFLD and the ultimate progression of liver diseases are the leading causes of HCC. NASH leads to the hepatocytes' death and compensatory proliferation, and converts the mild fibrosis to advanced fibrosis with elevated levels of TNFα, TGF-β1 and IL-18, which increase the risks of HCC (Anstee et al., 2019). The tumor microenvironment comprises cancer cells, immune cells and their mediators, and pro-inflammatory cytokines and chemokines (Gallimore and Simon, 2008). In the liver, the pathogenesis of NASH-associated HCC exclusively depends on the intrahepatic inflammatory and immune responses, autophagy, oxidative stress and DNA damage (Wolf et al., 2014; Anstee et al., 2019). Immune evasion of cancer cells is regulated by various immune suppressor mechanisms, which involve different subsets of immune cells and contribute to the initiation and progression of HCC (Greten et al., 2015). Meanwhile, lymphocytic infiltrate at the tumor site decreases the risk of a recurrent tumor and increases the overall survival rate (Greten et al., 2013). However, the role of T cells, especially Tregs, in NASH-associated HCC is not understood well. Striking evidence advocated that the Tregs population increases in peripheral blood and tumor tissues collected from HCC individuals (Guo et al., 2014). Therefore, researchers believe that increase in Tregs may exert adverse effects on the HCC disease prognosis (Wilke et al., 2010), and an augmented ratio of effector $CD4^+$/Treg cells represent a better prognosis for HCC (Kalathil et al., 2019).

Several studies have determined the critical role of $CD4^+$ T cells in the initiation and progression of HCC. $CD4^+$ T cells generally impede the HCC initiation and progression, thereby contributing to the tumor regression (Rakhra et al., 2010). Tregs adversely affect the local immune microenvironment (Nishida and Kudo, 2017). $FOXP3^+$ Tregs exert immunosuppressive functions in the tumor settings by restricting the development and activation of anti-tumor effector cells and facilitating the tumor immune escape (Khazaie and von Boehmer, 2006). Thus, elevated population of $CD4^+$ $CD25^+$ $FoxP3^+$ Tregs promotes the disease initiation and progression by impairing the functions of effector $CD8^+$ cells (Fu et al., 2007; Gao et al., 2007; Shi et al., 2018). Earlier studies have indicated that Tregs could regulate the differentiation and development of T cells by secreting anti-inflammatory IL-10 and TGF-β1 or repressing the IFN-γ and the T cells proliferation, thereby inhibiting their immune function (Bergmann et al., 2011). Meanwhile, HCC tumors themselves secret TGF-β1, which serves as the foremost vital source of TGF-β1 in HCC patients (Wang et al., 2016). This TGF-β1 could be a major factor in activating the regulatory phenotype of Tregs and maintaining their biological functions (Marie et al., 2005).

The functional heterogenicity of $CD4^+$ T cells embraces Teff and Treg cell functions depending on their differentiation (Sallusto and Lanzavecchia, 2009). HCC pathogenesis greatly depends on the selective loss of hepatic resident $CD4^+$ T cells, which accelerates the progression of HCC from NAFLD liver (Ma et al., 2016). It is obvious that IFN-γ secreting cytotoxic $CD4^+$ Th1 cells monitor and clear the premalignant senescent hepatocytes (Kang et al., 2011). However, $FOXP3^+$ Tregs inhibit the proliferation and function of Th1 and other Teffs. Therefore, despite the decreased population of total $CD4^+$ in NASH liver, an enhanced population of Tregs has been observed, which aggravates the inflammation in NASH by establishing the pro-tumorigenic settings and leading to the initiation of the NASH-HCC malignant process (Wang et al., 2021).

Yes-associated protein-1 (YAP1) is a transcriptional coactivator and downstream effector of the Hippo signaling pathway (Manmadhan and Ehmer, 2019). Several studies have reported the positive correlation of YAP1 with the severity of hepatocyte injury and the progression of NAFLD and NASH (Chen et al., 2018; Salloum et al., 2021). Moreover, inhibiting the YAP1, reported as an independent prognostic marker and associated with the disease-free survival HCC patients (Xu et al., 2009), restores hepatocyte differentiation, and reduces the tumor development and the advancement of HCC (Fitamant et al., 2015). Besides the direct evidence of YAP in the development of NAFLD-HCC, it was investigated that YAP is

necessary for the differentiation and immunosuppressive functions of Treg cells (Fan et al., 2017; Ni et al., 2018).

Mounting evidence indicated the potential role of gut microbiota in modulating T-cell immunity directly or *via* their metabolites, including SCFAs (Asarat et al., 2016). Microbial dysbiosis leads to the generation of excessive amounts of SCFA, especially butyrate, which aid in setting the tumor microenvironment (Singh et al., 2018). An ex-vivo investigation showed a positive correlation between Treg and butyrate. It indicated that gut microbiota in the NAFLD-HCC model expands the population of total and effector IL-10$^+$ Tregs while decreasing the expansion of CD8$^+$ cells (Behary et al., 2021). Similarly, it has been verified that IL-2 plays important role in the activation and expansion of CD8$^+$ T cells (Chinen et al., 2016). The presence of peripheral Tregs consumes the IL-2, thereby attenuating the functions of CD8$^+$ T cells (Chinen et al., 2016). Previous ex-vivo investigation implied that this T cell expression profile is triggered by the gut-microbiota isolated from NAFLD-HCC patients, but not cirrhosis, and demonstrated the microbiota and metabolites-specific regulatory effects on T cells in NAFLD-HCC (Behary et al., 2021). Together, all these investigations highlight the role of Treg in the onset of NAFLD-HCC. However, detailed mechanistic studies are still required to thoroughly understand the functions of Tregs in the pre-tumor process from NAFLD to HCC.

Conclusion

Chronic hepatic diseases, such as ALD and NAFLD, are emerging as the foremost cause of liver cancer and morbidity worldwide. It has been estimated that the prevalence of NAFLD-associated HCC will drastically increase (up to 45%–130%) in a decade (Grgurevic et al., 2021). The progression of hepatic diseases largely depends on several factors, such as ROS, oxidative stress, lipotoxicity, IR and gut microbiota. In the hepatic microenvironment, numerous hepatic parenchymal, non-parenchymal, innate immune cells, adaptive immune cells, and inflammatory cytokines and chemokines interact with each other to maintain immune homeostasis. However, factors contributing to metabolic steatosis disrupt this homeostasis which greatly affects the population of T cells in the hepatic microenvironment and leads to the CD4$^+$ T cell infiltration in the liver. Numerous CD4$^+$ T cell subsets participate in regulating the ALD, NAFLD, and NAFLD-related HCC disease progression. Among these T cells, FOXP3$^+$ Tregs play pivotal roles in the progression of steatohepatitis and in creating pre-tumor microenvironment settings. It was suggested that during the hepatic injury, factors contributing to the disease progression reduce the activation and development of FOXP3$^+$ Tregs. However, an increase in Tregs is involved in tempering the features of ALD and NAFLD by reducing the steatohepatitis and fibrosis, and exerting the immunosuppressive effects by hindering the inflammatory cellular immunity (Albano, 2012; Ikeno et al., 2020). Meanwhile, an increased population of Tregs promotes tumor development by setting a premalignant stage for the progression of HCC in the NASH-associated liver (Wang et al., 2021). It is noteworthy that various parenchymal and non-parenchymal cells in the liver interact to induce an immune response. Increasing evidence demonstrates that various factors influence the activity and function of Tregs. However, studies reporting the impacts of Tregs on the hepatic parenchymal and non-parenchymal cells are not illustrated.

Tregs-targeted therapy is regarded as a prospective HCC therapeutic strategy. So far, numerous innovative therapeutic strategies have been reported to target Tregs in clinical trials. These strategies, including using small molecules or antibodies, disrupt the function or differentiation of Tregs (Tsung and Norton, 2015). Despite advancements in biological and cancer research, little attention has been paid to developing strategies targeting the Tregs, especially in NAFLD-associated HCC. Various effective therapies, including monoclonal antibodies (anti-PD-1, anti-PD-L1, and anti-CTLA-4), have shown favorable prognosis and improved overall survival rates in patients with solid tumous (Saleh and Elkord, 2020a). However, studies demonstrating the effect of these monoclonal antibody usages in treating NAFLD-associated HCC are limited and need attention. More importantly, considering the side effects and low efficacy of these monoclonal antibodies, it is important to identify new anti-tumor drugs and validate the efficacy of newly identified drugs alone or in combination with other immune checkpoint inhibitors. Treg-targeted therapy represses tumor growth by enhancing the infiltration of CD8$^+$ cells at the site of the tumor, improving the functions of APCs and minimizing the infiltration of myeloid suppressive cells in TME (Saleh and Elkord, 2019; Saleh and Elkord, 2020b). Thus, realizing the significant effects of the adoptive transfer of Tregs in the NAFLD and NAFLD-related HCC (Van Herck et al., 2020), it is important to evaluate the efficacy of natural or engineered Tregs along with other immune checkpoint inhibitors in NAFLD, premalignant NAFLD and NAFLD-related HCC. Meanwhile, it is worthwhile to understand the effects and function of Tregs in HCC tumors induced by different causative agents and varying disease stages and histological grades.

In conclusion, the cell-cell interactions, production of inflammatory cytokines and chemokines, and antigens in the tumor microenvironment diversify the functional studies of Tregs in HCC tumors. A comprehensive and clear mechanistic understanding of the biology of hepatic Treg cells in the steatosis and premalignant process may lead to inventing novel therapeutic approaches that target Tregs to restrict and

treat chronic hepatic diseases, metabolic steatosis and NAFLD-associated HCC.

Author contributions

FR and PW prepared the draft. FP outlined the topic and edited it.

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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

Albano, E. (2012). Role of adaptive immunity in alcoholic liver disease. *Int. J. Hepatol.* 2012, 893026. doi:10.1155/2012/893026

Ali, A. H., and Lindor, K. D. (2016). Obeticholic acid for the treatment of primary biliary cholangitis. *Expert Opin. Pharmacother.* 17 (13), 1809–1815. doi:10.1080/14656566.2016.1218471

Almeida, J., Polvorosa, M. A., Gonzalez-Quintela, A., Marcos, M., Pastor, I., Hernandez Cerceno, M. L., et al. (2013). Decreased peripheral blood CD4+/CD25+ regulatory T cells in patients with alcoholic hepatitis. *Alcohol. Clin. Exp. Res.* 37 (8), 1361–1369. doi:10.1111/acer.12095

An, L., Wirth, U., Koch, D., Schirren, M., Drefs, M., Koliogiannis, D., et al. (2021). The role of gut-derived lipopolysaccharides and the intestinal barrier in fatty liver diseases. *J. Gastrointest. Surg.* 26, 671–683. doi:10.1007/s11605-021-05188-7

Angelin, A., Gil-de-Gomez, L., Dahiya, S., Jiao, J., Guo, L., Levine, M. H., et al. (2017). Foxp3 reprograms T cell metabolism to function in low-glucose, high-lactate environments. *Cell. Metab.* 25 (6), 1282–1293. e7. doi:10.1016/j.cmet.2016.12.018

Anstee, Q. M., Reeves, H. L., Kotsiliti, E., Govaere, O., and Heikenwalder, M. (2019). From NASH to HCC: Current concepts and future challenges. *Nat. Rev. Gastroenterol. Hepatol.* 16 (7), 411–428. doi:10.1038/s41575-019-0145-7

Arab, J. P., Karpen, S. J., Dawson, P. A., Arrese, M., and Trauner, M. (2017). Bile acids and nonalcoholic fatty liver disease: Molecular insights and therapeutic perspectives. *Hepatology* 65 (1), 350–362. doi:10.1002/hep.28709

Arpaia, N., Campbell, C., Fan, X., Dikiy, S., van der Veeken, J., deRoos, P., et al. (2013). Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* 504 (7480), 451–455. doi:10.1038/nature12726

Arpaia, N., Green, J. A., Moltedo, B., Arvey, A., Hemmers, S., Yuan, S., et al. (2015). A distinct function of regulatory T cells in tissue protection. *Cell.* 162 (5), 1078–1089. doi:10.1016/j.cell.2015.08.021

Asarat, M., Apostolopoulos, V., Vasiljevic, T., and DOnkOr, O. (2016). Short-chain fatty acids regulate cytokines and Th17/treg cells in human peripheral blood mononuclear cells *in vitro*. *Immunol. Investig.* 45 (3), 205–222. doi:10.3109/08820139.2015.1122613

Azizov, V., Dietel, K., Steffen, F., Durholz, K., Meidenbauer, J., Lucas, S., et al. (2020). Ethanol consumption inhibits T(FH) cell responses and the development of autoimmune arthritis. *Nat. Commun.* 11 (1), 1998. doi:10.1038/s41467-020-15855-z

Bala, S., Marcos, M., Gattu, A., Catalano, D., and Szabo, G. (2014). Acute binge drinking increases serum endotoxin and bacterial DNA levels in healthy individuals. *PloS one* 9 (5), e96864. doi:10.1371/journal.pone.0096864

Balmer, M. L., Slack, E., de Gottardi, A., Lawson, M. A. E., Hapfelmeier, S., Miele, L., et al. (2014). The liver may act as a firewall mediating mutualism between the host and its gut commensal microbiota. *Sci. Transl. Med.* 6 (237), 237ra66. doi:10.1126/scitranslmed.3008618

Bamboat, Z. M., Stableford, J. A., Plitas, G., Burt, B. M., Nguyen, H. M., Welles, A. P., et al. (2009). Human liver dendritic cells promote T cell hyporesponsiveness. *J. Immunol.* 182 (4), 1901–1911. doi:10.4049/jimmunol.0803404

Behary, J., Amorim, N., Jiang, X. T., Raposo, A., Gong, L., McGovern, E., et al. (2021). Gut microbiota impact on the peripheral immune response in non-alcoholic fatty liver disease related hepatocellular carcinoma. *Nat. Commun.* 12 (1), 187. doi:10.1038/s41467-020-20422-7

Ben Ya'acov, A., Lichtenstein, Y., Zolotarov, L., and Ilan, Y. (2015). The gut microbiome as a target for regulatory T cell-based immunotherapy: Induction of regulatory lymphocytes by oral administration of anti-LPS enriched colostrum alleviates immune mediated colitis. *BMC Gastroenterol.* 15, 154. doi:10.1186/s12876-015-0388-x

Ben-Shoshan, J., Maysel-Auslender, S., Mor, A., Keren, G., and George, J. (2008). Hypoxia controls CD4+CD25+ regulatory T-cell homeostasis via hypoxia-inducible factor-1alpha. *Eur. J. Immunol.* 38 (9), 2412–2418. doi:10.1002/eji.200838318

Bergmann, C., Wild, C. A., Narwan, M., Lotfi, R., Lang, S., and Brandau, S. (2011). Human tumor-induced and naturally occurring Treg cells differentially affect NK cells activated by either IL-2 or target cells. *Eur. J. Immunol.* 41 (12), 3564–3573. doi:10.1002/eji.201141532

Bettelli, E., Carrier, Y., Gao, W., Korn, T., Strom, T. B., Oukka, M., et al. (2006). Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 441 (7090), 235–238. doi:10.1038/nature04753

Bishehsari, F., Magno, E., Swanson, G., Desai, V., Voigt, R. M., Forsyth, C. B., et al. (2017). Alcohol and gut-derived inflammation. *Alcohol Res.* 38 (2), 163–171.

Blanco, P., Palucka, A. K., Pascual, V., and Banchereau, J. (2008). Dendritic cells and cytokines in human inflammatory and autoimmune diseases. *Cytokine Growth Factor Rev.* 19 (1), 41–52. doi:10.1016/j.cytogfr.2007.10.004

Bluestone, J. A., Mackay, C. R., O'Shea, J. J., and Stockinger, B. (2009). The functional plasticity of T cell subsets. *Nat. Rev. Immunol.* 9 (11), 811–816. doi:10.1038/nri2654

Breitkopf, K., Nagy, L. E., Beier, J. I., Mueller, S., Weng, H., and Dooley, S. (2009). Current experimental perspectives on the clinical progression of alcoholic liver disease. *Alcohol. Clin. Exp. Res.* 33 (10), 1647–1655. doi:10.1111/j.1530-0277.2009.01015.x

Burghardt, S., Claass, B., Erhardt, A., Karimi, K., and Tiegs, G. (2014). Hepatocytes induce Foxp3$^+$ regulatory T cells by Notch signaling. *J. Leukoc. Biol.* 96 (4), 571–577. doi:10.1189/jlb.2AB0613-342RR

Burzyn, D., Kuswanto, W., Kolodin, D., Shadrach, J. L., Cerletti, M., Jang, Y., et al. (2013). A special population of regulatory T cells potentiates muscle repair. *Cell.* 155 (6), 1282–1295. doi:10.1016/j.cell.2013.10.054

Cabrera, R., Tu, Z., Xu, Y., Firpi, R. J., Rosen, H. R., Liu, C., et al. (2004). An immunomodulatory role for CD4(+)CD25(+) regulatory T lymphocytes in hepatitis C virus infection. *Hepatology* 40 (5), 1062–1071. doi:10.1002/hep.20454

Cai, X., Young, G. M., and Xie, W. (2021). The xenobiotic receptors PXR and CAR in liver physiology, an update. *Biochim. Biophys. Acta. Mol. Basis Dis.* 1867 (6), 166101. doi:10.1016/j.bbadis.2021.166101

Cairoli, V., De Matteo, E., Rios, D., Lezama, C., Galoppo, M., Casciato, P., et al. (2021). Hepatic lymphocytes involved in the pathogenesis of pediatric and adult non-alcoholic fatty liver disease. *Sci. Rep.* 11 (1), 5129. doi:10.1038/s41598-021-84674-z

Campbell, C., McKenney, P. T., Konstantinovsky, D., Isaeva, O. I., Schizas, M., Verter, J., et al. (2020). Bacterial metabolism of bile acids promotes generation of peripheral regulatory T cells. *Nature* 581 (7809), 475–479. doi:10.1038/s41586-020-2193-0

Cao, Z., Wara, A. K., Icli, B., Sun, X., Packard, R. R. S., Esen, F., et al. (2009). Kruppel-like factor KLF10 targets transforming growth factor-beta1 to regulate CD4(+)CD25(-) T cells and T regulatory cells. *J. Biol. Chem.* 284 (37), 24914–24924. doi:10.1074/jbc.M109.000059

Chang, M. P., and Norman, D. C. (1999). Ethanol impairs major histocompatibility complex (MHC) class II molecule-mediated but not MHC class I molecule-mediated T cell response in alcohol-consuming mice. *Immunopharmacol. Immunotoxicol.* 21 (1), 65–87. doi:10.3109/08923979909016395

Chaudhry, A., Samstein, R. M., Treuting, P., Liang, Y., Pils, M. C., Heinrich, J. M., et al. (2011). Interleukin-10 signaling in regulatory T cells is required for suppression of Th17 cell-mediated inflammation. *Immunity* 34 (4), 566–578. doi:10.1016/j.immuni.2011.03.018

Chedid, A., Mendenhall, C. L., Moritz, T. E., French, S. W., Chen, T. S., Morgan, T. R., et al. (1993). Cell-mediated hepatic injury in alcoholic liver disease. Veterans Affairs Cooperative Study Group 275. *Gastroenterology* 105 (1), 254–266. doi:10.1016/0016-5085(93)90034-a

Chen, P., Luo, Q., Huang, C., Gao, Q., Li, L., Chen, J., et al. (2018). Pathogenesis of non-alcoholic fatty liver disease mediated by YAP. *Hepatol. Int.* 12 (1), 26–36. doi:10.1007/s12072-017-9841-y

Chen, R. C., Xu, L. M., Du, S. J., Huang, S. S., Wu, H., Dong, J. J., et al. (2016). Lactobacillus rhamnosus GG supernatant promotes intestinal barrier function, balances Treg and TH17 cells and ameliorates hepatic injury in a mouse model of chronic-binge alcohol feeding. *Toxicol. Lett.* 241, 103–110. doi:10.1016/j.toxlet.2015.11.019

Chen, Y. Y., Jeffery, H. C., Hunter, S., Bhogal, R., Birtwistle, J., Braitch, M. K., et al. (2016). Human intrahepatic regulatory T cells are functional, require IL-2 from effector cells for survival, and are susceptible to Fas ligand-mediated apoptosis. *Hepatology* 64(1), 138–150. doi:10.1002/hep.28517

Chiang, J. Y. L., and Ferrell, J. M. (2019). Bile acids as metabolic regulators and nutrient sensors. *Annu. Rev. Nutr.* 39, 175–200. doi:10.1146/annurev-nutr-082018-124344

Chinen, T., Kannan, A. K., Levine, A. G., Fan, X., Klein, U., Zheng, Y., et al. (2016). An essential role for the IL-2 receptor in T(reg) cell function. *Nat. Immunol.* 17 (11), 1322–1333. doi:10.1038/ni.3540

Cipolletta, D., Feuerer, M., Li, A., Kamei, N., Lee, J., Shoelson, S. E., et al. (2012). PPAR-γ is a major driver of the accumulation and phenotype of adipose tissue Treg cells. *Nature* 486 (7404), 549–553. doi:10.1038/nature11132

Clambey, E. T., McNamee, E. N., Westrich, J. A., Glover, L. E., Campbell, E. L., Jedlicka, P., et al. (2012). Hypoxia-inducible factor-1 alpha-dependent induction of FoxP3 drives regulatory T-cell abundance and function during inflammatory hypoxia of the mucosa. *Proc. Natl. Acad. Sci. U. S. A.* 109 (41), E2784–E2793. doi:10.1073/pnas.1202366109

Cottrez, F., Hurst, S. D., Coffman, R. L., and Groux, H. (2000). T regulatory cells 1 inhibit a Th2-specific response *in vivo*. *J. Immunol.* 165 (9), 4848–4853. doi:10.4049/jimmunol.165.9.4848

Dallio, M., Sangineto, M., Romeo, M., Villani, R., Romano, A. D., Loguercio, C., et al. (2021). Immunity as cornerstone of non-alcoholic fatty liver disease: The contribution of oxidative stress in the disease progression. *Int. J. Mol. Sci.* 22 (1), E436. doi:10.3390/ijms22010436

Davies, S. P., Reynolds, G. M., Wilkinson, A. L., Li, X., Rose, R., Leekha, M., et al. (2019). Hepatocytes delete regulatory T cells by enclysis, a CD4(+) T cell engulfment process. *Cell. Rep.* 29 (6), 1610–1620. doi:10.1016/j.celrep.2019.09.068

Delli Bovi, A. P., Marciano, F., Mandato, C., Siano, M. A., Savoia, M., and Vajro, P. (2021). Oxidative stress in non-alcoholic fatty liver disease. An updated mini review. *Front. Med.* 8, 595371. doi:10.3389/fmed.2021.595371

Demirkiran, A., Kok, A., Kwekkeboom, J., Kusters, J. G., Metselaar, H. J., Tilanus, H. W., et al. (2006). Low circulating regulatory T-cell levels after acute rejection in liver transplantation. *Liver Transpl.* 12 (2), 277–284. doi:10.1002/lt.20612

Drysdale, B. E., Howard, D. L., and Johnson, R. J. (1996). Identification of a lipopolysaccharide inducible transcription factor in murine macrophages. *Mol. Immunol.* 33 (11-12), 989–998. doi:10.1016/s0161-5890(96)00043-0

Dunham, R. M., Thapa, M., Velazquez, V. M., Elrod, E. J., Denning, T. L., Pulendran, B., et al. (2013). Hepatic stellate cells preferentially induce Foxp3+ regulatory T cells by production of retinoic acid*J. Immunol.* 190 (5), 2009–2016. doi:10.4049/jimmunol.1201937

Dywicki, J., Buitrago-Molina, L. E., Noyan, F., Davalos-Misslitz, A. C., Hupa-Breier, K. L., Lieber, M., et al. (2022). The detrimental role of regulatory T cells in nonalcoholic steatohepatitis. *Hepatol. Commun.* 6 (2), 320–333. doi:10.1002/hep4.1807

Eken, A., Ortiz, V., and Wands, J. R. (2011). Ethanol inhibits antigen presentation by dendritic cells. *Clin. Vaccine Immunol.* 18 (7), 1157–1166. doi:10.1128/CVI.05029-11

Fan, Y., Gao, Y., Rao, J., Wang, K., Zhang, F., and Zhang, C. (2017). YAP-1 promotes Tregs differentiation in hepatocellular carcinoma by enhancing TGFBR2 transcription. *Cell. Physiol. biochem.* 41 (3), 1189–1198. doi:10.1159/000464380

Farber, J. M. (1992). A collection of mRNA species that are inducible in the RAW 264.7 mouse macrophage cell line by gamma interferon and other agents. *Mol. Cell. Biol.* 12 (4), 1535–1545. doi:10.1128/mcb.12.4.1535

Feuerer, M., Herrero, L., Cipolletta, D., Naaz, A., Wong, J., Nayer, A., et al. (2009). Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. *Nat. Med.* 15 (8), 930–939. doi:10.1038/nm.2002

Fiorucci, S., Distrutti, E., Carino, A., Zampella, A., and Biagioli, M. (2021). Bile acids and their receptors in metabolic disorders. *Prog. Lipid Res.* 82, 101094. doi:10.1016/j.plipres.2021.101094

Fitamant, J., Kottakis, F., Benhamouche, S., Tian, H. S., Chuvin, N., Parachoniak, C. A., et al. (2015). YAP inhibition restores hepatocyte differentiation in advanced HCC, leading to tumor regression. *Cell. Rep.* 10 (10), 1692–1707. doi:10.1016/j.celrep.2015.02.027

Fontenot, J. D., Gavin, M. A., and Rudensky, A. Y. (2003). Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat. Immunol.* 4 (4), 330–336. doi:10.1038/ni904

Fu, J., Xu, D., Liu, Z., Shi, M., Zhao, P., Fu, B., et al. (2007). Increased regulatory T cells correlate with CD8 T-cell impairment and poor survival in hepatocellular carcinoma patients. *Gastroenterology* 132 (7), 2328–2339. doi:10.1053/j.gastro.2007.03.102

Gallimore, A. M., and Simon, A. K. (2008). Positive and negative influences of regulatory T cells on tumour immunity. *Oncogene* 27 (45), 5886–5893. doi:10.1038/onc.2008.269

Gao, B., Ahmad, M. F., Nagy, L. E., and Tsukamoto, H. (2019). Inflammatory pathways in alcoholic steatohepatitis. *J. Hepatol.* 70 (2), 249–259. doi:10.1016/j.jhep.2018.10.023

Gao, Q., Qiu, S. J., Fan, J., Zhou, J., Wang, X. Y., Xiao, Y. S., et al. (2007). Intratumoral balance of regulatory and cytotoxic T cells is associated with prognosis of hepatocellular carcinoma after resection. *J. Clin. Oncol.* 25 (18), 2586–2593. doi:10.1200/JCO.2006.09.4565

Gerriets, V. A., Kishton, R. J., Johnson, M. O., Cohen, S., Siska, P. J., Nichols, A. G., et al. (2016). Foxp3 and Toll-like receptor signaling balance T(reg) cell anabolic metabolism for suppression. *Nat. Immunol.* 17 (12), 1459–1466. doi:10.1038/ni.3577

Gershon, R. K., and Kondo, K. (1970). Cell interactions in the induction of tolerance: The role of thymic lymphocytes. *Immunology* 18 (5), 723–737.

Goddard, S., Youster, J., Morgan, E., and Adams, D. H. (2004). Interleukin-10 secretion differentiates dendritic cells from human liver and skin. *Am. J. Pathol.* 164 (2), 511–519. doi:10.1016/S0002-9440(10)63141-0

Gregg, R., Smith, C. M., Clark, F. J., Dunnion, D., Kha, N. N., Chak, R. R., et al. (2005). The number of human peripheral blood CD4+ CD25high regulatory T cells increases with age. *Clin. Exp. Immunol.* 140 (3), 540–546. doi:10.1111/j.1365-2249.2005.02798.x

Greten, T. F., Duffy, A. G., and Korangy, F. (2013). Hepatocellular carcinoma from an immunologic perspective. *Clin. Cancer Res.* 19 (24), 6678–6685. doi:10.1158/1078-0432.CCR-13-1721

Greten, T. F., Wang, X. W., and Korangy, F. (2015). Current concepts of immune based treatments for patients with HCC: From basic science to novel treatment approaches. *Gut* 64 (5), 842–848. doi:10.1136/gutjnl-2014-307990

Grgurevic, I., Bozin, T., Mikus, M., Kukla, M., and O'Beirne, J. (2021). Hepatocellular carcinoma in non-alcoholic fatty liver disease: From epidemiology to diagnostic approach. *Cancers* 13 (22), 5844. doi:10.3390/cancers13225844

Guo, C. L., Yang, X. H., Cheng, W., Xu, Y., Li, J. B., Sun, Y. X., et al. (2014). Expression of Fas/FasL in CD8+ T and CD3+ Foxp3+ Treg cells--relationship with apoptosis of circulating CD8+ T cells in hepatocellular carcinoma patients. *Asian pac. J. Cancer Prev.* 15 (6), 2613–2618. doi:10.7314/apjcp.2014.15.6.2613

Hang, S., Paik, D., Yao, L., Kim, E., Trinath, J., Lu, J., et al. (2019). Bile acid metabolites control T(H)17 and T(reg) cell differentiation. *Nature* 576 (7785), 143–148. doi:10.1038/s41586-019-1785-z

He, B., Wu, L., Xie, W., Shao, Y., Jiang, J., Zhao, Z., et al. (2017). The imbalance of Th17/Treg cells is involved in the progression of nonalcoholic fatty liver disease in mice. *BMC Immunol.* 18 (1), 33. doi:10.1186/s12865-017-0215-y

Heinz, R., and Waltenbaugh, C. (2007). Ethanol consumption modifies dendritic cell antigen presentation in mice. *Alcohol. Clin. Exp. Res.* 31 (10), 1759–1771. doi:10.1111/j.1530-0277.2007.00479.x

Herkel, J., Jagemann, B., Wiegard, C., Lazaro, J. F. G., Lueth, S., Kanzler, S., et al. (2003). MHC class II-expressing hepatocytes function as antigen-presenting cells and activate specific CD4 T lymphocytes. *Hepatology* 37 (5), 1079–1085. doi:10.1053/jhep.2003.50191

Hori, S., Nomura, T., and Sakaguchi, S. (2003). Control of regulatory T cell development by the transcription factor Foxp3. *Science* 299 (5609), 1057–1061. doi:10.1126/science.1079490

Ikeno, Y., Ohara, D., Takeuchi, Y., Watanabe, H., Kondoh, G., Taura, K., et al. (2020). Foxp3+ regulatory T cells inhibit CCl(4)-induced liver inflammation and fibrosis by regulating tissue cellular immunity. *Front. Immunol.* 11, 584048. doi:10.3389/fimmu.2020.584048

Jeffery, H. C., Braitch, M. K., Brown, S., and Oo, Y. H. (2016). Clinical potential of regulatory T cell therapy in liver diseases: An overview and current perspectives. *Front. Immunol.* 7, 334. doi:10.3389/fimmu.2016.00334

Jenne, C. N., and Kubes, P. (2013). Immune surveillance by the liver. *Nat. Immunol.* 14 (10), 996–1006. doi:10.1038/ni.2691

Kalathil, S. G., Hutson, A., Barbi, J., Iyer, R., and Thanavala, Y. (2019). Augmentation of IFN-γ+ CD8+ T cell responses correlates with survival of HCC patients on sorafenib therapy. *JCI Insight* 4 (15), 130116. doi:10.1172/jci.insight.130116

Kanamori, M., Nakatsukasa, H., Okada, M., Lu, Q., and Yoshimura, A. (2016). Induced regulatory T cells: Their development, stability, and applications. *Trends Immunol.* 37 (11), 803–811. doi:10.1016/j.it.2016.08.012

Kang, T. W., Yevsa, T., Woller, N., Hoenicke, L., Wuestefeld, T., Dauch, D., et al. (2011). Senescence surveillance of pre-malignant hepatocytes limits liver cancer development. *Nature* 479 (7374), 547–551. doi:10.1038/nature10599

Kasztelan-Szczerbińska, B., Surdacka, A., Celinski, K., Rolinski, J., Zwolak, A., Miacz, S., et al. (2015). Prognostic significance of the systemic inflammatory and immune balance in alcoholic liver disease with a focus on gender-related differences. *PLoS One* 10 (6), e0128347. doi:10.1371/journal.pone.0128347

Keitel, V., Stindt, J., and Häussinger, D. (2019). Bile acid-activated receptors: GPBAR1 (TGR5) and other G protein-coupled receptors. *Handb. Exp. Pharmacol.* 256, 19–49. doi:10.1007/164_2019_230

Khanam, A., Trehanpati, N., and Sarin, S. K. (2019). Increased interleukin-23 receptor (IL-23R) expression is associated with disease severity in acute-on-chronic liver failure. *Liver Int.* 39 (6), 1062–1070. doi:10.1111/liv.14015

Khazaie, K., and von Boehmer, H. (2006). The impact of CD4+CD25+ Treg on tumor specific CD8+ T cell cytotoxicity and cancer. *Semin. Cancer Biol.* 16 (2), 124–136. doi:10.1016/j.semcancer.2005.11.006

Kubes, P., and Jenne, C. (2018). Immune responses in the liver. *Annu. Rev. Immunol.* 36, 247–277. doi:10.1146/annurev-immunol-051116-052415

Lai, W. K., Curbishley, S. M., Goddard, S., Alabraba, E., Shaw, J., Youster, J., et al. (2007). Hepatitis C is associated with perturbation of intrahepatic myeloid and plasmacytoid dendritic cell function. *J. Hepatol.* 47 (3), 338–347. doi:10.1016/j.jhep.2007.03.024

Lan, R. Y., Cheng, C., Lian, Z. X., Tsuneyama, K., Yang, G. X., Moritoki, Y., et al. (2006). Liver-targeted and peripheral blood alterations of regulatory T cells in primary biliary cirrhosis. *Hepatology* 43 (4), 729–737. doi:10.1002/hep.21123

Land, S. C., and Tee, A. R. (2007). Hypoxia-inducible factor 1alpha is regulated by the mammalian target of rapamycin (mTOR) via an mTOR signaling motif. *J. Biol. Chem.* 282 (28), 20534–20543. doi:10.1074/jbc.M611782200

Laso, F. J., Vaquero, J. M., Almeida, J., Marcos, M., and Orfao, A. (2007). Chronic alcohol consumption is associated with changes in the distribution, immunophenotype, and the inflammatory cytokine secretion profile of circulating dendritic cells. *Alcohol. Clin. Exp. Res.* 31 (5), 846–854. doi:10.1111/j.1530-0277.2007.00377.x

Lau, A. H., Abe, M., and Thomson, A. W. (2006). Ethanol affects the generation, cosignaling molecule expression, and function of plasmacytoid and myeloid dendritic cell subsets *in vitro* and *in vivo*. *J. Leukoc. Biol.* 79 (5), 941–953. doi:10.1189/jlb.0905517

Lee, G. R. (2018). The balance of Th17 versus Treg cells in autoimmunity. *Int. J. Mol. Sci.* 19 (3), 730. doi:10.3390/ijms19030730

Liu, Z.-M., Wang, K. P., Ma, J., and Guo Zheng, S. (2015). The role of all-trans retinoic acid in the biology of Foxp3+ regulatory T cells. *Cell. Mol. Immunol.* 12 (5), 553–557. doi:10.1038/cmi.2014.133

Longhi, M. S., Ma, Y., Bogdanos, D. P., Cheeseman, P., Mieli-Vergani, G., and Vergani, D. (2004). Impairment of CD4(+)CD25(+) regulatory T-cells in autoimmune liver disease. *J. Hepatol.* 41 (1), 31–37. doi:10.1016/j.jhep.2004.03.008

Lu, L., Barbi, J., and Pan, F. (2017). The regulation of immune tolerance by FOXP3. *Nat. Rev. Immunol.* 17 (11), 703–717. doi:10.1038/nri.2017.75

Lu, L., Lan, Q., Li, Z., Zhou, X., Gu, J., Li, Q., et al. (2014). Critical role of all-trans retinoic acid in stabilizing human natural regulatory T cells under inflammatory conditions. *Proc. Natl. Acad. Sci. U. S. A.* 111 (33), E3432–E3440. doi:10.1073/pnas.1408780111

Luck, M. E., Li, X., Herrnreiter, C. J., and Choudhry, M. A. (2021). Ethanol intoxication and burn injury increases intestinal regulatory T cell population and regulatory T cell suppressive capability. *Shock* 57, 230. doi:10.1097/SHK.0000000000001853

Ma, C., Kesarwala, A. H., Eggert, T., Medina-Echeverz, J., Kleiner, D. E., Jin, P., et al. (2016). NAFLD causes selective CD4(+) T lymphocyte loss and promotes hepatocarcinogenesis. *Nature* 531 (7593), 253–257. doi:10.1038/nature16969

Ma, X., Hua, J., Mohamood, A. R., Hamad, A. R. A., Ravi, R., and Li, Z. (2007). A high-fat diet and regulatory T cells influence susceptibility to endotoxin-induced liver injury. *Hepatology* 46 (5), 1519–1529. doi:10.1002/hep.21823

Malehmir, M., Pfister, D., Gallage, S., Szydlowska, M., Inverso, D., Kotsiliti, E., et al. (2019). Platelet GPIbα is a mediator and potential interventional target for NASH and subsequent liver cancer. *Nat. Med.* 25 (4), 641–655. doi:10.1038/s41591-019-0379-5

Manco, M. (2017). Insulin resistance and NAFLD: A dangerous liaison beyond the genetics. *Child. (Basel, Switz.* 4 (8), 74. doi:10.3390/children4080074

Manmadhan, S., and Ehmer, U. (2019). Hippo signaling in the liver - a long and ever-expanding story. *Front. Cell. Dev. Biol.* 7, 33. doi:10.3389/fcell.2019.00033

Mansouri, A., Gattolliat, C. H., and Asselah, T. (2018). Mitochondrial dysfunction and signaling in chronic liver diseases. *Gastroenterology* 155 (3), 629–647. doi:10.1053/j.gastro.2018.06.083

Marie, J. C., Letterio, J. J., Gavin, M., and Rudensky, A. Y. (2005). TGF-beta1 maintains suppressor function and Foxp3 expression in CD4+CD25+ regulatory T cells. *J. Exp. Med.* 201 (7), 1061–1067. doi:10.1084/jem.20042276

Martínez-Blanco, M., Lozano-Ojalvo, D., Perez-Rodriguez, L., Benede, S., Molina, E., and Lopez-Fandino, R. (2021). Retinoic acid induces functionally suppressive Foxp3(+)RORγt(+) T cells *in vitro*. *Front. Immunol.* 12, 675733. doi:10.3389/fimmu.2021.675733

Matos, L. C., Batista, P., Monteiro, N., Ribeiro, J., Cipriano, M. A., Henriques, P., et al. (2013). Lymphocyte subsets in alcoholic liver disease. *World J. Hepatol.* 5 (2), 46–55. doi:10.4254/wjh.v5.i2.46

McGlynn, K. A., Petrick, J. L., and London, W. T. (2015). Global epidemiology of hepatocellular carcinoma: An emphasis on demographic and regional variability. *Clin. Liver Dis.* 19 (2), 223–238. doi:10.1016/j.cld.2015.01.001

Miller, A. M., Horiguchi, N., Jeong, W. I., Radaeva, S., and Gao, B. (2011). Molecular mechanisms of alcoholic liver disease: Innate immunity and cytokines. *Alcohol. Clin. Exp. Res.* 35 (5), 787–793. doi:10.1111/j.1530-0277.2010.01399.x

Mucida, D., Pino-Lagos, K., Kim, G., Nowak, E., Benson, M. J., Kronenberg, M., et al. (2009). Retinoic acid can directly promote TGF-beta-mediated Foxp3(+) Treg cell conversion of naive T cells. *Immunity* 30 (4), 471–472. author reply 472-3. doi:10.1016/j.immuni.2009.03.008

Mutlu, E. A., Gillevet, P. M., Rangwala, H., Sikaroodi, M., Naqvi, A., Engen, P. A., et al. (2012). Colonic microbiome is altered in alcoholism. *Am. J. Physiol. Gastrointest. Liver Physiol.* 302 (9), G966–G978. doi:10.1152/ajpgi.00380.2011

Nagy, L. E. (2015). The role of innate immunity in alcoholic liver disease. *Alcohol Res.* 37 (2), 237–250.

Newton, R., Priyadharshini, B., and Turka, L. A. (2016). Immunometabolism of regulatory T cells. *Nat. Immunol.* 17 (6), 618–625. doi:10.1038/ni.3466

Ni, X., Tao, J., Barbi, J., Chen, Q., Park, B. V., Li, Z., et al. (2018). YAP is essential for treg-mediated suppression of antitumor immunity. *Cancer Discov.* 8 (8), 1026–1043. doi:10.1158/2159-8290.CD-17-1124

Nishida, N., and Kudo, M. (2017). Immunological microenvironment of hepatocellular carcinoma and its clinical implication. *Oncology* 92 (Suppl. 1), 40–49. doi:10.1159/000451015

O'Garra, A., and Murphy, K. (1994). Role of cytokines in determining T-lymphocyte function. *Curr. Opin. Immunol.* 6 (3), 458–466. doi:10.1016/0952-7915(94)90128-7

O'Garra, A., and Vieira, P. (2004). Regulatory T cells and mechanisms of immune system control. *Nat. Med.* 10 (8), 801–805. doi:10.1038/nm0804-801

O'Neill, L. A., Kishton, R. J., and Rathmell, J. (2016). A guide to immunometabolism for immunologists. *Nat. Rev. Immunol.* 16 (9), 553–565. doi:10.1038/nri.2016.70

O'Shea, R. S., Dasarathy, S., and McCullough, A. J. (2010). Alcoholic liver disease. *Hepatology* 51 (1), 307–328. doi:10.1002/hep.23258

Oo, Y. H., Weston, C. J., Lalor, P. F., Curbishley, S. M., Withers, D. R., Reynolds, G. M., et al. (2010). Distinct roles for CCR4 and CXCR3 in the recruitment and positioning of regulatory T cells in the inflamed human liver. *J. Immunol.* 184 (6), 2886–2898. doi:10.4049/jimmunol.0901216

Ortiz, V., and Wands, J. R. (2014). Chronic ethanol diet increases regulatory T-cell activity and inhibits hepatitis C virus core-specific cellular immune responses in mice. *Hepatol. Res.* 44 (7), 788–797. doi:10.1111/hepr.12173

Paquissi, F. C. (2016). Immune imbalances in non-alcoholic fatty liver disease: From general biomarkers and neutrophils to interleukin-17 Axis Activation and new therapeutic targets. *Front. Immunol.* 7, 490. doi:10.3389/fimmu.2016.00490

Parker, G. A., and Picut, C. A. (2012). Immune functioning in non lymphoid organs: The liver. *Toxicol. Pathol.* 40 (2), 237–247. doi:10.1177/0192623311428475

Peterson, R. A. (2012). Regulatory T-cells: Diverse phenotypes integral to immune homeostasis and suppression. *Toxicol. Pathol.* 40 (2), 186–204. doi:10.1177/0192623311430693

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., et al. (2017). Oxidative stress: Harms and benefits for human health. *Oxidative Med. Cell. Longev.* 2017, 8416763.

Rakhra, K., Bachireddy, P., Zabuawala, T., Zeiser, R., Xu, L., Kopelman, A., et al. (2010). CD4(+) T cells contribute to the remodeling of the microenvironment required for sustained tumor regression upon oncogene inactivation. *Cancer Cell.* 18 (5), 485–498. doi:10.1016/j.ccr.2010.10.002

Rao, R. K., and Samak, G. (2013). Protection and restitution of gut barrier by probiotics: Nutritional and clinical implications. *Curr. Nutr. Food Sci.* 9 (2), 99–107. doi:10.2174/1573401311309020004

Rau, M., Schilling, A. K., Meertens, J., Hering, I., Weiss, J., Jurowich, C., et al. (2016). Progression from nonalcoholic fatty liver to nonalcoholic steatohepatitis is marked by a higher frequency of Th17 cells in the liver and an increased Th17/resting regulatory T cell ratio in peripheral blood and in the liver. *J. Immunol.* 196 (1), 97–105. doi:10.4049/jimmunol.1501175

Ribeiro, S. R., Covre, L. P., Stringari, L. L., da Penha Zago-Gomes, M., Gomes, D. C. O., and Pereira, F. E. L. (2017). Peripheral blood CD4(+)/CD25(+) regulatory T cells in alcoholic patients with Strongyloides stercoralis infection. *Parasitol. Res.* 116 (3), 1071–1074. doi:10.1007/s00436-016-5355-0

Roh, Y. S., Kim, J. W., Park, S., Shon, C., Kim, S., Eo, S. K., et al. (2018). Toll-Like receptor-7 signaling promotes nonalcoholic steatohepatitis by inhibiting regulatory T cells in mice. *Am. J. Pathol.* 188 (11), 2574–2588. doi:10.1016/j.ajpath.2018.07.011

Russell, D. W. (2003). The enzymes, regulation, and genetics of bile acid synthesis. *Annu. Rev. Biochem.* 72, 137–174. doi:10.1146/annurev.biochem.72.121801.161712

Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M., and Toda, M. (1995). Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol.* 155 (3), 1151–1164.

Saleh, R., and Elkord, E. (2020). Acquired resistance to cancer immunotherapy: Role of tumor-mediated immunosuppression. *Semin. Cancer Biol.* 65, 13–27. doi:10.1016/j.semcancer.2019.07.017

Saleh, R., and Elkord, E. (2020). FoxP3(+) T regulatory cells in cancer: Prognostic biomarkers and therapeutic targets. *Cancer Lett.* 490, 174–185. doi:10.1016/j.canlet.2020.07.022

Saleh, R., and Elkord, E. (2019). Treg-mediated acquired resistance to immune checkpoint inhibitors. *Cancer Lett.* 457, 168–179. doi:10.1016/j.canlet.2019.05.003

Salloum, S., Jeyarajan, A. J., Kruger, A. J., Holmes, J. A., Shao, T., Sojoodi, M., et al. (2021). Fatty acids activate the transcriptional coactivator YAP1 to promote liver fibrosis via p38 mitogen-activated protein kinase. *Cell. Mol. Gastroenterol. Hepatol.* 12 (4), 1297–1310. doi:10.1016/j.jcmgh.2021.06.003

Sallusto, F., and Lanzavecchia, A. (2009). Heterogeneity of CD4+ memory T cells: Functional modules for tailored immunity. *Eur. J. Immunol.* 39 (8), 2076–2082. doi:10.1002/eji.200939722

Scheinecker, C., Göschl, L., and Bonelli, M. (2020). Treg cells in health and autoimmune diseases: New insights from single cell analysis. *J. Autoimmun.* 110, 102376. doi:10.1016/j.jaut.2019.102376

Schubert, K., Olde Damink, S. W. M., von Bergen, M., and Schaap, F. G. (2017). Interactions between bile salts, gut microbiota, and hepatic innate immunity. *Immunol. Rev.* 279 (1), 23–35. doi:10.1111/imr.12579

Segers, M. E., and Lebeer, S. (2014). Towards a better understanding of Lactobacillus rhamnosus GG--host interactions. *Microb. Cell. Fact.* 13 (Suppl. 1), S7. doi:10.1186/1475-2859-13-S1-S7

Shah, R. A., and Kowdley, K. V. (2020). Obeticholic acid for the treatment of nonalcoholic steatohepatitis. *Expert Rev. Gastroenterol. Hepatol.* 14 (5), 311–321. doi:10.1080/17474124.2020.1748498

Shi, C., Chen, Y., Chen, Y., Yang, Y., Bing, W., and Qi, J. (2018). CD4(+) CD25(+) regulatory T cells promote hepatocellular carcinoma invasion via TGF-β1-induced epithelial-mesenchymal transition. *Onco. Targets. Ther.* 12, 279–289. doi:10.2147/OTT.S172417

Shin, D. J., and Wang, L. (2019). Bile acid-activated receptors: A review on FXR and other nuclear receptors. *Handb. Exp. Pharmacol.* 256, 51–72. doi:10.1007/164_2019_236

Singal, A. K., and Anand, B. S. (2013). Recent trends in the epidemiology of alcoholic liver disease. *Clin. Liver Dis.* 2 (2), 53–56. doi:10.1002/cld.168

Singh, V., Yeoh, B. S., Chassaing, B., Xiao, X., Saha, P., Aguilera Olvera, R., et al. (2018). Dysregulated microbial fermentation of soluble fiber induces cholestatic liver cancer. *Cell.* 175 (3), 679–694. e22. doi:10.1016/j.cell.2018.09.004

Slevin, E., Baiocchi, L., Wu, N., Ekser, B., Sato, K., Lin, E., et al. (2020). Kupffer cells: Inflammation pathways and cell-cell interactions in alcohol-associated liver disease. *Am. J. Pathol.* 190 (11), 2185–2193. doi:10.1016/j.ajpath.2020.08.014

Smith, P. M., Howitt, M. R., Panikov, N., Michaud, M., Gallini, C. A., Bohlooly-Y, M., et al. (2013). The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Sci. (New York, N.Y.)* 341 (6145), 569–573. doi:10.1126/science.1241165

Song, X., Sun, X., Oh, S. F., Wu, M., Zhang, Y., Zheng, W., et al. (2020). Microbial bile acid metabolites modulate gut RORγ(+) regulatory T cell homeostasis. *Nature* 577 (7790), 410–415. doi:10.1038/s41586-019-1865-0

Stacchiotti, V., Rezzi, S., Eggersdorfer, M., and Galli, F. (2021). Metabolic and functional interplay between gut microbiota and fat-soluble vitamins. *Crit. Rev. Food Sci. Nutr.* 61 (19), 3211–3232. doi:10.1080/10408398.2020.1793728

Starley, B. Q., Calcagno, C. J., and Harrison, S. A. (2010). Nonalcoholic fatty liver disease and hepatocellular carcinoma: A weighty connection. *Hepatology* 51 (5), 1820–1832. doi:10.1002/hep.23594

Szabo, G. (1999). Consequences of alcohol consumption on host defence. *Alcohol Alcohol* 34 (6), 830–841. doi:10.1093/alcalc/34.6.830

Szabo, G., and Mandrekar, P. (2009). A recent perspective on alcohol, immunity, and host defense. *Alcohol. Clin. Exp. Res.* 33 (2), 220–232. doi:10.1111/j.1530-0277.2008.00842.x

Szabo, G., Mandrekar, P., Petrasek, J., and Catalano, D. (2011). The unfolding web of innate immune dysregulation in alcoholic liver injury. *Alcohol. Clin. Exp. Res.* 35 (5), 782–786. doi:10.1111/j.1530-0277.2010.01398.x

Tang, Y., Bian, Z., Zhao, L., Liu, Y., Liang, S., Wang, Q., et al. (2011). Interleukin-17 exacerbates hepatic steatosis and inflammation in non-alcoholic fatty liver disease. *Clin. Exp. Immunol.* 166 (2), 281–290. doi:10.1111/j.1365-2249.2011.04471.x

Tian, J., Dang, H. N., Yong, J., Chui, W. S., Dizon, M. P. G., Yaw, C. K. Y., et al. (2011). Oral treatment with γ-aminobutyric acid improves glucose tolerance and insulin sensitivity by inhibiting inflammation in high fat diet-fed mice. *PLoS One* 6 (9), e25338. doi:10.1371/journal.pone.0025338

Tosello-Trampont, A. C., Landes, S. G., Nguyen, V., Novobrantseva, T. I., and Hahn, Y. S. (2012). Kuppfer cells trigger nonalcoholic steatohepatitis development in diet-induced mouse model through tumor necrosis factor-α production. *J. Biol. Chem.* 287 (48), 40161–40172. doi:10.1074/jbc.M112.417014

Tsung, K., and Norton, J. A. (2015). An immunological view of chemotherapy. *Immunotherapy* 7 (9), 941–943. doi:10.2217/imt.15.62

Unitt, E., Rushbrook, S. M., Marshall, A., Davies, S., Gibbs, P., Morris, L. S., et al. (2005). Compromised lymphocytes infiltrate hepatocellular carcinoma: The role of T-regulatory cells. *Hepatology* 41 (4), 722–730. doi:10.1002/hep.20644

Van Herck, M. A., Vonghia, L., Kwanten, W. J., Vanwolleghem, T., Ebo, D. G., Michielsen, P. P., et al. (2020). Adoptive cell transfer of regulatory T cells exacerbates hepatic steatosis in high-fat high-fructose diet-fed mice. *Front. Immunol.* 11, 1711. doi:10.3389/fimmu.2020.01711

Vignali, D. A., Collison, L. W., and Workman, C. J. (2008). How regulatory T cells work. *Nat. Rev. Immunol.* 8 (7), 523–532. doi:10.1038/nri2343

Viitala, K., MaKKonen, K., Israel, Y., LehTimaki, T., JaakkOla, O., Koivula, T., et al. (2000). Autoimmune responses against oxidant stress and acetaldehyde-derived epitopes in human alcohol consumers. *Alcohol. Clin. Exp. Res.* 24 (7), 1103–1109. doi:10.1111/j.1530-0277.2000.tb04656.x

Visekruna, A., and Luu, M. (2021). The role of short-chain fatty acids and bile acids in intestinal and liver function, inflammation, and carcinogenesis. *Front. Cell. Dev. Biol.* 9, 703218. doi:10.3389/fcell.2021.703218

von Haefen, C., Mei, W., Menk, M., Klemz, R., Jones, A., Wernecke, K. D., et al. (2011). Ethanol changes gene expression of transcription factors and cytokine production of CD4+ T-cell subsets in PBMCs stimulated with LPS. *Alcohol. Clin. Exp. Res.* 35 (4), 621–631. doi:10.1111/j.1530-0277.2010.01376.x

Wagner, N. M., Brandhorst, G., Czepluch, F., Lankeit, M., Eberle, C., Herzberg, S., et al. (2013). Circulating regulatory T cells are reduced in obesity and may identify subjects at increased metabolic and cardiovascular risk. *Obes. (Silver Spring)* 21 (3), 461–468. doi:10.1002/oby.20087

Wang, A., Luan, H. H., and Medzhitov, R. (2019). An evolutionary perspective on immunometabolism. *Science* 363 (6423), eaar3932. doi:10.1126/science.aar3932

Wang, H., Zhang, H., Wang, Y., Brown, Z. J., Xia, Y., Huang, Z., et al. (2021). Regulatory T-cell and neutrophil extracellular trap interaction contributes to carcinogenesis in non-alcoholic steatohepatitis. *J. Hepatol.* 75 (6), 1271–1283. doi:10.1016/j.jhep.2021.07.032

Wang, Y., Kirpich, I., Liu, Y., Ma, Z., Barve, S., McClain, C. J., et al. (2011). Lactobacillus rhamnosus GG treatment potentiates intestinal hypoxia-inducible factor, promotes intestinal integrity and ameliorates alcohol-induced liver injury. *Am. J. Pathol.* 179 (6), 2866–2875. doi:10.1016/j.ajpath.2011.08.039

Wang, Y., Liu, T., Tang, W., Deng, B., Chen, Y., Zhu, J., et al. (2016). Hepatocellular carcinoma cells induce regulatory T cells and lead to poor prognosis via production of transforming growth factor-β1. *Cell. Physiol. biochem.* 38 (1), 306–318. doi:10.1159/000438631

Wara, A. K., Wang, S., Wu, C., Fang, F., Haemmig, S., Weber, B. N., et al. (2020). KLF10 deficiency in CD4(+) T cells triggers obesity, insulin resistance, and fatty liver. *Cell. Rep.* 33 (13), 108550. doi:10.1016/j.celrep.2020.108550

Wiechers, C., Zou, M., Galvez, E., Beckstette, M., Ebel, M., Strowig, T., et al. (2021). The microbiota is dispensable for the early stages of peripheral regulatory T cell induction within mesenteric lymph nodes. *Cell. Mol. Immunol.* 18 (5), 1211–1221. doi:10.1038/s41423-021-00647-2

Wiegard, C., Frenzel, C., Herkel, J., Kallen, K. J., Schmitt, E., and Lohse, A. W. (2005). Murine liver antigen presenting cells control suppressor activity of CD4+CD25+ regulatory T cells. *Hepatology* 42 (1), 193–199. doi:10.1002/hep.20756

Wilke, C. M., Wu, K., Zhao, E., Wang, G., and Zou, W. (2010). Prognostic significance of regulatory T cells in tumor. *Int. J. Cancer* 127 (4), 748–758. doi:10.1002/ijc.25464

Wolf, M. J., Adili, A., Piotrowitz, K., Abdullah, Z., Boege, Y., Stemmer, K., et al. (2014). Metabolic activation of intrahepatic CD8+ T cells and NKT cells causes nonalcoholic steatohepatitis and liver cancer via cross-talk with hepatocytes. *Cancer Cell.* 26 (4), 549–564. doi:10.1016/j.ccell.2014.09.003

Wu, H.-J., and Wu, E. (2012). The role of gut microbiota in immune homeostasis and autoimmunity. *Gut microbes* 3 (1), 4–14. doi:10.4161/gmic.19320

Xu, M. Z., Yao, T. J., Lee, N. P. Y., Ng, I. O. L., Chan, Y. T., Zender, L., et al. (2009). Yes-associated protein is an independent prognostic marker in hepatocellular carcinoma. *Cancer* 115 (19), 4576–4585. doi:10.1002/cncr.24495

Yan, A. W., Fouts, D. E., Brandl, J., Starkel, P., Torralba, M., Schott, E., et al. (2011). Enteric dysbiosis associated with a mouse model of alcoholic liver disease. *Hepatology* 53 (1), 96–105. doi:10.1002/hep.24018

Younossi, Z., Anstee, Q. M., Marietti, M., Hardy, T., Henry, L., Eslam, M., et al. (2018). Global burden of NAFLD and NASH: Trends, predictions, risk factors and prevention. *Nat. Rev. Gastroenterol. Hepatol.* 15 (1), 11–20. doi:10.1038/nrgastro.2017.109

Younossi, Z. M., Koenig, A. B., Abdelatif, D., Fazel, Y., Henry, L., and Wymer, M. (2016). Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 64 (1), 73–84. doi:10.1002/hep.28431

Zeng, H., and Chi, H. (2015). Metabolic control of regulatory T cell development and function. *Trends Immunol.* 36 (1), 3–12. doi:10.1016/j.it.2014.08.003

Zheng, Z., and Wang, B. (2021). The gut-liver Axis in health and disease: The role of gut microbiota-derived signals in liver injury and regeneration. *Front. Immunol.* 12, 775526. doi:10.3389/fimmu.2021.775526

Zhou, X., Kong, N., Wang, J., Fan, H., Zou, H., Horwitz, D., et al. (2010). Cutting edge: All-trans retinoic acid sustains the stability and function of natural regulatory T cells in an inflammatory milieu. *J. Immunol.* 185 (5), 2675–2679. doi:10.4049/jimmunol.1000598

Zhu, Q., Wang, H., Jiang, B., Ni, X., Jiang, L., Li, C., et al. (2018). Loss of ATF3 exacerbates liver damage through the activation of mTOR/p70S6K/HIF-1α signaling pathway in liver inflammatory injury. *Cell. Death Dis.* 9 (9), 910. doi:10.1038/s41419-018-0894-1

Ziegler, S. F. (2006). FOXP3: Of mice and men. *Annu. Rev. Immunol.* 24, 209–226. doi:10.1146/annurev.immunol.24.021605.090547

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Chimeric Antigen Receptor T Cells Targeting Cell Surface GRP78 to Eradicate Acute Myeloid Leukemia

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Acute myeloid leukemia (AML) is a serious, life-threatening hematological malignancy. The treatment outcome of relapsed or refractory AML patients remains dismal, and new treatment options are needed. Chimeric antigen receptor (CAR) T cells have been successful in improving the prognosis for B-lineage acute lymphoblastic leukemia and lymphoma by targeting CD19. However, CAR T-cell therapy for AML is still elusive, owing to the lack of a tumor-specific cell surface antigen and spare hematopoietic stem cells (HSCs). This study generated a novel CAR construction that targets the cell surface protein glucose-regulated protein 78 (GRP78) (csGRP78). We confirmed that GRP78-CAR T cells demonstrate an anti-tumor effect against human AML cells *in vitro*. In xenograft models, GRP78-CAR T cells effectively eliminate AML cells and protect mice against systemic leukemia, in the meanwhile, prolonging survival. In addition, GRP78-CAR T cells also specifically eradicate the primary AML patient-derived blast. In particular, GRP78-CAR T cells spare normal HSCs, highlighting that GRP78-CAR is a promising approach for the therapy of AML.

Keywords: GRP78, CAR T cell, acute myeloid leukemia (AML), hematopoietic stem cells (HSCS), cell surface

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INTRODUCTION

In recent years, chimeric antigen receptor (CAR) T-cell treatment has achieved great success in clinical trials, especially those in which CAR T cells targeting CD19 have shown excellent response against B-cell lineage hematological malignancies (Scheuermann and Racila, 1995; Porter et al., 2011; Maude et al., 2014; Sommermeyer et al., 2017; Park et al., 2018). However, other subtypes of hematological malignancies, including acute myeloid leukemia (AML) with deficiency of a specific target on the cell surface lack effective therapy.

AML, the most common acute leukemia in adults, is characterized by a clonal expansion of myeloid blasts in the bone marrow, blood, and other tissues. The prognosis of AML patients is still poor, and the 5-year survival rate remains below 50% owing to resistance to the treatments and relapse of the disease. Unlike the B-cell malignancies with antigens that are exclusively expressed in B-cell lineages and B-cell aplasia, which is clinically tolerable, CAR-targeted antigens for myeloid cells are shared with normal hematopoietic stem cells (HSCs); thus, no ideal surface marker can distinguish between normal and tumor cells (Mardiana and Gill, 2020). Several CARs targeting CD33 (Wang et al., 2015) or CD123 (Ruella et al., 2016) show potency in the pre-clinical model; however, these CAR T cells are targeting both normal HSCs

and leukemic cells, which lead to myeloablation and bone marrow failure and induce significant cytokine release syndrome (Ehninger et al., 2014; Wang et al., 2015). To prevent the severe side effect induced by the CAR T-cell treatment, several approaches were utilized including limiting the persistence of CAR T cells through engineering a "safety switch" (Di Stasi et al., 2011); novel developed CAR T cells targeting CLL-1 or CD70 are efficient against AML without being toxic to normal HSCs (Tashiro et al., 2017; Wang et al., 2018; Sauer et al., 2021). In the past decades, numerous tumor antigens such as CD33, CD123, CLL1, and CD38 have been explored as target antigens for AML treatment (Jin et al., 2009; Walter et al., 2012; Wang et al., 2018; Cui et al., 2021), and the clinical trials for these CAR T-cell therapies reported improved outcomes. A phase I clinical trial showed that CLL1 CAR T-cell therapy has high efficacy and limited toxicity in relapsed and refractory AML patients (Zhang et al., 2021). Another clinical trial reported that four out of six (66.7%) relapsed patients achieved complete remission (CR) or CR with incomplete count recovery (CRi) after CD38-CAR T-cell infusion, and the clinically adverse effects of patients are manageable (Cui et al., 2021). Since the number of cases in these studies is limited, the safety and efficacy of CAR T-cell therapy for patients with relapsed/refractory AML requires further investigations.

Glucose-regulated protein 78 (GRP78; also known as Bip) as an endoplasmic reticulum (ER) chaperone protein is essential for protein quality control (Quinones et al., 2008). The accumulation of evidence shows that GRP78 contains anti-apoptotic function through activation of UPR and blocking caspase activation to enhance cell survival and contribute to tumor progression (Fu et al., 2007; Casas, 2017). In particular, GRP78 is expressed and located mainly within the ER lumen. However, a small fraction of GRP78 could re-locate to the cell surface on a certain type of cells, particularly tumor cells (Shin et al., 2003; Arap et al., 2004; Gonzalez–Gronow et al., 2009; Zhang et al., 2010). GRP78 on the cell surface of tumor cells promotes cell proliferation, metastasis, and resistance to drug therapy (Lee, 2007; Li and Li, 2012).

A proof-of-concept study reported that the cell surface GRP78 could be specifically targeted by peptidic ligands to induce tumor cell death in prostate and breast cancer models (Arap et al., 2004; Kim et al., 2006). The GRP78 monoclonal antibody MAb159 specifically shows inhibition of tumor cell proliferation and metastasis and leads to tumor regression (Liu et al., 2013). The inhibition of cell surface GRP78 by polyclonal N-20 antibody can suppress glioma cancer cell survival and proliferation (Kang et al., 2016). GRP78 was proven to be highly expressed on the cell surface of AML patients' peripheral blood cells, and it is also over-expressed in chronic lymphocytic leukemia patients' cells than in normal B cells, but not in T cells (Huergo-Zapico et al., 2012; Staquicini et al., 2018). Therefore, the overexpression of GRP78 on the plasma membrane of leukemia cells potentially provides a novel target for the therapy of hematological malignancies.

Here, we developed CAR T cells targeting cell surface GRP78 to specifically eradicate AMLs cells *in vitro* and *in vivo*.

METHODS

Cell Lines and Primary Samples

KG1a, HL-60, and U937 cell lines were from ATCC and were purchased from the China National Collection of Authenticated Cell Cultures and Conservation Genetics CAS Kunming Cell Bank. HL-60 and U937 cells were cultured in RPMI (Gibco) supplemented with 10% FBS, 100 U/ml penicillin, and 100 mg/ml streptomycin sulfate at 37°C with 5% CO_2. KG1a cells were cultured in RPMI (Gibco) supplemented with 20% FBS, 100 U/ml penicillin, and 100 mg/ml streptomycin sulfate at 37°C with 5% CO_2. Primary human AML blood samples were obtained from the West China Hospital of Sichuan University with the approval of patients. Blood and bone marrow from healthy donors were provided by the West China Hospital of Sichuan University with the approval of donors (#2022151).

Chimeric Antigen Receptor T-Cell Production

Primary T cells were isolated from peripheral blood of healthy donors using the RosetteSep Human T Cell Enrichment Cocktail (Stemcell Technologies) according to the manufacturer's protocol. The isolated T cells were confirmed by flow cytometry using phycoerythrin-conjugated anti-human CD3 (BioLegend, 300408). T cells were cultured in Advanced RPMI1640 (Gibco) supplemented with 10% FBS, 100 U/ml penicillin, 100 mg/ml streptomycin sulfate, and 200 U/ml IL2 (PeproTech). T cells were counted and stimulated by CD3/CD28 beads (Life Technologies) for 72 h and infected with lentiviral particles at an MOI of 100.

Cytotoxic T Lymphocyte Assay

The specific cytotoxicity of the CAR T cells was measured against the CFSE-labeled target cancer cells at indicated effector/target (E/T) ratios in triplicate wells. After 24 h of culture in Advanced RPMI 1640 (Gibco) supplemented with 10% FBS, 100 U/ml penicillin, and 100 mg/ml streptomycin sulfate, total cells were harvested, and dead cells were labeled with PI following flow cytometry analysis.

Cytokine Production Assay

Effector cells and target cells were cultured at varied E/T ratios in Advanced RPMI 1640 (Gibco) media for 24 or 48 h. Human interferon gamma (INF-γ; #KHC4021, Invitrogen), tumor necrosis factor (TNF; #550610, BD Biosciences), and granzyme B (#ab235635, Abcam) in the supernatant of culture were analyzed using a commercial assay according to the manufacturer's instructions.

Immunofluorescence

A total of 0.5×10^6 cells were collected and washed once with ice-cold PBS (pH 7.4) and incubated with anti-GRP78 (#PA1-014A, Thermo Fisher Scientific; 1:200 in 2% FBS PBS) for 60 min at 4°C. Cells were centrifuged and washed once with PBS followed by fixation in ice-cold methanol (100%) for 10 min at 4°C and washed once again with PBS. Cells were incubated in blocking buffer (2.5% BSA/10% goat serum/

0.1% Tween-20) for 30 min at RT. Incubation with goat anti-rabbit IgG (H+L) secondary antibody conjugated with Cyanine3 (#A10520, ThermoFisher Scientific, 1:200) in blocking buffer for 1 h at RT. After washing once with PBS, cell nuclei were stained with DAPI in PBS for 10 min in the dark. The cells were centrifuged and washed once with PBS, resuspended in 30 μl PBS and dropped on the coverslip, mounted with Aqua-Polymount on the slide (#1860620, Polysciences Inc.).

Xenograft Animal Model

Six-week-old female NOD-Prkdcem26ll2rgem26Nju (NCG) mice were purchased from GemPharmatech Co., Ltd. (Jiangsu, China) and housed in a pathogen-free animal facility. Then, 1.0×10^6 KG1a-Luci or U937-EGFP-Luci cells were intravenously injected into NCG mice *via* the tail vein 1 week after arriving at the local animal facility. Moreover, 5.0×10^6 Non-CAR, MOCK-CAR, and GRP78-CAR T cells were intravenously injected via the tail vein on day 6 for U937-EGFP-Luci–injected mice and day 7 for KG1a-Luci–injected mice after leukemia cells were transplanted, followed by serial bioluminescence imaging to quantify the progression of the tumor. The bioluminescence images were captured using an IVIS imaging system and quantified by Living Image software 4.1 (PerkinElmer). Mice were injected intraperitoneally with Avertin (375 mg/kg) and D-luciferin (150 mg/kg) and imaged under anesthesia. Mice were sacrificed when they were in the moribund state or when they showed signs of hind limb paralysis or at end of the experiment time point (50 days post leukemia cell injection for U937-EGFP-Luci or 100 days for KG1a-Luci).

Flow Cytometry

Primary AML cells were isolated through density gradient centrifuge, and then the remaining red blood cells were lysed. For the cell lysis analysis of $CD34^+$ progenitor/stem cell subsets, CD34-FITC mAbs (Clone 4H11, Invitrogen) were stained, and the positive subset was analyzed using the percentage of lysis after staining with PI. The transduction rate of CAR into T cells was detected using mKate signal. Flow cytometry was performed on a BD Fortesa flow cytometer, and results were analyzed using the software FlowJo v10.

RESULTS

Glucose-Regulated Protein 78–Chimeric Antigen Receptor T Cells Demonstrated Robust Cytotoxicity Against Leukemia Cells *In Vitro*

To access the expression of GRP78 on the cell surface, we performed live-cell staining with GRP78, observing enhanced GRP78 expression on the plasma membrane of multiple AML cell lines (**Figure 1A**; **Supplementary Figure S1**). Thus, the enriched GRP78 on the cell surface provides the opportunity to be targeted.

We constructed a Pep42 peptide into a second-generation CAR construct to generate GRP78-CAR that contains 4-1BB and CD3ζ costimulatory signaling domains (**Supplementary Figure S2A**). Healthy T cells were expanded and transduced by a GRP78-CAR lentivirus, and Non-CAR, MOCK-CAR, and CD19-CAR were used as controls. The CAR lentivirus transduction is efficient in that more than 50% of T cells expressed CAR (**Supplementary Figure S2B**). The cytotoxicity of CAR T cells was assessed by co-culturing with leukemia cells *in vitro* for 24 h. It was observed that GRP78-CAR T cells robustly elicited specific cytotoxicity against all three AML cell lines (**Figure 1B**; GRP78-CAR vs. Non-CAR, MOCK-CAR, and CD19-CAR). Next, we evaluated the dose-dependent cytotoxicity of the CAR T cells by progressively increasing the E/T ratio from 0.5: 1 to 5:1. GRP78-CAR T cells exhibited robust lysis against AML cells, even at the 0.5:1 ratio for KG1a and U937 cells (**Figure 1C**). Considerable amounts of released cytokines including IFN-γ, TNF-α, and granzyme B were detected in the supernatant of the medium after GRP78-CAR T cells were co-cultured with AML cells (**Figure 1D**). Together, the result of the *in vitro* cytotoxicity assay provides compelling evidence that GRP78-CAR T cells can effectively kill AML cells *in vitro*.

Glucose-Regulated Protein 78–Chimeric Antigen Receptor T Cells Eliminate Human Acute Myeloid Leukemia *In Vivo* in Xenograft Mouse Models

The anti-leukemia cytotoxicity *in vivo* was validated through the human AML xenograft mouse model, in which 1×10^6 U937-EGFP-Luci leukemia cells were intravenously injected into NSG mice, followed by 5.0×10^6 Non-CAR T cells, MOCK-CAR T cells, and GRP78-CAR T cells injection at day 6 (**Figure 2A**). The burden of tumor cells was monitored over a series of days by luminescence imaging.

The mice injected with Non-CAR T cells and MOCK-CAR T cells showed first signs of disease, such as reduced mobility, hypothermia, and scrubby fur, within 17 days post injection, strikingly compared to 34 days for the mice injected with GRP78-CAR T cells (**Figure 2B**). Control mice that received Non-CAR T or MOCK-CAR T-cell injection displayed rapid progression of leukemia that 50% of animals succumbed 19 days post leukemia cell injection. In sharp contrast, among GRP78-CAR T-cell–treated mice, 50% survived more than 45 days after leukemia cell injection (**Figure 2D**). Moreover, three out of seven mice survived healthy for 50 days after tumor cell challenges, and tumor burden imaging confirmed that the vast majority of the leukemia cells were eliminated *in vivo* (**Figures 2C,D**).

We also tested the therapeutic efficacy of GRP78-CAR T cells by intravenous injection of AML KG1a-Luci cells into NSG mice, and the groups of mice treated with Non-CAR T cells, MOCK-CAR T cells, or GRP78-CAR T cells were monitored by bioluminescence at indicated days post injection (**Supplementary Figure S3A**). The AML burden in mice was

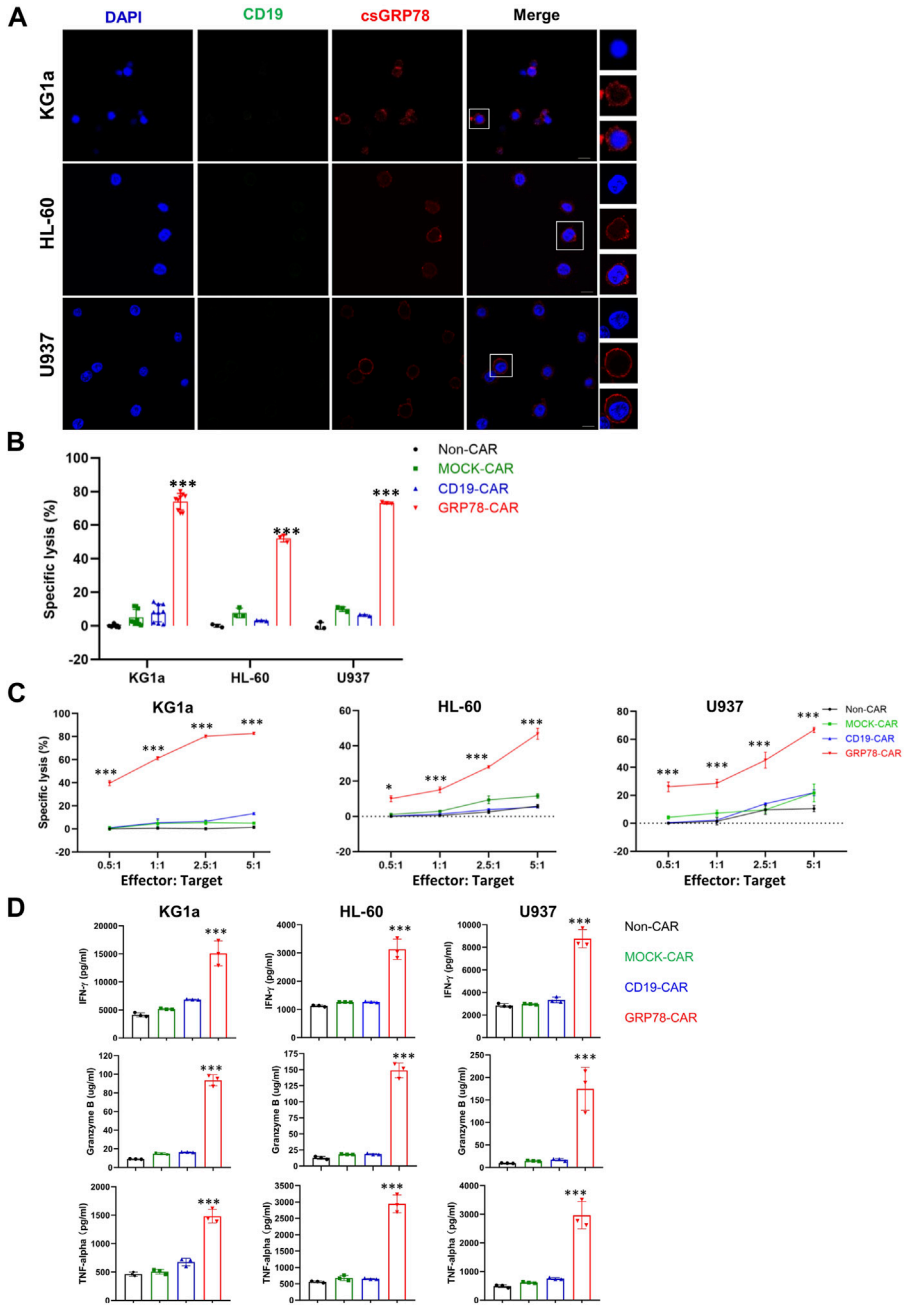

FIGURE 1 | Glucose-regulated protein 78 (GRP78)–chimeric antigen receptor (CAR) T cells demonstrate anti-leukemic effect against acute myeloid leukemia cells. **(A)** Confocal image of representative immunofluorescence staining of cell surface GRP78 (red) and CD19 (green) on indicated leukemia cell lines. The denoted cells are zoomed on the right. The bars indicated 10 μm length. **(B)** cytotoxic assay measuring the specific lysis of target cells. CFSE-labeled AML cell lines KG1a, HL-60, and U937 cells were co-incubated with Non-CAR T, MOCK-CAR T, CD19-CAR T, and GRP78-CAR T cells at an effector/target (E/T) ratio of 5:1 for 24 h. Experiments were repeated with at least triplicate samples. Data represent means ± SDs. Two-sided Student's t-test was performed between GRP78-CAR and Non-CAR, MOCK-CAR, and CD19-CAR, $^{***}p < 0.01$. **(C)** leukemia cells were incubated with Non-CAR T, MOCK-CAR T, CD19-CAR T, and GRP78-CAR T cells at various (E/T) ratios as indicated. Percentages of lysis cells are shown as means ± SDs. All experiments were repeated with at least triplicate samples. GRP78-CAR vs. Non-CAR, MOCK-CAR, and CD19-CAR T cells with the two-sided Student's t-test, $^{***}p < 0.01$. **(D)** ELISA measurements of cytokines interferon gamma (IFN-γ), tumor necrosis factor–α (TNF-α), and granzyme B in the cell supernatant were determined following 24-h incubation of target cells with specified CAR-T cells. Two-side Student's t-test was performed between GRP78-CAR T cell treatment and Non-CAR, MOCK-CAR, and CD19-CAR T cells. All experiments were repeated with at least triplicate samples. Data represent means ± SDs. $^{***}p < 0.01$.

quantified and surrogated as bioluminescence, and the group of mice with GRP78-CAR T-cell injection exhibited strikingly lower bioluminescence signal than mice treated with Non-CAR T cells and MOCK-CAR T cells, indicating that the vast majority of KG1a leukemia cells were eradicated by GRP78-CAR T cells *in vivo* (**Supplementary Figures S3B,C**). After analysis of lymphoid organs of sacrificed mice, the residual leukemia cells in lymphoid organs are rarely detectable in mice injected with GRP78-CAR T cells (**Supplementary Figures S3D,E**).

Altogether, the *in vivo* AML xenograft mouse experiment is consistent with the *in vitro* cytotoxicity assay that GRP78-CAR T cells can eliminate leukemia cells both *in vitro* and *in vivo*.

Glucose-Regulated Protein 78–Chimeric Antigen Receptor T Cells Eradicated Primary Acute Myeloid Leukemia Blast

Next, we determined whether GRP78-CAR T cells could elicit cytotoxicity against primary AML blasts. A panel of normal and primary AML blood samples obtained from healthy donors and AML patients were co-cultured with CAR T cells at an E/T ratio of 10:1 for 48 h. Cytotoxicity induced by CAR T cells against normal or patients' samples *in vitro* was determined by cytotoxic T lymphocyte assay. In total, 24 patients' blood samples were collected, and the fraction of blasts in peripheral blood cells varied from 18% to 99% including variable FAB subtypes (**Supplementary Table S1**). GRP78-CAR T cells induced significantly higher cytotoxicity against AML blast samples (6 out of 24, 25%), but no cytotoxicity to normal samples (**Figure 3A**). In contrast to control CAR T cells, GRP78-CAR T-cell treatments released significantly higher levels of cytokine IFN-γ to the supernatant, which indicates that GRP78-CAR T cells specifically elicit immune cytotoxicity to patients' AML blasts (**Figure 3B**). Furthermore, immunofluorescence staining was performed on GRP78-CAR T-cell response samples and confirmed that GRP78 is enriched on the plasma membrane of patients' tumor samples (**Supplementary Figure S4**).

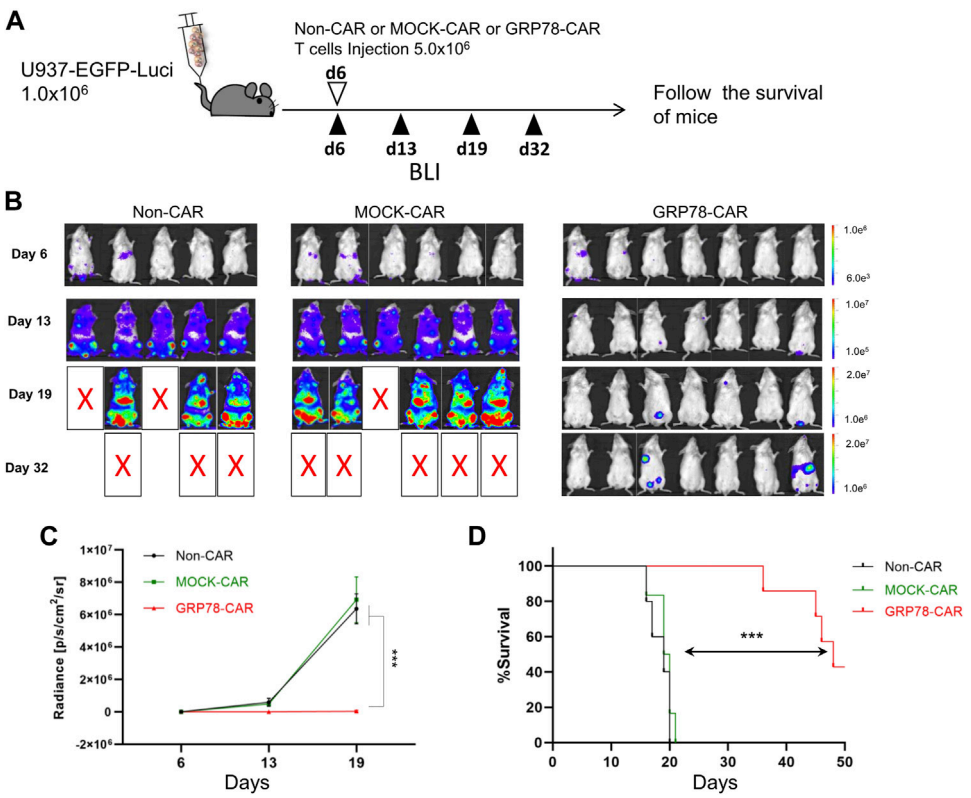

FIGURE 2 | Glucose-regulated protein 78 (GRP78)–chimeric antigen receptor (CAR) T cells eliminate human acute myeloid leukemia (AML) *in vivo* in xenograft mouse models. **(A)** Schematic of the U937 leukemia cell xenograft model. NSG mice were injected *via* the tail vein with 1 × 10^6 U937-EGFP luciferase (U937-EGFP-Luc) on day 0. Bioluminescent imaging (BLI) was performed on day 6 to quantify engraftment and for randomization of treatment groups. GRP78-CAR T cells (5 × 10^6), MOCK-CAR T cells (5 × 10^6), or Non-CAR T cells (5 × 10^6) were injected via the tail vein on day 6, followed with serial BLI. BLI radiance was measured as a surrogate quantification of tumor burden. **(B)** tumor burden was visualized by BLI on days 6, 13, and 19 following U937-EGFP-Luc cell transplantation. **(C)** bioluminescent signal for each treatment group over time. Data represent mean values of each group ± SEMs. The number of mice in each group is listed in **(B)**. Two-way ANOVA test was performed between GRP78-CAR T-cell treatment and Non-CAR and MOCK-CAR T cells. ***$p < 0.01$. **(D)** Kaplan–Meier analysis of survival. Log-rank (Mantel–Cox) tests were used to perform statistical analyses of survival between groups.

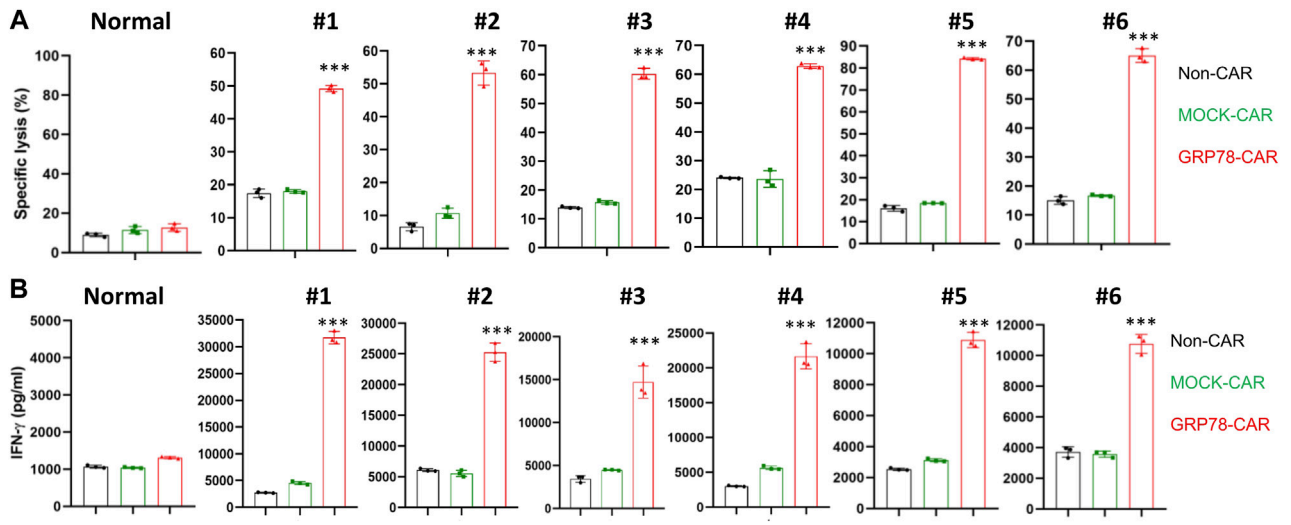

FIGURE 3 | Glucose-regulated protein 78 (GRP78)–chimeric antigen receptor (CAR) T cells eradicate primary acute myeloid leukemia (AML) blasts. **(A)** cytotoxic assay measuring the specific lysis of target cells. CFSE-labeled primary AML patient PBMC were co-incubated with Non-CAR T, MOCK-CAR T, and GRP78-CAR T cells at an effector/target ratio of 5:1 for 48 h. The normal PBMC sample was collected from the blood of the healthy donors. Experiments were repeated with at least triplicate samples. Data represent means ± SDs. GRP78-CAR vs. Non-CAR, MOCK-CAR, and CD19-CAR. ***$p < 0.01$ (two-sided Student's t-test). **(B)** ELISA measurement of cytokine interferon (IFN) gamma in the cell supernatant was determined following 48-h incubation of primary PBMC cells with specified CAR T cells. Two side Student's t-test was performed between GRP78-CAR T cell treatments with Non-CAR and MOCK-CAR. Data represent means ± SDs. GRP78-CAR vs. Non-CAR, MOCK-CAR, CD19-CAR. ***$p < 0.01$ (two-sided Student's t-test).

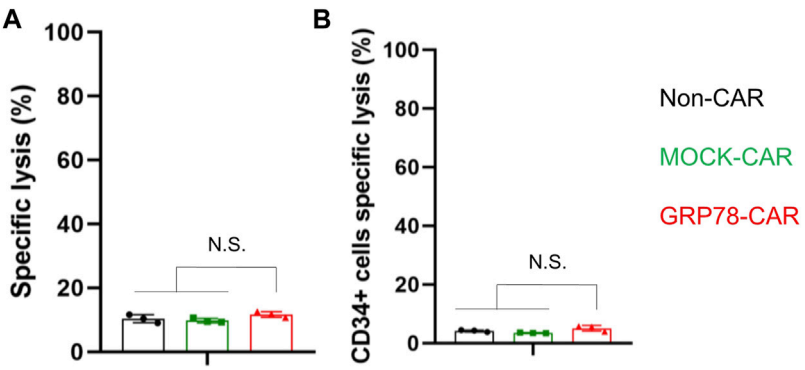

FIGURE 4 | Hematopoietic stem cells are spared by glucose-regulated protein 78 (GRP78)–chimeric antigen receptor (CAR) T cells. **(A)** cytotoxic assay measuring the specific lysis of bone marrow cells from healthy donors. CFSE-labeled primary normal bone marrow cells were co-incubated with Non-CAR T, MOCK-CAR T, and GRP78-CAR T cells at an effector/target ratio of 5:1 for 24 h. Experiments were repeated with at least triplicate wells. Data represent means ± SDs. ***$p < 0.01$ (two-sided Student's t-test). **(B)** cytotoxicity of Non-CAR, MOCK-CAR, and GRP78-CAR T cells against hematopoietic stem cells ($CD34^+$) derived from the bone marrow of healthy donors. The cells were co-cultured at an effector/target ratio of 5:1 for 24 h. Data represent mean values of triplicate wells ± SDs. ***$p < 0.01$ (two-sided Student's t-test).

Hematopoietic Stem Cells Are Spared by Glucose-Regulated Protein 78–Chimeric Antigen Receptor T Cells

The limitation of CAR T-cell treatment for AML is because of the lack of a tumor-specific antigen enriched in leukemia cells but not in normal cells and HSCs. Therefore, we isolated cells from the bone marrow of healthy donors after lysis of red blood cells to co-incubate with CAR T cells. GRP78-CAR T cells exhibit a comparable level of cytotoxicity with Non-CAR and MOCK-CAR T cells (**Figure 4A**). Next, the cytotoxicity of engineered CAR T was tested on the HSCs ($CD34^+$) derived from the bone marrow of healthy donors. After 24 h of co-culture of CAR-T cells with the cell mixture, no increment of cell lysis was observed on the $CD34^+$ cell subpopulation (**Figure 4B**). Another sample from healthy donors showed a consistent result (**Supplementary Figure**

S5). Overall, stem cells derived from the hematopoietic system are not targeted by GRP78-CAR T cells, suggesting that the engineered GRP78-CAR T cell is a safe therapy for AML without the potential toxicity.

Regarding CAR lentivirus transfection that may lead to CAR T cells fratricide via self-targeting, we tested the GRP78-CAR T cells' induced cytotoxicity to primary T cells. It was confirmed that no significant cytotoxicity was induced by GRP78-CAR T cells in comparison to control CAR T cells and accompanied by a similar level of cytokine production (**Supplementary Figures S6A,B**). GRP78-CAR lentivirus transfection also exhibits no impairment to the T-cell proliferation (**Supplementary Figure S6C**), which facilitates the expansion and preparation of GRP78-CAR T cells *in vitro*. Furthermore, GRP78 was barely detected on the cell surface of normal T cells by immunostaining, which is consistent with the above observation (**Supplementary Figure S6D**).

DISCUSSION

AML is the most common acute leukemia in adults. Although for AML patients, CR is achieved in 60–80% of younger adults and 40–60% of older adults after standard chemotherapy, in the majority of cases, AML will relapse within 3 years (Döhner et al., 2010; Döhner et al., 2015; Döhner et al., 2017). CAR T-cell therapy is an emerged technology that is developed and proven to be an effective treatment for hematological malignancies, such as CAR T cells targeting CD19 for B-cell malignancies. However, the lack of effective CAR-T for the therapy of AML is because of the short of specificity. Previous studies reported that CAR-T cells targeted CD33/CD123, which antigens targeted both leukemia cells and hematological stem cells, indicating CD33/CD123 CAR-T treatment might result in the bone marrow failure (Pizzitola et al., 2014).

Current CAR T cells targeting CLL-1 and CD70 demonstrated cytotoxicity to tumor cells and spared $CD34^+$ stem cells, indicating the potency of clinical implications for these two CAR T-cell treatments. However, it is well known that AML is featured with genetic heterogeneity and variable fusion genes as tumorigenesis drivers. Only a fraction of AML cells expresses CLL-1 or CD70 on the plasma membrane as the target for CAR T cells. Furthermore, relapse after CAR T-cell therapy remains a challenge owing to the mutation or down-regulation of the antigen on the tumor cell surface, which leads to the tumor escaping from CAR targeting and consequently results in resistance to therapy (Ruella and Maus, 2016; Shah and Fry, 2019). To overcome tumor antigen escape, the CAR is designed to simultaneously target multiple cell surface antigens, such as CD22 and CD20 (Zah et al., 2016; Fry et al., 2018). Therefore, it underscores the necessity of developing novel CAR T cells that target diverse antigens to combine or complement the current CAR T-cell therapy for AML and provide more options for patients.

GRP78, a chaperone protein that belongs to the heat-shock protein family, plays a crucial role in maintaining cellular homeostasis. It is an evolutionarily conserved protein that presents in multiple subcellular positions and exerts distinct functions: retains in the ER to produce unfolded protein response, binds to mitochondria to interact with apoptotic executors, and resides in the plasma membrane to transduce proliferative signal as a receptor (Zhang and Zhang, 2010). The plasma membrane GRP78 has been uncovered in various human cancers, including leukemia (Staquicini et al., 2018), melanoma (de Ridder et al., 2011), prostate cancer (Arap et al., 2004), and colorectal cancer (Shin et al., 2003; Li et al., 2013). In the tumor cells, the cell surface GRP78 has a crucial role in protection from apoptosis, promotion of proliferation, evasion from immune surveillance, and resistance to various therapies (Gonzalez–Gronow et al., 2009; Zhang and Zhang, 2010). However, the mechanisms by which GRP78 is translocated to the cell surface remain elusive. Noting that the tumor cells exhibit a high level of GRP78 on the plasma membrane, targeting GRP78 to induce cytotoxicity of tumor cells becomes an attractive method. Several studies designed GRP78-binding peptides conjugated with cytotoxic drugs to precisely target cancer cells, which demonstrated promising results *in vitro* and *in vivo* (Arap et al., 2004; Kim et al., 2006; Yoneda et al., 2008). Jiang et al. (2019) constructed GRP78-targeted nanocage to specifically target and kill hepatocellular carcinoma and suppress lung metastasis. Here, we cloned Pep42, a cyclic oligopeptide that specifically targets cell surface GRP78, into a second-generation CAR construct to generate GRP78-CAR and demonstrated that GRP78-CAR T cells can effectively induce cytotoxicity of AML cells *in vitro* and eradicate explanted leukemia cells *in vivo*. Furthermore, in a cohort of 24 AML patients, we found that GRP78-CAR T cells effectively eradicated AML blasts in a fraction of AML specimens (25%) with different FAB subtypes, supporting its promising application for AML treatment.

In normal cells, previous studies reported that the functions of GRP78 are binding to polypeptides in the ER and activating unfolded protein response when polypeptides are overproduced. Thus, GRP78 resides in the lumen of the ER to maintain intracellular homeostasis. Besides, several studies have uncovered that GRP78 can also express on the plasma membrane of various cancer cells and endothelial cells (Davidson et al., 2005; Lee, 2007; Gonzalez–Gronow et al., 2009). Therefore, GRP78 serves as a suitable target for CAR T-cell therapy. In principle, CAR T cells targeting the cell surface of GRP78 have the advantage of sparing normal cells and specifically inducing cytotoxicity of tumor cells. In particular, for the therapy of AML, which requires greater cancer cell specificity, in pioneer studies, CAR T cells targeting antigens CD33, CD44, and CD123 have been developed (Jin et al., 2006; Jin et al., 2009; Walter et al., 2012). However, these treatments exhibit side effects when normal HSCs are targeted accompanied by bone marrow toxicity, which leads to myeloablation. Our result supports that GRP78-CAR T cells are not targeting the normal cells and avoid cytotoxicity to HSCs, which ensures the safety of GRP78-CAR T-cell therapy.

Aligned with our study, a recent study by Hebbar et al. (2022) also reported that GRP78 is a promising target for CAR

T cells in the therapy of AML. Both studies highlighted that GRP78-CAR T cells are able to eradicate the malignant cells *in vitro* and *in vivo* and the same peptide (Pep42) was chosen to construct CAR. Both studies confirmed that GRP78-CAR T cells are safe and would not impair the HSCs. In our study, we confirmed that 6 out of 24 fresh AML primary blasts can be eradicated by GRP78-CAR T cells, while they measured the expression of GRP78 in 14 primary AML samples without testing the cytotoxicity of GRP78-CAR T cells. Thus, we might provide more direct evidence of GRP78-CAR T cells' induced cytotoxicity to primary AML samples. In their study, flow cytometry was performed and demonstrated that GRP78 is expressed on the cell surface of more than half of primary AML samples using an antibody for ER retention sequence (KDEL) and a biotin-conjugated peptide, but the antibody used in the study is no longer supplied by the company. In our study, we performed immunostaining to assess the expression and visualize the localization of GRP78 for living AML cells and primary samples. We observed that GRP78 is expressed on the plasma membrane of three AML cell lines and 25% of primary AML samples, which is consistent with the cytotoxicity induced by GRP78-CAR T cells. The lower percentage of GRP78 we detected might be because of the difference in antibodies, more stringent criteria used in immunofluorescence staining, and the sizes of samples. Furthermore, the GRP78-CAR T cells generated in our study might be more effective than those generated by Hebbar et al. (2022). Their study proved the anti-AML activity of GRP78-CAR T cells *in vivo* by injection of 5×10^3 MOLM13 AML cells following 3×10^6 CAR T cells on day 7, whereas our study demonstrated that 5×10^6 GRP78-CAR T cells can eradicate explanted 1×10^6 of U937 or KG1a AML cells in the xenograft mouse models. Together, both studies uncover the anti-AML activity of GRP78-CAR, thus highlighting GRP78-CAR T-cell therapy as a promising approach in the clinic.

In this study, we constructed the GRP78-CAR and demonstrated that GRP78-CAR T cells manifested high cytolytic effects against AML cells *in vitro* and significantly eradicated the tumor in xenograft mouse models. Moreover, GRP78-CAR T cells could notably eliminate primary AML blasts, which indicates a substantial fraction of AML patients could benefit from GRP78-CAR T-cell therapy in the future. GRP78-CAR T cells showed no cytotoxicity on normal bone marrow cells and HSCs. Together, GRP78-CAR T cells can effectively eradicate AML and have the potency to be applied in clinical therapy.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, and further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committees of West China Hospital of Sichuan University (#2022151). The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by the Animal Ethics Committee of West China Hospital of Sichuan University.

AUTHOR CONTRIBUTIONS

WY, TL, and XZ conceived and designed the experiments. WY, HZ, and JT performed the experiments. YC provided the CAR plasmids. HZ collected the normal and patients' samples. XC provided the clinical data. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcell.2022.928140/full#supplementary-material

REFERENCES

Arap, M. A., Lahdenranta, J., Mintz, P. J., Hajitou, A., Sarkis, Á. S., Arap, W., et al. (2004). Cell Surface Expression of the Stress Response Chaperone GRP78 Enables Tumor Targeting by Circulating Ligands. *Cancer Cell* 6, 275–284. doi:10.1016/j.ccr.2004.08.018

Casas, C. (2017). GRP78 at the Centre of the Stage in Cancer and Neuroprotection. *Front. Neurosci.* 11, 177. doi:10.3389/fnins.2017.00177

Cui, Q., Qian, C., Xu, N., Kang, L., Dai, H., Cui, W., et al. (2021). CD38-directed CAR-T Cell Therapy: a Novel Immunotherapy Strategy for Relapsed Acute Myeloid Leukemia after Allogeneic Hematopoietic Stem Cell Transplantation. *J. Hematol. Oncol.* 14, 82. doi:10.1186/s13045-021-01092-4

Davidson, D. J., Haskell, C., Majest, S., Kherzai, A., Egan, D. A., Walter, K. A., et al. (2005). Kringle 5 of Human Plasminogen Induces Apoptosis of Endothelial and Tumor Cells through Surface-Expressed Glucose-Regulated Protein 78. *Cancer Res.* 65, 4663–4672. doi:10.1158/0008-5472.CAN-04-3426

de Ridder, G. G., Gonzalez-Gronow, M., Ray, R., and Pizzo, S. V. (2011). Autoantibodies against Cell Surface GRP78 Promote Tumor Growth in a Murine Model of Melanoma. *Melanoma Res.* 21, 35–43. doi:10.1097/CMR.0b013e3283426805

Di Stasi, A., Tey, S.-K., Dotti, G., Fujita, Y., Kennedy-Nasser, A., Martinez, C., et al. (2011). Inducible Apoptosis as a Safety Switch for Adoptive Cell Therapy. *N. Engl. J. Med.* 365, 1673–1683. doi:10.1056/NEJMoa1106152

Döhner, H., Estey, E., Grimwade, D., Amadori, S., Appelbaum, F. R., Büchner, T., et al. (2017). Diagnosis and Management of AML in Adults: 2017 ELN Recommendations from an International Expert Panel. *Blood* 129, 424–447. doi:10.1182/blood-2016-08-733196

Döhner, H., Estey, E. H., Amadori, S., Appelbaum, F. R., Büchner, T., Burnett, A. K., et al. (2010). Diagnosis and Management of Acute Myeloid Leukemia in Adults: Recommendations from an International Expert Panel, on Behalf of the European LeukemiaNet. *Blood* 115, 453–474. doi:10.1182/blood-2009-07-235358

Döhner, H., Weisdorf, D. J., and Bloomfield, C. D. (2015). Acute Myeloid Leukemia. *N. Engl. J. Med.* 373, 1136–1152. doi:10.1056/NEJMra1406184

Ehninger, A., Kramer, M., Röllig, C., Thiede, C., Bornhäuser, M., von Bonin, M., et al. (2014). Distribution and Levels of Cell Surface Expression of CD33 and CD123 in Acute Myeloid Leukemia. *Blood Cancer J.* 4, e218. doi:10.1038/bcj.2014.39

Fry, T. J., Shah, N. N., Orentas, R. J., Stetler-Stevenson, M., Yuan, C. M., Ramakrishna, S., et al. (2018). CD22-targeted CAR T Cells Induce Remission in B-ALL that Is Naive or Resistant to CD19-Targeted CAR Immunotherapy. *Nat. Med.* 24, 20–28. doi:10.1038/nm.4441

Fu, Y., Li, J., and Lee, A. S. (2007). GRP78/BiP Inhibits Endoplasmic Reticulum BIK and Protects Human Breast Cancer Cells against Estrogen Starvation-Induced Apoptosis. *Cancer Res.* 67, 3734–3740. doi:10.1158/0008-5472.CAN-06-4594

Gonzalez–Gronow, M., Selim, M. A., Papalas, J., and Pizzo, S. V. (2009). GRP78: a Multifunctional Receptor on the Cell Surface. *Antioxidants Redox Signal.* 11, 2299–2306. doi:10.1089/ARS.2009.2568

Hebbar, N., Epperly, R., Vaidya, A., Thanekar, U., Moore, S. E., Umeda, M., et al. (2022). CAR T Cells Redirected to Cell Surface GRP78 Display Robust Anti-acute Myeloid Leukemia Activity and Do Not Target Hematopoietic Progenitor Cells. *Nat. Commun.* 13, 587. doi:10.1038/s41467-022-28243-6

Huergo-Zapico, L., Gonzalez-Rodriguez, A. P., Contesti, J., Gonzalez, E., López-Soto, A., Fernandez-Guizan, A., et al. (2012). Expression of ERp5 and GRP78 on the Membrane of Chronic Lymphocytic Leukemia Cells: Association with Soluble MICA Shedding. *Cancer Immunol. Immunother.* 61, 1201–1210. doi:10.1007/s00262-011-1195-z

Jiang, B., Zhang, R., Zhang, J., Hou, Y., Chen, X., Zhou, M., et al. (2019). GRP78-targeted Ferritin Nanocaged Ultra-high Dose of Doxorubicin for Hepatocellular Carcinoma Therapy. *Theranostics* 9, 2167–2182. doi:10.7150/thno.30867

Jin, L., Hope, K. J., Zhai, Q., Smadja-Joffe, F., and Dick, J. E. (2006). Targeting of CD44 Eradicates Human Acute Myeloid Leukemic Stem Cells. *Nat. Med.* 12, 1167–1174. doi:10.1038/nm1483

Jin, L., Lee, E. M., Ramshaw, H. S., Busfield, S. J., Peoppl, A. G., Wilkinson, L., et al. (2009). Monoclonal Antibody-Mediated Targeting of CD123, IL-3 Receptor α Chain, Eliminates Human Acute Myeloid Leukemic Stem Cells. *Cell Stem Cell* 5, 31–42. doi:10.1016/j.stem.2009.04.018

Kang, B. R., Yang, S.-H., Chung, B.-R., Kim, W., and Kim, Y. (2016). Cell Surface GRP78 as a Biomarker and Target for Suppressing Glioma Cells. *Sci. Rep.* 6, 34922. doi:10.1038/srep34922

Kim, Y., Lillo, A. M., Steiniger, S. C. J., Liu, Y., Ballatore, C., Anichini, A., et al. (2006). Targeting Heat Shock Proteins on Cancer Cells: Selection, Characterization, and Cell-Penetrating Properties of a Peptidic GRP78 Ligand. *Biochemistry* 45, 9434–9444. doi:10.1021/bi060264j

Lee, A. S. (2007). GRP78 Induction in Cancer: Therapeutic and Prognostic Implications: Figure 1. *Cancer Res.* 67, 3496–3499. doi:10.1158/0008-5472.CAN-07-0325

Li, Z., and Li, Z. (2012). Glucose Regulated Protein 78: a Critical Link between Tumor Microenvironment and Cancer Hallmarks. *Biochimica Biophysica Acta (BBA) - Rev. Cancer* 1826, 13–22. doi:10.1016/j.bbcan.2012.02.001

Li, Z., Zhang, L., Zhao, Y., Li, H., Xiao, H., Fu, R., et al. (2013). Cell-surface GRP78 Facilitates Colorectal Cancer Cell Migration and Invasion. *Int. J. Biochem. Cell Biol.* 45, 987–994. doi:10.1016/j.biocel.2013.02.002

Liu, R., Li, X., Gao, W., Zhou, Y., Wey, S., Mitra, S. K., et al. (2013). Monoclonal Antibody against Cell Surface GRP78 as a Novel Agent in Suppressing PI3K/AKT Signaling, Tumor Growth, and Metastasis. *Clin. Cancer Res.* 19, 6802–6811. doi:10.1158/1078-0432.CCR-13-1106

Mardiana, S., and Gill, S. T. (2020). CAR T Cells for Acute Myeloid Leukemia: State of the Art and Future Directions. *Front. Oncol.* 10, 697. doi:10.3389/fonc.2020.00697

Maude, S. L., Frey, N., Shaw, P. A., Aplenc, R., Barrett, D. M., Bunin, N. J., et al. (2014). Chimeric Antigen Receptor T Cells for Sustained Remissions in Leukemia. *N. Engl. J. Med.* 371, 1507–1517. doi:10.1056/NEJMoa1407222

Park, J. H., Rivière, I., Gonen, M., Wang, X., Sénéchal, B., Curran, K. J., et al. (2018). Long-Term Follow-Up of CD19 CAR Therapy in Acute Lymphoblastic Leukemia. *N. Engl. J. Med.* 378, 449–459. doi:10.1056/NEJMoa1709919

Pizzitola, I., Anjos-Afonso, F., Rouault-Pierre, K., Lassailly, F., Tettamanti, S., Spinelli, O., et al. (2014). Chimeric Antigen Receptors against CD33/CD123 Antigens Efficiently Target Primary Acute Myeloid Leukemia Cells *In Vivo*. *Leukemia* 28, 1596–1605. doi:10.1038/leu.2014.62

Porter, D. L., Levine, B. L., Kalos, M., Bagg, A., and June, C. H. (2011). Chimeric Antigen Receptor-Modified T Cells in Chronic Lymphoid Leukemia. *N. Engl. J. Med.* 365, 725–733. doi:10.1056/NEJMoa1103849

Quinones, Q. J., de Ridder, G. G., and Pizzo, S. V. (2008). GRP78: a Chaperone with Diverse Roles beyond the Endoplasmic Reticulum. *Histol. Histopathol.* 23, 1409–1416. doi:10.14670/HH-23.1409

Ruella, M., Barrett, D. M., Kenderian, S. S., Shestova, O., Hofmann, T. J., Perazzelli, J., et al. (2016). Dual CD19 and CD123 Targeting Prevents Antigen-Loss Relapses after CD19-Directed Immunotherapies. *J. Clin. Invest.* 126, 3814–3826. doi:10.1172/JCI87366

Ruella, M., and Maus, M. V. (2016). Catch Me if You Can: Leukemia Escape after CD19-Directed T Cell Immunotherapies. *Comput. Struct. Biotechnol. J.* 14, 357–362. doi:10.1016/j.csbj.2016.09.003

Sauer, T., Parikh, K., Sharma, S., Omer, B., Sedloev, D., Chen, Q., et al. (2021). CD70-specific CAR T Cells Have Potent Activity against Acute Myeloid Leukemia without HSC Toxicity. *Blood* 138, 318–330. doi:10.1182/blood.2020008221

Scheuermann, R. H., and Racila, E. (1995). CD19 Antigen in Leukemia and Lymphoma Diagnosis and Immunotherapy. *Leukemia Lymphoma* 18, 385–397. doi:10.3109/10428199509059636

Shah, N. N., and Fry, T. J. (2019). Mechanisms of Resistance to CAR T Cell Therapy. *Nat. Rev. Clin. Oncol.* 16, 372–385. doi:10.1038/s41571-019-0184-6

Shin, B. K., Wang, H., Yim, A. M., Le Naour, F., Brichory, F., Jang, J. H., et al. (2003). Global Profiling of the Cell Surface Proteome of Cancer Cells Uncovers an Abundance of Proteins with Chaperone Function. *J. Biol. Chem.* 278, 7607–7616. doi:10.1074/jbc.M210455200

Sommermeyer, D., Hill, T., Shamah, S. M., Salter, A. I., Chen, Y., Mohler, K. M., et al. (2017). Fully Human CD19-specific Chimeric Antigen Receptors for T-Cell Therapy. *Leukemia* 31, 2191–2199. doi:10.1038/leu.2017.57

Staquicini, D. I., D'Angelo, S., Ferrara, F., Karjalainen, K., Sharma, G., Smith, T. L., et al. (2018). Therapeutic Targeting of Membrane-Associated GRP78 in Leukemia and Lymphoma: Preclinical Efficacy *In Vitro* and Formal Toxicity Study of BMTP-78 in Rodents and Primates. *Pharmacogenomics J.* 18, 436–443. doi:10.1038/tpj.2017.46

Tashiro, H., Sauer, T., Shum, T., Parikh, K., Mamonkin, M., Omer, B., et al. (2017). Treatment of Acute Myeloid Leukemia with T Cells Expressing Chimeric Antigen Receptors Directed to C-type Lectin-like Molecule 1. *Mol. Ther.* 25, 2202–2213. doi:10.1016/j.ymthe.2017.05.024

Walter, R. B., Appelbaum, F. R., Estey, E. H., and Bernstein, I. D. (2012). Acute Myeloid Leukemia Stem Cells and CD33-Targeted Immunotherapy. *Blood* 119, 6198–6208. doi:10.1182/blood-2011-11-325050

Wang, J., Chen, S., Xiao, W., Li, W., Wang, L., Yang, S., et al. (2018). CAR-T Cells Targeting CLL-1 as an Approach to Treat Acute Myeloid Leukemia. *J. Hematol. Oncol.* 11, 7. doi:10.1186/s13045-017-0553-5

Wang, Q.-s., Wang, Y., Lv, H.-y., Han, Q.-w., Fan, H., Guo, B., et al. (2015). Treatment of CD33-Directed Chimeric Antigen Receptor-Modified T Cells in One Patient with Relapsed and Refractory Acute Myeloid Leukemia. *Mol. Ther.* 23, 184–191. doi:10.1038/mt.2014.164

Yoneda, Y., Steiniger, S. C. J., Čapková, K., Mee, J. M., Liu, Y., Kaufmann, G. F., et al. (2008). A Cell-Penetrating Peptidic GRP78 Ligand for Tumor Cell-

specific Prodrug Therapy. *Bioorg. Med. Chem. Lett.* 18, 1632–1636. doi:10.1016/j.bmcl.2008.01.060

Zah, E., Lin, M.-Y., Silva-Benedict, A., Jensen, M. C., and Chen, Y. Y. (2016). T Cells Expressing CD19/CD20 Bispecific Chimeric Antigen Receptors Prevent Antigen Escape by Malignant B Cells. *Cancer Immunol. Res.* 4, 498–508. doi:10.1158/2326-6066.CIR-15-0231

Zhang, H., Wang, P., Li, Z., He, Y., Gan, W., and Jiang, H. (2021). Anti-CLL1 Chimeric Antigen Receptor T-Cell Therapy in Children with Relapsed/Refractory Acute Myeloid Leukemia. *Clin. Cancer Res.* 27, 3549–3555. doi:10.1158/1078-0432.CCR-20-4543

Zhang, L.-H., and Zhang, X. (2010). Roles of GRP78 in Physiology and Cancer. *J. Cell. Biochem.* 110, 1299–1305. doi:10.1002/jcb.22679

Zhang, Y., Liu, R., Ni, M., Gill, P., and Lee, A. S. (2010). Cell Surface Relocalization of the Endoplasmic Reticulum Chaperone and Unfolded Protein Response Regulator GRP78/BiP. *J. Biol. Chem.* 285, 15065–15075. doi:10.1074/jbc.M109.087445

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Peritoneal resident macrophages in tumor metastasis and immunotherapy

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Macrophages residing in various tissues play crucial roles in innate immunity, tissue repair, and immune homeostasis. The development and differentiation of macrophages in non-lymphoid tissues are highly regulated by the tissue microenvironment. Peritoneum provides a unique metastatic niche for certain types of tumor cells. As the dominant immune cell type in peritoneal cavity, macrophages control the immune response to tumor and influence the efficacy of anti-tumor therapy. Considering the heterogeneity of macrophages in origin, metabolism, and function, it is always challenging to define the precise roles of macrophages in tumor microenvironment. We review here recent progresses in peritoneal resident macrophage research in the context of physiological and metastatic tumor conditions, which may benefit the development of new anti-tumor therapies through targeting macrophages.

KEYWORDS

resident macrophage, peritoneal metastasis, tumor immunity, immunotherapy, ontogeny, immune evasion, tumor microenvironment, tumor associated macrophage

1 Introduction

Macrophages are critical for immunity, tissue repair, and organ regeneration. Tissue resident macrophages are observed in multiple tissues and defined as a population genetically and developmentally distinct from inflammatory macrophages (Cox et al., 2021). Unlike blood monocytes or differentiated inflammatory macrophages, tissue resident macrophages maintain their population in-site *via* self-renewal without the contribution of blood monocytes and hardly move to other tissues (Yona et al., 2013; Dick et al., 2019). Genetic fate-mapping strategies in mouse developmental model indicates most of the tissue resident macrophages originate from colony-stimulating factor 1 receptor (*Csf1r*)-expressing erythromyeloid progenitors (EMPs) which arise at approximately embryonic day 8 (E8) from the yolk sac hemogenic endothelium (Gomez Perdiguero et al., 2015). At about E10, hematopoietic stem cells (HSCs) migrate into the fetal liver, which serves as the major organ of HSC differentiation until birth (Gekas et al., 2005). Meanwhile, HSCs progenitors replenish part of tissue resident macrophages under physiological and pathological contexts (Hashimoto et al., 2013; Bain et al., 2014). Although tissue resident macrophages in different tissues share

certain core activities, they display functional diversity to support the homeostasis of each tissue. Dysfunction of tissue resident macrophages causes severe and often fatal developmental disorders as reported in recent experimental works in mice (De Schepper et al., 2019; Oosterhof et al., 2019). Understanding their diversity and contribution to pathophysiological processes may provide new therapeutic targets for human diseases, especially tumor.

Tumorigenesis is mediated by mutated genes which not only support uncontrolled tumor cell growth but also protect them from immune surveillance. Tumor associated macrophages (TAMs) represent the dominant myeloid cell population in most types of solid tumors of both humans and mice. TAMs contribute to the immunosuppressive tumor microenvironment (TME) which protects tumor cells from anti-tumor immune response (Pittet et al., 2022). However, the connection between tissue resident macrophages and TAMs in different solid tumors is still not well understood. Monocytes have been considered as the precursors of TAMs for many years since CCL2/CCR2 signaling which recruits monocytes from bone marrow to tumor facilitates breast cancer metastasis (Qian et al., 2011). Furthermore, detailed research points out monocyte-derived macrophages, but not mammary tissue macrophages, promote the breast cancer development (Franklin et al., 2014). Recently, new research revealed the pro-tumoral role of tissue resident macrophages, similar to monocytic macrophages, in tumorigenesis and tumor growth among certain types of tumors. For example, the pancreatic tissue resident macrophages originate from embryonic hematopoiesis and promote pancreatic ductal adenocarcinoma (PDAC) progression (Zhu et al., 2017). Consistently, the lung resident interstitial macrophages contribute to the pool of TAMs together with CCR2-dependent recruited macrophages (Loyher et al., 2018). Moreover, we and other groups demonstrate that the peritoneal resident macrophages promote ovarian cancer development and metastasis into peritoneal cavity (Casanova-Acebes et al., 2020; Etzerodt et al., 2020; Xia et al., 2020). Therefore, the involvement of tissue resident macrophages may decide the fate of tumor progression.

Peritoneal cavity is a fluid-filled space located between the wall of the abdomen and the organs found in the abdomen. Peritoneal resident macrophages are well-studied as mouse primary macrophages in peritoneal cavity and share very similar characters with macrophages obtained from both pleural cavity and pericardial cavity. Peritoneal resident macrophages maintain serosal homeostasis and provide immune surveillance in the etiology of pathologies including peritonitis, endometriosis, and metastatic cancers. Here, we summarize the new concepts related to the development and differentiation of cavity-resident macrophages, especially peritoneal resident macrophages, and their roles in tumor metastasis and immunotherapy.

2 The basic biology of peritoneal resident macrophages in steady-state mouse

2.1 The phenotypes of peritoneal resident macrophages

Peritoneal macrophages have been used as the source of primary mouse resting macrophages for almost 60 years since Cohn and collaborators started to collect and analyze them in the 1960s (Steinman and Moberg, 1994). At that time, these cells were considered as an individual macrophage population since they are characterized by the classical mouse macrophage markers CD11b and F4/80. Further, peritoneal resident macrophages have been divided into large peritoneal macrophages (LPMs) and small peritoneal macrophages (SPMs) based on their size (Ghosn et al., 2010). In the meantime, the two subsets display phenotypic differences that LPMs have been found to be F4/80^{High}MHC-II^{Low} while SPMs to be F4/80^{Low}MHC-II^{High}. The LPMs are the long-lived macrophages since they express more mature markers as CD40, CD80, and CD86 (Ghosn et al., 2010). Some unique markers are specifically expressed on each subsets, for example T-cell immunoglobulin and mucin domain containing 4 (TIM4) and Intercellular adhesion molecule 2 (ICAM2) expressed on LPMs while CCR2 and CD226 expressed on SPMs (Kim et al., 2016) (Table 1). In steady-state mouse, LPMs are the dominant population (more than 90%) with very identical profiles while SPMs are a very small (less than 10%) and heterogeneous population consisted with several monocytic subsets (Bain et al., 2016; Kim et al., 2016).

2.2 The determinants of peritoneal resident macrophage differentiation

Several critical factors determine the formation of macrophage niches which control the size of the macrophage population and imprint their tissue-specific identity in peritoneal cavity, including the ontogeny, intrinsic factors, and local environment (Figure 1).

2.2.1 Ontogeny

It is important to understand how the tissue macrophages establish their compartment during mammalian development and aging. The population of tissue resident macrophages in different location and at different time points can arise from three distinct waves of precursors: early yolk sac macrophages, fetal liver monocytes, or bone marrow derived monocytes (Bleriot et al., 2020). In the steady state condition, most tissue resident macrophages in mice and humans originate from the embryonic stage and are maintained by self-renew but not differentiated from adult hematopoiesis (Ginhoux and

TABLE 1 The phenotypic difference between LPMs and SPMs in steady-state mice.

Surface markers	Peritoneal resident macrophages (LPMs)	Monocyte derive macrophages (SPMs)	References
CCR2	–	+	(Kim et al., 2016; Xia et al., 2020)
CD9	High	Low	Rosas et al. (2014)
CD11b	High	Low	(Ghosn et al., 2010; Gautier et al., 2012)
CD11c	+	+/–	(Ghosn et al., 2010; Bain et al., 2016)
CD24	+	–	Rosas et al. (2014)
CD40	High	Low	Ghosn et al. (2010)
CD49f	+	–	Okabe and Medzhitov, (2014)
CD64	+	–	Gautier et al. (2012)
CD73	+	–	(Okabe and Medzhitov, 2014; Rosas et al., 2014)
CD80	High	Low	Ghosn et al. (2010)
CD86	High	Low	Ghosn et al. (2010)
CD93	+	–	(Okabe and Medzhitov, 2014; Rosas et al., 2014)
CD102 (ICAM2)	+	–	(Okabe and Medzhitov, 2014; Kim et al., 2016; Bain et al., 2020)
CD206	–	+	Accarias et al. (2016)
CD209b	High	Low	Rosas et al. (2014)
CD226	–	+	Kim et al. (2016)
CRIg	+	–	(Rosas et al., 2014; Xia et al., 2020)
F4/80	High	Low	(Ghosn et al., 2010; Gautier et al., 2012; Okabe and Medzhitov, 2014; Bain et al., 2016)
MerTK	+	–	Gautier et al. (2012)
MHC-II	Low	High	(Ghosn et al., 2010; Okabe and Medzhitov, 2014; Bain et al., 2016; Kim et al., 2016)
TIM4	+	–	(Rosas et al., 2014; Bain et al., 2016; Xia et al., 2020)
TLR4	High	Low	Ghosn et al. (2010)

Level of expression: + positive; – negative.

Guilliams, 2016). Indeed, the fate-mapping studies demonstrate the precursors of LPMs migrate and reside in the peritoneal cavity during embryonic stage and are shaped by the tissue microenvironment (Yona et al., 2013; Bain et al., 2016). In the young mice, embryonic derived LPMs depend on self-proliferation to sustain their numbers. However, bone marrow derived macrophages can differentiate and acquire key characteristics of the embryonic population in peritoneal cavity and finally replenish the embryonic derived LPMs with aging (Bain et al., 2016).

2.2.2 Intrinsic factors

In addition to ontogeny, intrinsic factors also have an important effect on the peritoneal macrophage differentiation, such as genetic background, sex difference, and phagocytosis. Systematic genetic and epigenetic analyses of tissue resident macrophages from five diverse strains of mice discovered the macrophage identity was linked to more than 100 transcription factors that in turn bind to hundreds of connected cis-regulatory domains (Link et al., 2018). Therefore, mutation of these regulatory elements might disturb the specific macrophage identity. For example, deletion of a specific enhancer of *Csf1r* gene, the fms-intronic regulatory element (FIRE), selectively affects peritoneal resident macrophage populations (Rojo et al., 2019). In addition, CCAAT/enhancer binding protein (C/EBP)-β, functioning as macrophage restricted lineage determining transcription factor, plays an intrinsic role in the generation of LPMs (Cain et al., 2013). Sex differences modulate the immune response (Wilkinson et al., 2022). Sex has been proposed to affect brain microglia differentiation (Thion et al., 2018). Similarly, peritoneal resident macrophages exhibit sexually dimorphic traits that their replenishment from the bone marrow is higher in males compared to that in females, which is driven by changes in the local microenvironment that arise upon sexual maturation (Bain et al., 2020). Phagocytosis, the phagocytes restricted activity to uptake the particles discovered by Ilya Metchnikoff in starfish larvae in 1880s (Epelman et al., 2014), transiently decides the resident macrophage identity by polarizing the cell toward an anti-inflammatory phenotype (A-Gonzalez et al., 2017). But the abilities of phagocytosis between LPMs and SPMs are not the same. At the early stage of infection, LPMs are the major bacterial

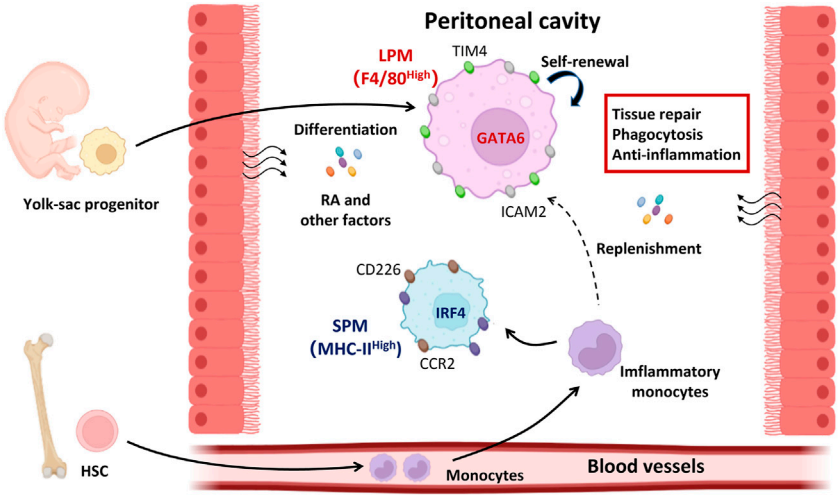

FIGURE 1
The determinants of peritoneal resident macrophage differentiation. Under steady state, both LPMs and SPMs represent the peritoneal resident macrophages. LPMs are dominant in the peritoneal cavity and function in tissue repair, phagocytosis, and anti-inflammation. LPMs are differentiated from the yolk-sac progenitors and maintain their numbers by self-renewal. Local environment supports the LPMs differentiation by secreting the retinoic acid and other factors. Different from LPMs, SPMs are differentiated from HSC derived monocytes and expand themselves in peritoneal cavity once inflammation occurs. The differentiation of LPMs is mediated by transcriptional factor GATA6 while that of SPMs is IRF4. GATA6, GATA binding protein six; HSC, hematopoietic stem cells; IRF4, interferon regulatory factor 4; LPMs, large peritoneal macrophages; RA, retinoic acid; SPMs, small peritoneal macrophages.

phagocytic cells beyond SPMs (Ghosn et al., 2010). Consistently, it is convinced in the context of apoptotic cells clearance by peritoneal and pleural resident macrophages which is shown the transcription factors KLF2 and KLF4 initiate the apoptotic cell clearance program in tissue residential macrophages (Roberts et al., 2017). This type of program in peritoneal resident macrophages is context dependent and reversible.

2.2.3 Local microenvironment

The local microenvironment, so-called "niche of residence" (Guilliams and Scott, 2017), provides signals necessary for the maturation of functional resident macrophages from any precursor in a time-dependent manner. The transcriptional factor GATA binding protein 6 (GATA6) is responsible for the development and identity of peritoneal resident macrophage, specifically expressed in LPMs but not SPMs (Gautier et al., 2012; Gautier et al., 2014; Okabe and Medzhitov, 2014; Rosas et al., 2014). Retinoic acid, a metabolite of vitamin A, induces tissue-specific localization and functional polarization of LPMs through the induction of GATA6 expression (Gautier et al., 2014; Okabe and Medzhitov, 2014). The omentum which is formed by a double layer of mesothelial cells in peritoneal cavity provides continuous retinoic acid and other factors to support the LPMs profiles (Okabe and Medzhitov, 2014). Furthermore, transcription factor Wilms' tumor 1 (WT1) positive mesothelial and fibroblastic stromal cells induce retinoic acid-dependent and -independent hallmark genes of GATA6$^+$ macrophages by expressing two rate-limiting enzymes, aldehyde dehydrogenases (ALDH)-1 and -2, in retinol metabolism (Buechler et al., 2019). Hence, serous cavity restricts the peritoneal resident macrophage identity.

2.3 The homoeostasis of peritoneal resident macrophages

In newborn mouse, LPMs expand *via* local proliferation which is considerably reduced and most likely provides homeostatic control of cell numbers in the adult (Davies et al., 2011). The proliferative capacity of LPMs is determined by signals from the local microenvironment rather than their genetic heterogeneity and origin (Bain et al., 2016; Bain et al., 2020). CSF1-Fc or IL-4c is known to drive proliferation of peritoneal macrophages in steady state or TH-2 inflammation (Jenkins et al., 2011; Jenkins et al., 2013). During the resolution of inflammation, LPMs survive and then undergo a transient and intense proliferative burst *in situ* to repopulate the tissue which is M-CSF dependent and highly decided by the expression of GATA6. Selective GATA6 deficiency resulted in dysregulated peritoneal macrophage proliferative renewal during homeostasis and in response to inflammation, which was associated with delays in the resolution of inflammation (Rosas et al., 2014). In addition, GATA6-deficient macrophages are vulnerable to death and lead to a reduction of peritoneal resident macrophages by

lacking functional aspartoacylase (Gautier et al., 2014). Accordingly, mammalian target of rapamycin complex 2 (mTORC2) has been found negatively regulate the GATA6 expression by controlling forkhead box O1 (FOXO1) activation. Hence, mTORC2 deficiency enhances the generation of tissue-resident peritoneal cells through increased proliferation and cell survival (Oh et al., 2017).

Notably, the proliferation activity of peritoneal macrophage displays a sexual dimorphism that LPMs proliferate faster at the resolution stage of inflammation in male mice than that in female mice. Consistently, the signature of male peritoneal macrophages was dominated by cell cycle-associated genes compared to genes associated with lipid uptake and transport as well as immune response in female (Bain et al., 2020). Interestingly, peritoneal macrophages express estrogen receptors which mediated enhanced proliferation in response to exogenous estrogen. Furthermore, ovariectomy leads to a reduction in the number of macrophages which imply the sex maturation influences the biology of the peritoneal resident macrophages (Pepe et al., 2017).

2.4 The metabolic profiles of peritoneal resident macrophages

The metabolism of macrophage is highly linked with their activation, polarization, and function (Leone and Powell, 2020). Macrophages in tissue establish metabolic adaptation to support homeostatic tissue function and facilitate wound healing. The peritoneal resident macrophages express the GATA6 associated gene *Aspa*, which encodes the hydrolase enzyme aspartoacylase to catalyze the deacylation of N-acetylaspartate into aspartate and acetate (Gautier et al., 2014). The peritoneal cavity is enriched for N-acetylaspartate relative to its abundance in serum. Considering the acetate is the precursor of acetyl-CoA which has been used as fuel for the tricarboxylic acid (TCA) cycle, peritoneal resident macrophages displays higher level of mitochondrial oxygen-consumption rates compared with that in bone marrow derived macrophages *in vitro* (Davies et al., 2017). In addition, the concentration of glutamate is higher in the peritoneal cavity than in serum which is able to supplement glutamine to maintain respiratory burst during phagocytosis *via* enhancing mitochondrial complex-II metabolism in peritoneal resident macrophages (Davies et al., 2017). Consistently, peritoneal resident macrophages increase TCA cycle-associated genes and mitochondrial mass compared with monocyte derive macrophages. Inhibition of mTORC2 increases TCA cycle-associated genes expression and mitochondrial mass which may be due to the elevated GATA6 expression (Oh et al., 2017). Hence, the diversity of metabolites in peritoneal cavity may determine the metabolic profiles through modulating GATA6 expression in peritoneal resident macrophage.

3 The role of peritoneal resident macrophages in tumor metastasis into the cavity

TAMs are a key component of the TME which promote angiogenesis, tumor metastasis, and immune evasion (Mantovani et al., 2017). The serous cavity is a common metastatic site for a variety of malignant cancers, including colorectal cancer (Ceelen et al., 2020), gastric cancer (Song et al., 2019), ovarian cancer (Etzerodt et al., 2020; Xia et al., 2020), and lung cancer (Chow et al., 2021). When tumors metastasize into the peritoneal cavity, resident macrophages not only support tumor cell colonization and proliferation but also suppress the anti-tumor immune response (Figure 2).

3.1 The metastasis of tumors into peritoneal cavity relies on macrophages in omentum

The omentum is an adipose tissue layer containing certain milky spots (clusters of leukocytes) which are mainly composed of macrophages and B1 cells, resembling the cellular composition found in the peritoneal cavity. However, the phenotype of omental macrophages is different from peritoneal residential macrophages (Louwe et al., 2022). CD169 and LYVE1 are used to identify three omental macrophage subsets, in which the $CD169^{High}LYVE1^{+}$ subset can be further divided into four subpopulations based on the expression of CD163 and TIM4. Interestingly, many of the $CD163^{+}TIM4^{+}$ cells are embryonic origin and support the growth of metastatic ovarian cancer (Etzerodt et al., 2020). In peritoneal metastasis, the colonization of omentum is always associated with poor prognosis which provides a critical basement for tumor metastasis into the peritoneal cavity (Coccolini et al., 2013). Consistent with this observation, omentectomy inhibits tumor growth in the peritoneal cavity through a surgical procedure to remove the omentum (Etzerodt et al., 2020). Mechanically, ovarian cancer frequently colonizes the omentum which is observed passively through the milky spots (Rangel-Moreno et al., 2009), or fascinated by neutrophil extracellular traps (Lee et al., 2019), or recruited by the chemokines like IL-8 and CXCL12 (Nieman et al., 2011; Kasagi et al., 2016). Macrophages play an important role to assist the tumor cell colonization into omentum. It is reported that omental macrophages promote the migration and colonization of ovarian cancer cells to the omentum through the secretion of chemokine ligands that interact with chemokine receptor 1 (CCR1), and inhibition of CCR1 reduces ovarian cancer colonization (Krishnan et al., 2020). In addition, macrophages are supposed to form the immune suppressive microenvironment in the milky spots which are selectively invaded by tumor cells (Oosterling et al., 2006; Clark et al., 2013).

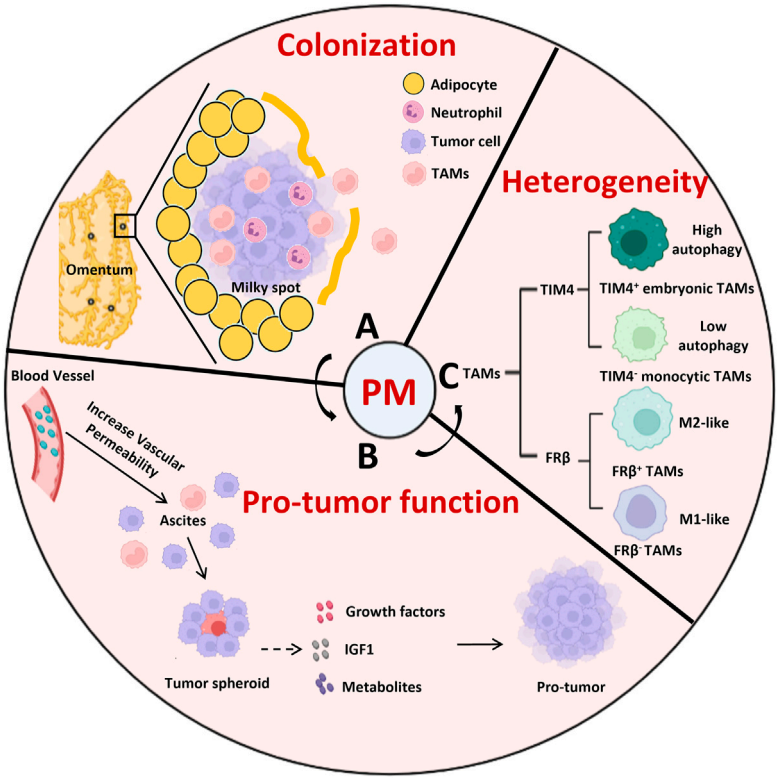

FIGURE 2
The role of peritoneal resident macrophages in the tumor metastasis into the cavity. **(A)** The colonization of omentum is a critical step for tumor metastasis, which is occurred passively through the milky spots. **(B)** TAMs promote the tumor peritoneal metastasis through gathering tumor cells to form the spheroid and secreting growth factors, IGF1, metabolites to support the tumor growth. **(C)** The heterogenicity of TAMs distinguished by TIM4 or FRβ in peritoneal metastasis. Compared to TIM4$^-$ monocytic TAMs, TIM4$^+$ embryonic TAMs express more OXPHOS genes and adapt to autophagy to support their survival. FRβ$^-$ TAMs display a round shape that was more monocytic in appearance and consistent with M1 phenotype while FRβ$^+$ TAMs exhibited an elongated cell shape with a M2-polarized pro-tumor profiles. IGF1, insulin-like growth factor 1; PM, peritoneal metastasis; TAMs, tumor associated macrophages.

3.2 The promotion of tumor metastasis by peritoneal resident macrophages

3.2.1 Support tumor growth and ascites formation

TAMs directly promote the development of metastases in ovarian cancer through the production of cytokines IL-6 (Isobe et al., 2015), vascular endothelial growth factor (VEGF) (Song et al., 2019), and transforming growth factor β (TGFβ) (Rodriguez et al., 2001), among others. In addition, insulin-like growth factor 1 (IGF1) expressed by TAMs increased the proliferation and migration of ID8 mouse ovarian cancer cells; while blockade of the IGF1 pathway in ID8 cells with an IGF1 neutralizing antibody effectively inhibited the ID8 caused tumor growth (Liu et al., 2018). Moreover, TAMs derived metabolites also contribute to the tumor growth. In peritoneal metastasis, GATA6$^+$ residential macrophages elicit a fatty acid oxidation mediated an increase in oxidative phosphorylation (OXPHOS). Itaconic acid, a peritoneal resident macrophage specific metabolite which is produced by immune responsive gene 1 (IRG1) mediated catabolism of mitochondrial cis-aconitate, can promote tumor progression into the peritoneum. Knockdown of IRG1 significantly reduced peritoneal tumors with reductions in OXPHOS and ROS in TAMs and ROS-mediated MAPK activation in tumor cells (Weiss et al., 2018).

Patients with advanced peritoneal tumors often develop malignant ascites with fluid accumulation in the peritoneal cavity. The formation of peritoneal ascites is associated with increased vascular permeability and obstructed lymphatic drainage. The overexpression of VEGF causes the increased vascular permeability (Herr et al., 2012) accompanied with IL-6 and IL-10, as well as CXCL12 in ascites (Zeng et al., 2019). As the major source of VEGF, macrophages resided in peritoneal cavity promote the ascites formation in ovarian cancer and gastric cancer peritoneal metastasis (Song et al., 2019). The CSF1 antibody blockade and clodronate liposomes depletion inhibit peritoneal resident macrophage accumulation and

prevent ascites formation in ovarian cancer (Robinson-Smith et al., 2007; Moughon et al., 2015). Ascites-derived spheroids in ovarian cancer facilitate tumor growth and progression. At early stages of transcoelomic metastasis of mouse epithelial ovarian cancer, M2 macrophage-like TAMs formed spheroids and secreted EGF, which upregulated αMβ2 integrin on TAMs and ICAM-1 on tumor cells to enhance association between tumor cells and TAMs, then to support tumor cell proliferation and migration (Yin et al., 2016).

3.2.2 Facilitate immune evasion

In TME, TAMs have been summarized to be the dominant suppressive cells associated with immune evasion (Mantovani et al., 2017; Pathria et al., 2019). It is known that macrophages can directly target and eliminate the tumor cells. However, tumor expressed CD24 in ovarian cancer prevents macrophage-mediated phagocytic tumor clearance and promotes immune evasion by interacting with the inhibitory receptor sialic-acid-binding Ig-like lectin 10 (Siglec-10) expressed on TAMs (Barkal et al., 2019). Meanwhile, the presence of tumor infiltrating $CD8^+$ T lymphocytes is highly associated with longer survival in ovarian cancer (Zhang et al., 2003; Peng et al., 2015). However, it is observed that the level of cavity resident macrophages is associated with reduced numbers of $CD8^+$ T cells in pleural effusions and peritoneal ascites from patients with cancers. Mechanistically, TIM4 on the surface of cavity-resident macrophages interacts with its receptor phosphatidylserine (PS) upregulated on the surface of activated cytotoxic $CD8^+$ T cells, which leads to $CD8^+$ T cells sequestrated away from tumor targets and proliferation suppression by $TIM4^+$ macrophages (Chow et al., 2021). In addition, canonical autophagy in peritoneal resident macrophages shows a suppressive effect on their IFNγ pathway activation (Wang et al., 2020). In mouse ID8 ovarian cancer metastasis, the depletion of autophagy related gene FIP200 in peritoneal TAMs induced T cell mediated anti-tumor response which may be due to the spontaneous IFNγ mediated immune activation in autophagy deficient TAMs (Xia et al., 2020). Hence, increased autophagy in peritoneal TAMs promotes ovarian cancer immune evasion.

3.3 The polarization of peritoneal tumor-associated macrophages remodeled by tumor cells

The plasticity of TAMs makes them polarized into two functional types, M1 (pro-inflammatory with anti-tumor activity) and M2 (anti-inflammatory with pro-tumor activity) macrophages, determined by their local TME (Murray et al., 2014; Muller et al., 2017). It is reported ovarian cancer cells polarize macrophages toward an M2 phenotype *in vivo* which gradually gain expression of M2-like marker genes, such as CD206, Arg1, and CD163, at 4–8 weeks after tumor injection (Yin et al., 2016). The homeobox A9 (HOXA9) expression in ovarian cancer cells stimulated chemotaxis of peritoneal macrophages and induced macrophages to acquire M2-like features (Ko et al., 2014). In a mouse ovarian metastatic model, tumor cells promote membrane-cholesterol efflux and depletion of lipid rafts from macrophages which promotes IL-4-mediated reprogramming but inhibits IFNγ-induced gene expression to accelerate tumor progression (Goossens et al., 2019). In addition, tumor-derived ubiquitin protein ligase E3 component N-recognin 5 (UBR5), an E3 ligase overexpressed in human ovarian cancer associated with poor prognosis, promotes TAMs recruitment and polarization *via* key chemokines and cytokines (Song et al., 2020). Meanwhile, M2-like TAM polarization could be repressed by sorbin and SH3 domain containing 2 (SORBS2) through stabilizing WAP four-disulfide core domain 1 (WFDC1) and IL-17D in ovarian cancer (Zhao et al., 2018). Furthermore, IFNγ secreted by tumor-infiltrating lymphocyte (TIL) is critical for M1 TAMs polarization. However, epigenetic silencing of CCL5 expression through DNA methylation in ovarian cancer cells leads to the TIL desertification and reduced IFNγ polarized M1 TAMs (Dangaj et al., 2019). Hence, the conversion of M2 TAMs to M1 TAMs provides an approach to improve the anti-tumor response by targeting TAMs.

3.4 The accumulation and discrepancy of peritoneal resident macrophage subsets in peritoneal metastasis

The accumulation of TAMs in peritoneal cavity reflects the development of peritoneal metastasis. Tumor derived factors, such as M-CSF and IL-4, promote the peritoneal residential macrophages self-proliferation or differentiation from monocytes. Due to the different ontogeny of peritoneal resident macrophages, the accumulation of peritoneal TAMs may originate from different precursors. In our work, the expression of TIM4 can be used to distinguish $TIM4^+$ embryonic TAMs from $TIM4^-$ monocytic TAMs which share very similar gene signatures with LPMs and SPMs in tumor free mice (Xia et al., 2020). Both TAM subsets expand in ID8 metastatic models. Further, we observe $TIM4^+$ embryonic TAMs only depend on the self-proliferation while most of $TIM4^-$ monocytic TAMs differentiate from infiltrated monocytes which were recruited by the tumor caused inflammation in peritoneal cavity. Compared to $TIM4^-$ monocytic TAMs, $TIM4^+$ embryonic TAMs express more OXPHOS genes and adapt to autophagy to support their survival in ovarian cancer metastasis. As the dominant TAMs in early tumor stage, $TIM4^+$ embryonic macrophages promote the tumor metastasis (Xia et al., 2020). Similarly, surface expression of folate receptor (FR) β also can be used to identify two different peritoneal TAM subsets. $FR\beta^-$ TAMs display a round shape that is more monocytic in appearance and consistent with

M1 phenotype. However, FRβ^{+} TAMs exhibits an elongated cell shape with a M2-polarized protumor profiles (Rodriguez-Garcia et al., 2021). In addition, the monocytic TAMs increase their percentage in total TAMs following the tumor growth and show some effects on tumor metastasis. At the advanced tumor stage, monocytic TAMs accumulate a lot from blood and elicit pro-tumor effect. For example, F4/80^{Low} SPMs can directly enhance ovarian cancer cell growth with expression of protumor and proangiogenic molecular mediators upregulated by IL-17 (Rei et al., 2014). Transcription factor ZEB1 expressed in F4/80^{Low} TAMs enhances tumor progression with the induction of a CCR2-MMP9-CCL2 positive loop between TAMs and cancer cells (Cortes et al., 2017).

Noticeably, the macrophages also reside within mesothelial membranes lining the peritoneal cavity which can be divided into LYVE1^{High}MHC-II^{Low} and LYVE1$^{Low/-}$MHC-II^{High} subsets. LYVE1^{High} macrophages predominantly originate from embryonic-derived progenitors and promote epithelial ovarian tumor growth. The LYVE1^{High} mesothelial macrophages express similar profiles with ovarian tumor-associated macrophages previously described in the omentum. These data reveal that the peritoneal compartment contains other two resident macrophage populations and that LYVE1^{High} mesothelial macrophages drive tumor growth (Zhang et al., 2021).

4 Targeting peritoneal resident macrophages to enhance anti-tumor immunotherapy

Harnessing the immune system provides an important approach to combating cancer. Several strategies have been developed to manipulate the immune response, including immune checkpoint blockade (ICB), chimeric antigen receptor T cells (CAR T cells), dendritic cell vaccines, and cytokine therapies. Peritoneal resident macrophages assist tumors to suppress the immune response which should be a good target to improve the effect of immunotherapy (Duan and Luo, 2021). Several strategies have been developed to target the TAMs in peritoneal cavity:

4.1 Myeloid checkpoint blockade

Blocking the PD-1/PD-L1 or CTLA-4 pathways with antibodies relieves the cytotoxic function of T cells to control the tumor growth (Zou et al., 2016). However, patients with peritoneal metastasis are hardly responsive to the PD-1/PD-L1 blockade indicating that there are other checkpoints involved to hinder the effect of ICB in peritoneal metastasis. Along with the immune checkpoints on T cells, several checkpoints that are mainly associated with macrophages have been discovered. TIM4^{+} cavity TAMs sequester and impair proliferation of CD8^{+} T cells under the ICB treatment through the interaction between TIM4 and PS. Hence, TIM4 blockade abrogates this sequestration and proliferation suppression and enhances anti-tumor efficacy in models of anti-PD-1 therapy and adoptive T cell therapy in mice (Chow et al., 2021). CD47 on the tumor surface acts as a "don't eat me" signal and prevents macrophage-mediated phagocytosis through its direct interaction with signal regulatory protein-α (SIRPα). Blockade of CD47 signaling by using targeted monoclonal antibodies restores the phagocytic capacity of TAMs which enables macrophage phagocytosis of ovarian cancer cells (Willingham et al., 2012; Liu R. et al., 2017). Furthermore, a phase I trial of an anti-CD47 antibody Hu5F9-G4 demonstrated two patients with ovarian/fallopian tube cancers had partial remissions for 5.2 and 9.2 months (Sikic et al., 2019). In addition, the CD24/Siglec-10 signal also provide a target for restoring the phagocytosis of peritoneal resident TAMs in ovarian cancer (Barkal et al., 2019).

4.2 Tumor-associated macrophages depletion

Emerging evidence show the loss of TAMs destroy the suppressive TME and promote anti-tumor immune response. Targeting the differentiation and proliferation of TAMs always influence their population. Administration of GW2580, an inhibitor of CSF1 receptor, reduced infiltration of M2-like macrophages and dramatically decreased ascites volume in the late stages of ovarian cancer metastasis (Moughon et al., 2015). Depletion of GATA6 induces peritoneal residential macrophage apoptosis which dramatically decreases their numbers (Gautier et al., 2014; Rosas et al., 2014). Blocking the induction of GATA6 or inhibiting its transcriptional activity should strongly impair the identity of peritoneal resident macrophages and reduce their numbers. For example, retinoid X receptors (RXRs) control mouse serous-macrophage identity by regulating chromatin accessibility and the transcriptional regulation of canonical macrophage genes, partially through GATA6. RXRs deficiency impairs neonatal expansion of the LPM pool and reduces the survival of adult LPMs through excess lipid accumulation. Depletion of RXR diminishes LPMs accumulation in ovarian cancer and strongly reduces tumor progression in mice which indicates targeting RXR signaling may improve ovarian cancer outcomes *via* interfering the maintenance of the serous macrophage pool (Casanova-Acebes et al., 2020). In addition, the peritoneal resident TAMs highly relied on the autophagy for survival compared to the migrated TAMs. Autophagy can be used as a target to selectively reduce the TIM4^{+} residential TAMs and tumor growth (Xia et al., 2020).

Some artificial materials are developed to destroy the peritoneal TAMs. Clodronate liposome elicits toxic effects on

macrophages *via* phagocytosis. The administration of clodronate liposome reduces the number of peritoneal TAMs and metastasis in ovarian cancer (Etzerodt et al., 2020; Xia et al., 2020). CAR T cells are a type of immunotherapy that involves T cells genetically modified to express receptors that recognize cancer-specific antigens (Kershaw et al., 2013). Interestingly, Rodriguez-Garcia et al. (2021) developed mouse and human FRβ-specific CAR T cells which recognize and deplete the FRβ$^+$ TAMs in ovarian cancer metastasis. Furthermore, pre-treatment of the TME with anti-TAM CAR T cells improves the efficacy of tumor-specific CAR T cells against ovarian cancer metastasis. Similarly, G5-methotrexate (G5-MTX) nanoparticles restrict tumor growth by targeting and depleting the FRβ$^+$ TAMs in ascites models of ovarian cancer (Penn et al., 2018).

4.3 Metabolic remodeling

It is well known that aggressively proliferated tumor cells consume amount of nutrients and fulfill the TME with their metabolic products which crosstalk with TAMs and form immune suppressive TME (Chang et al., 2015). This immunosuppressive remodeling seems not because of direct nutrient competition since cell-intrinsic programs drive the preferential acquisition of glucose and glutamine by immune and cancer cells (Reinfeld et al., 2021). Cancer cells show the highest uptake of glutamine which is not only their fuel but also drives M2-like macrophage polarization *via* epigenetic modifications (Liu P. S. et al., 2017). In addition, certain tumor derived metabolites function to promote the polarization of M2 TAMs phenotype, including lactic acid (Colegio et al., 2014), succinate (Wu et al., 2020), and so on. Hence, alteration of the tumor or macrophage metabolism provides an approach to convert the anti-inflammatory TAMs into pro-inflammatory TAMs. For example, genetic depletion of glutamine synthetase skews macrophages toward an M1-like phenotype and inhibits tumor metastasis (Palmieri et al., 2017).

In the context of peritoneal grafted cancer models, it is observed that the fatty acid oxidation (FAO), a way to break down a fatty acid into acetyl-CoA, increase in peritoneal resident macrophages (Weiss et al., 2018). Lipid metabolism is critical for the identity and homeostasis of LPMs and RXR-deficient peritoneal TIM4$^+$ LPMs leads to lipid accumulation and apoptosis (Casanova-Acebes et al., 2020). Considering the LPMs represent the dominant peritoneal TAMs, interrupting their lipid metabolism may have the effect to control the ovarian cancer metastasis by inducing their apoptosis. Moreover, hyaluronic acid secreted by ovarian cancer cells promotes plasma membrane cholesterol efflux in TAMs *via* binding to CD44, which enhances IL-4 receptor signaling *in vitro* and *in vivo* associated with reduced intracellular cholesterol, finally promoting the expression of the typical M2 marker Arg1 while inhibiting proinflammatory IL-12 expression (Goossens et al., 2019). Deletion of cholesterol efflux genes ATP-binding cassette transporter A1 (ABCA1) and ATP-binding cassette transporter G1 (ABCG1) in peritoneal TAMs has been found to significantly impair tumor progression (Goossens et al., 2019). In addition, mTORC2 is important for the metabolic reprogramming of tissue-resident macrophages (Oh et al., 2017) and TAMs (Huang et al., 2016), so mTORC2 can be a potential target to remodel TAMs from an anti-inflammatory to an anti-tumor phenotype. Hence, metabolic rewiring of peritoneal TAMs should be an alternative strategy to enhance the anti-tumor immunity.

5 The human counterpart of peritoneal resident macrophages in cavity metastasis

Human studies on peritoneal macrophage populations divide them into three distinct subsets based on the expression of CD14/CD16 (CD14^{++}CD16$^-$, CD14^{++}CD16$^+$, and CD14^{High}CD16^{High}). The CD14^{High}CD16^{High} subset represents the mature phenotype of steady-state human resident peritoneal macrophages based on the expression of GATA6, and other resident macrophage markers, such as CD206 and Slan (Ruiz-Alcaraz et al., 2016; Ruiz-Alcaraz et al., 2018). Consistent with mouse models, emerging evidence demonstrate that human peritoneal resident macrophages promote human cancer metastasis into cavity. For example, gastric cancer (GC) patients with peritoneal metastasis had increased levels of alternatively activated macrophages in the peritoneum compared to those without dissemination. Macrophages in the peritoneal cavity produce EGF and VEGF to enhance the angiogenesis in GC patients bearing peritoneal metastasis. Hence, patients bearing more macrophages in the peritoneum had a poorer prognosis (Song et al., 2019). The ascites provides a convenient way to isolate high amount of pure human macrophages which can be analyzed through their transcriptome and proteome. It is reported TAMs from human ovarian carcinoma ascites shared similar character with peritoneal resident macrophages, but not monocyte-derived macrophages. The elevated signature genes in human TAMs are highly related to extracellular matrix (ECM) remodeling which indicates the role for TAMs in cancer cell invasion and ovarian cancer progression (Finkernagel et al., 2016). Meanwhile, two subgroups of ascites macrophages have been identified in ovarian cancer patients. Subgroup A has a high expression of pro-tumor markers (CD163, PCOLCE2, IL-6) related to immune suppression and ECM remodeling while subgroup B has a low expression of pro-tumorigenic and immunosuppressive markers with an upregulation of genes linked to interferon signaling (Adhikary et al., 2017). Furthermore, the expression of complement receptor of the immunoglobulin superfamily (CRIg) and CCR2 have been used to define two phenotypically and functionally distinct human peritoneal

macrophage subpopulations (Irvine et al., 2016). CRIg^{High} cells are transcriptionally, metabolically, and functionally similar with the mouse F4/80^{High} resident peritoneal macrophages in cirrhosis (Irvine et al., 2016) and ovarian cancer patients (Xia et al., 2020).

6 Conclusion

As the dominant myeloid cells infiltrating TME, TAMs promote immune evasion through multiple routes, including triggering of inhibitory immune checkpoints in T cells. Understanding the ontogeny and modulation of those immunosuppressive cells is critical for overcoming their disadvantages. Different from monocytic macrophages, peritoneal resident macrophages originate from embryonic precursors which locate in peritoneal cavity during development and can self-maintain locally throughout life with tissue-specific levels of replacement by circulating precursors (Bain et al., 2016). After tumor cells infiltrate into the peritoneal cavity, peritoneal resident macrophages become the primary macrophages surrounding the tumor cells and promote the tumor growth at the early stage. Meanwhile, inflammatory macrophages differentiated from blood monocytes gradually increase in numbers as the tumor progresses, contributing to peritoneal metastasis at the late stage. Determining whether peritoneal resident TAMs are predictive biomarkers for early peritoneal metastasis is important for personalized patient care in clinical applications.

Targeting the peritoneal resident TAMs elicits anti-tumor immune response and controls tumor metastasis. The tumor spheroids, angiogenesis, and immune suppression microenvironments supported by peritoneal TAMs promote tumor survival and growth. Understanding the differentiation, polarization, and metabolism of peritoneal TAMs is beneficial for exploring different approaches to reduce or remodel TAMs. The development of new technologies, such as CAR-macrophage and CAR-T targeting to macrophages, extend the potential immunotherapy by modulating peritoneal macrophages. Given the peritoneal resident macrophages can be recruited to injured organs in peritoneal cavity, it is still unknown if the peritoneal resident macrophages have effects on the orthotopic tumor growth and initiate the tumor transformation and metastasis. In the future, more challenges need to be addressed to manipulate TAMs in peritoneal metastasis, including 1) to understand the metabolic consumption of peritoneal TAMs and the pro-tumor effects of TAMs related metabolites; 2) to discover peritoneal specific transcriptional factors and surface markers to distinguish pro- and anti-tumoral peritoneal TAM subsets; 3) to prevent the clearance of the tumor neo-antigens by peritoneal TAMs; 4) to reduce the interference of peritoneal TAMs in antigen presentation; 5) to explore peritoneal macrophage related epigenetic regulations of their profiles; 6) to avoid peritoneal TAMs mediated immunotherapy resistance. In summary, peritoneal resident macrophages promote tumor metastasis into peritoneal cavity and can be targeted to enhance T-cell immunity, to modify polarization of TAMs, and to enhance phagocytosis of cancer cells with or without other therapies.

Author contributions

YZ and HX wrote the text. YC and DO revised the content. All authors discussed on the contents of the manuscript.

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References

A-Gonzalez, N., Quintana, J. A., Garcia-Silva, S., Mazariegos, M., Gonzalez de la Aleja, A., Nicolas-Avila, J. A., et al. (2017). Phagocytosis imprints heterogeneity in tissue-resident macrophages. *J. Exp. Med.* 214 (5), 1281–1296. doi:10.1084/jem.20161375

Accarias, S., Genthon, C., Rengel, D., Boullier, S., Foucras, G., and Tabouret, G. (2016). Single-cell analysis reveals new subset markers of murine peritoneal macrophages and highlights macrophage dynamics upon *Staphylococcus aureus* peritonitis. *Innate Immun.* 22 (5), 382–392. doi:10.1177/1753425916651330

Adhikary, T., Wortmann, A., Finkernagel, F., Lieber, S., Nist, A., Stiewe, T., et al. (2017). Interferon signaling in ascites-associated macrophages is linked to a favorable clinical outcome in a subgroup of ovarian carcinoma patients. *Bmc Genomics* 18, 243. doi:10.1186/s12864-017-3630-9

Bain, C. C., Bravo-Blas, A., Scott, C. L., Perdiguero, E. G., Geissmann, F., Henri, S., et al. (2014). Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice. *Nat. Immunol.* 15 (10), 929–937. doi:10.1038/ni.2967

Bain, C. C., Gibson, D. A., Steers, N. J., Boufea, K., Louwe, P. A., Doherty, C., et al. (2020). Rate of replenishment and microenvironment contribute to the sexually dimorphic phenotype and function of peritoneal macrophages. *Sci. Immunol.* 5 (48), eabc4466. doi:10.1126/sciimmunol.abc4466

Bain, C. C., Hawley, C. A., Garner, H., Scott, C. L., Schridde, A., Steers, N. J., et al. (2016). Long-lived self-renewing bone marrow-derived macrophages displace embryo-derived cells to inhabit adult serous cavities. *Nat. Commun.* 7, ncomms11852. doi:10.1038/ncomms11852

Barkal, A. A., Brewer, R. E., Markovic, M., Kowarsky, M., Barkal, S. A., Zaro, B. W., et al. (2019). CD24 signalling through macrophage Siglec-10 is a target for cancer immunotherapy. *Nature* 572(7769), 392–396. doi:10.1038/s41586-019-1456-0

Bleriot, C., Chakarov, S., and Ginhoux, F. (2020). Determinants of resident tissue macrophage identity and function. *Immunity* 52 (6), 957–970. doi:10.1016/j.immuni.2020.05.014

Buechler, M. B., Kim, K. W., Onufer, E. J., Williams, J. W., Little, C. C., Dominguez, C. X., et al. (2019). A stromal niche defined by expression of the transcription factor WT1 mediates programming and homeostasis of cavity-resident macrophages. *Immunity* 51 (1), 119–130. e115. doi:10.1016/j.immuni.2019.05.010

Cain, D. W., O'Koren, E. G., Kan, M. J., Womble, M., Sempowski, G. D., Hopper, K., et al. (2013). Identification of a tissue-specific, C/EBPβ-dependent pathway of differentiation for murine peritoneal macrophages.. *J. Immunol.* 191 (9), 4665–4675. doi:10.4049/jimmunol.1300581

Casanova-Acebes, M., Menendez-Gutierrez, M. P., Porcuna, J., Alvarez-Errico, D., Lavin, Y., Garcia, A., et al. (2020). RXRs control serous macrophage neonatal expansion and identity and contribute to ovarian cancer progression. *Nat. Commun.* 11 (1), 1655. doi:10.1038/s41467-020-15371-0

Ceelen, W., Ramsay, R. G., Narasimhan, V., Heriot, A. G., and De Wever, O. (2020). Targeting the tumor microenvironment in colorectal peritoneal metastases. *Trends Cancer* 6 (3), 236–246. doi:10.1016/j.trecan.2019.12.008

Chang, C. H., Qiu, J., O'Sullivan, D., Buck, M. D., Noguchi, T., Curtis, J. D., et al. (2015). Metabolic competition in the tumor microenvironment is a driver of cancer progression. *Cell* 162 (6), 1229–1241. doi:10.1016/j.cell.2015.08.016

Chow, A., Schad, S., Green, M. D., Hellmann, M. D., Allaj, V., Ceglia, N., et al. (2021). Tim-4(+) cavity-resident macrophages impair anti-tumor CD8(+) T cell immunity. *Cancer Cell* 39 (7), 973–988.e9. doi:10.1016/j.ccell.2021.05.006

Clark, R., Krishnan, V., Schoof, M., Rodriguez, I., Theriault, B., Chekmareva, M., et al. (2013). Milky spots promote ovarian cancer metastatic colonization of peritoneal adipose in experimental models. *Am. J. Pathol.* 183 (2), 576–591. doi:10.1016/j.ajpath.2013.04.023

Coccolini, F., Gheza, F., Lotti, M., Virzi, S., Iusco, D., Ghermandi, C., et al. (2013). Peritoneal carcinomatosis. *World J. Gastroenterol.* 19 (41), 6979–6994. doi:10.3748/wjg.v19.i41.6979

Colegio, O. R., Chu, N. Q., Szabo, A. L., Chu, T., Rhebergen, A. M., Jairam, V., et al. (2014). Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. *Nature* 513 (7519), 559–563. doi:10.1038/nature13490

Cortes, M., Sanchez-Moral, L., de Barrios, O., Fernandez-Acenero, M. J., Martinez-Campanario, M. C., Esteve-Codina, A., et al. (2017). Tumor-associated macrophages (TAMs) depend on ZEB1 for their cancer-promoting roles. *EMBO J.* 36 (22), 3336–3355. doi:10.15252/embj.201797345

Cox, N., Pokrovskii, M., Vicario, R., and Geissmann, F. (2021). Origins, biology, and diseases of tissue macrophages. *Annu. Rev. Immunol.* 39, 313–344. doi:10.1146/annurev-immunol-093019-111748

Dangaj, D., Bruand, M., Grimm, A. J., Ronet, C., Barras, D., Duttagupta, P. A., et al. (2019). Cooperation between constitutive and inducible chemokines enables T cell engraftment and immune attack in solid tumors. *Cancer Cell* 35 (6), 885–900. doi:10.1016/j.ccell.2019.05.004

Davies, L. C., Rice, C. M., Palmieri, E. M., Taylor, P. R., Kuhns, D. B., and McVicar, D. W. (2017). Peritoneal tissue-resident macrophages are metabolically poised to engage microbes using tissue-niche fuels. *Nat. Commun.* 8 (1), 2074. doi:10.1038/s41467-017-02092-0

Davies, L. C., Rosas, M., Smith, P. J., Fraser, D. J., Jones, S. A., and Taylor, P. R. (2011). A quantifiable proliferative burst of tissue macrophages restores homeostatic macrophage populations after acute inflammation. *Eur. J. Immunol.* 41 (8), 2155–2164. doi:10.1002/eji.201141817

De Schepper, S., Verheijden, S., Aguilera-Lizarraga, J., Viola, M. F., Boesmans, W., Stakenborg, N., et al. (2019). Self-maintaining gut macrophages are essential for intestinal homeostasis. *Cell* 176 (3), 676. doi:10.1016/j.cell.2019.01.010

Dick, S. A., Macklin, J. A., Nejat, S., Momen, A., Clemente-Casares, X., Althagafi, M. G., et al. (2019). Self-renewing resident cardiac macrophages limit adverse remodeling following myocardial infarction. *Nat. Immunol.* 20 (1), 29–39. doi:10.1038/s41590-018-0272-2

Duan, Z., and Luo, Y. (2021). Targeting macrophages in cancer immunotherapy. *Signal Transduct. Target. Ther.* 6 (1), 127. doi:10.1038/s41392-021-00506-6

Epelman, S., Lavine, K. J., and Randolph, G. J. (2014). Origin and functions of tissue macrophages. *Immunity* 41 (1), 21–35. doi:10.1016/j.immuni.2014.06.013

Etzerodt, A., Moulin, M., Doktor, T. K., Delfini, M., Mossadegh-Keller, N., Bajenoff, M., et al. (2020). Tissue-resident macrophages in omentum promote metastatic spread of ovarian cancer. *J. Exp. Med.* 217 (4), e20191869. doi:10.1084/jem.20191869

Finkernagel, F., Reinartz, S., Lieber, S., Adhikary, T., Wortmann, A., Hoffmann, N., et al. (2016). The transcriptional signature of human ovarian carcinoma macrophages is associated with extracellular matrix reorganization. *Oncotarget* 7 (46), 75339–75352. doi:10.18632/oncotarget.12180

Franklin, R. A., Liao, W., Sarkar, A., Kim, M. V., Bivona, M. R., Liu, K., et al. (2014). The cellular and molecular origin of tumor-associated macrophages. *Science* 344 (6186), 921–925. doi:10.1126/science.1252510

Gautier, E. L., Ivanov, S., Williams, J. W., Huang, S. C., Marcelin, G., Fairfax, K., et al. (2014). Gata6 regulates aspartoacylase expression in resident peritoneal macrophages and controls their survival. *J. Exp. Med.* 211 (8), 1525–1531. doi:10.1084/jem.20140570

Gautier, E. L., Shay, T., Miller, J., Greter, M., Jakubzick, C., Ivanov, S., et al. (2012). Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. *Nat. Immunol.* 13 (11), 1118–1128. doi:10.1038/ni.2419

Gekas, C., Dieterlen-Lievre, F., Orkin, S. H., and Mikkola, H. K. A. (2005). The placenta is a niche for hematopoietic stem cells. *Dev. Cell* 8 (3), 365–375. doi:10.1016/j.devcel.2004.12.016

Ghosn, E. E., Cassado, A. A., Govoni, G. R., Fukuhara, T., Yang, Y., Monack, D. M., et al. (2010). Two physically, functionally, and developmentally distinct peritoneal macrophage subsets. *Proc. Natl. Acad. Sci. U. S. A.* 107 (6), 2568–2573. doi:10.1073/pnas.0915000107

Ginhoux, F., and Guilliams, M. (2016). Tissue-resident macrophage ontogeny and homeostasis. *Immunity* 44 (3), 439–449. doi:10.1016/j.immuni.2016.02.024

Gomez Perdiguero, E., Klapproth, K., Schulz, C., Busch, K., Azzoni, E., Crozet, L., et al. (2015). Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature* 518 (7540), 547–551. doi:10.1038/nature13989

Goossens, P., Rodriguez-Vita, J., Etzerodt, A., Masse, M., Rastoin, O., Gouirand, V., et al. (2019). Membrane cholesterol efflux drives tumor-associated macrophage reprogramming and tumor progression. *Cell Metab.* 29 (6), 1376–1389. doi:10.1016/j.cmet.2019.02.016

Guilliams, M., and Scott, C. L. (2017). Does niche competition determine the origin of tissue-resident macrophages? *Nat. Rev. Immunol.* 17 (7), 451–460. doi:10.1038/nri.2017.42

Hashimoto, D., Chow, A., Noizat, C., Teo, P., Beasley, M. B., Leboeuf, M., et al. (2013). Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. *Immunity* 38 (4), 792–804. doi:10.1016/j.immuni.2013.04.004

Herr, D., Sallmann, A., Bekes, I., Konrad, R., Holzheu, I., Kreienberg, R., et al. (2012). VEGF induces ascites in ovarian cancer patients via increasing peritoneal permeability by downregulation of Claudin 5. *Gynecol. Oncol.* 127 (1), 210–216. doi:10.1016/j.ygyno.2012.05.002

Huang, S. C., Smith, A. M., Everts, B., Colonna, M., Pearce, E. L., Schilling, J. D., et al. (2016). Metabolic reprogramming mediated by the mTORC2-IRF4 signaling Axis is essential for macrophage alternative activation. *Immunity* 45 (4), 817–830. doi:10.1016/j.immuni.2016.09.016

Irvine, K. M., Banh, X., Gadd, V. L., Wojcik, K. K., Ariffin, J. K., Jose, S., et al. (2016). CRIg-expressing peritoneal macrophages are associated with disease severity in patients with cirrhosis and ascites. *JCI Insight* 1 (8), e86914. doi:10.1172/jci.insight.86914

Isobe, A., Sawada, K., Kinose, Y., Ohyagi-Hara, C., Nakatsuka, E., Makino, H., et al. (2015). Interleukin 6 receptor is an independent prognostic factor and a potential therapeutic target of ovarian cancer. *PLoS One* 10 (2), e0118080. doi:10.1371/journal.pone.0118080

Jenkins, S. J., Ruckerl, D., Cook, P. C., Jones, L. H., Finkelman, F. D., van Rooijen, N., et al. (2011). Local macrophage proliferation, rather than recruitment from the

blood, is a signature of TH2 inflammation. *Science* 332 (6035), 1284–1288. doi:10.1126/science.1204351

Jenkins, S. J., Ruckerl, D., Thomas, G. D., Hewitson, J. P., Duncan, S., Brombacher, F., et al. (2013). IL-4 directly signals tissue-resident macrophages to proliferate beyond homeostatic levels controlled by CSF-1. *J. Exp. Med.* 210 (11), 2477–2491. doi:10.1084/jem.20121999

Kasagi, Y., Harada, Y., Morodomi, Y., Iwai, T., Saito, S., Yoshida, K., et al. (2016). Peritoneal dissemination requires an sp1-dependent CXCR4/CXCL12 signaling Axis and extracellular matrix-directed spheroid formation. *Cancer Res.* 76 (2), 347–357. doi:10.1158/0008-5472.CAN-15-1563

Kershaw, M. H., Westwood, J. A., and Darcy, P. K. (2013). Gene-engineered T cells for cancer therapy. *Nat. Rev. Cancer* 13 (8), 525–541. doi:10.1038/nrc3565

Kim, K. W., Williams, J. W., Wang, Y. T., Ivanov, S., Gilfillan, S., Colonna, M., et al. (2016). MHC II+ resident peritoneal and pleural macrophages rely on IRF4 for development from circulating monocytes. *J. Exp. Med.* 213 (10), 1951–1959. doi:10.1084/jem.20160486

Ko, S. Y., Ladanyi, A., Lengyel, E., and Naora, H. (2014). Expression of the homeobox gene HOXA9 in ovarian cancer induces peritoneal macrophages to acquire an M2 tumor-promoting phenotype. *Am. J. Pathol.* 184 (1), 271–281. doi:10.1016/j.ajpath.2013.09.017

Krishnan, V., Tallapragada, S., Schaar, B., Kamat, K., Chanana, A. M., Zhang, Y., et al. (2020). Omental macrophages secrete chemokine ligands that promote ovarian cancer colonization of the omentum via CCR1. *Commun. Biol.* 3 (1), 524. doi:10.1038/s42003-020-01246-z

Lee, W., Ko, S. Y., Mohamed, M. S., Kenny, H. A., Lengyel, E., and Naora, H. (2019). Neutrophils facilitate ovarian cancer premetastatic niche formation in the omentum. *J. Exp. Med.* 216 (1), 176–194. doi:10.1084/jem.20181170

Leone, R. D., and Powell, J. D. (2020). Metabolism of immune cells in cancer. *Nat. Rev. Cancer* 20 (9), 516–531. doi:10.1038/s41568-020-0273-y

Link, V. M., Duttke, S. H., Chun, H. B., Holtman, I. R., Westin, E., Hoeksema, M. A., et al. (2018). Analysis of genetically diverse macrophages reveals local and domain-wide mechanisms that control transcription factor binding and function. *Cell* 173 (7), 1796–1809. doi:10.1016/j.cell.2018.04.018

Liu, L., Wang, X., Li, X., Wu, X., Tang, M., and Wang, X. (2018). Upregulation of IGF1 by tumor-associated macrophages promotes the proliferation and migration of epithelial ovarian cancer cells. *Oncol. Rep.* 39 (2), 818–826. doi:10.3892/or.2017.6148

Liu, P. S., Wang, H. P., Li, X. Y., Chao, T., Christen, T. T. S., Christen, S., et al. (2017). α-ketoglutarate orchestrates macrophage activation through metabolic and epigenetic reprogramming.. *Nat. Immunol.* 18(9), 985–994. doi:10.1038/ni.3796

Liu, R., Wei, H. T., Gao, P., Yu, H., Wang, K., Fu, Z., et al. (2017). CD47 promotes ovarian cancer progression by inhibiting macrophage phagocytosis. *Oncotarget* 8 (24), 39021–39032. doi:10.18632/oncotarget.16547

Louwe, P. A., Forbes, S. J., Benezech, C., Pridans, C., and Jenkins, S. J. (2022). Cell origin and niche availability dictate the capacity of peritoneal macrophages to colonize the cavity and omentum. *Immunology* 166, 458–474. doi:10.1111/imm.13483

Loyher, P. L., Hamon, P., Laviron, M., Meghraoui-Kheddar, A., Goncalves, E., Deng, Z., et al. (2018). Macrophages of distinct origins contribute to tumor development in the lung. *J. Exp. Med.* 215 (10), 2536–2553. doi:10.1084/jem.20180534

Mantovani, A., Marchesi, F., Malesci, A., Laghi, L., and Allavena, P. (2017). Tumour-associated macrophages as treatment targets in oncology. *Nat. Rev. Clin. Oncol.* 14 (7), 399–416. doi:10.1038/nrclinonc.2016.217

Moughon, D. L., He, H., Schokrpur, S., Jiang, Z. K., Yaqoob, M., David, J., et al. (2015). Macrophage blockade using CSF1R inhibitors reverses the vascular leakage underlying malignant ascites in late-stage epithelial ovarian cancer. *Cancer Res.* 75 (22), 4742–4752. doi:10.1158/0008-5472.CAN-14-3373

Muller, S., Kohanbash, G., Liu, S. J., Alvarado, B., Carrera, D., Bhaduri, A., et al. (2017). Single-cell profiling of human gliomas reveals macrophage ontogeny as a basis for regional differences in macrophage activation in the tumor microenvironment. *Genome Biol.* 18, 234. doi:10.1186/s13059-017-1362-4

Murray, P. J., Allen, J. E., Biswas, S. K., Fisher, E. A., Gilroy, D. W., Goerdt, S., et al. (2014). Macrophage activation and polarization: Nomenclature and experimental guidelines. *Immunity* 41 (1), 14–20. doi:10.1016/j.immuni.2014.06.008

Nieman, K. M., Kenny, H. A., Penicka, C. V., Ladanyi, A., Buell-Gutbrod, R., Zillhardt, M. R., et al. (2011). Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth. *Nat. Med.* 17 (11), 1498–1503. doi:10.1038/nm.2492

Oh, M. H., Collins, S. L., Sun, I. H., Tam, A. J., Patel, C. H., Arwood, M. L., et al. (2017). mTORC2 signaling selectively regulates the generation and function of tissue-resident peritoneal macrophages. *Cell Rep.* 20 (10), 2439–2454. doi:10.1016/j.celrep.2017.08.046

Okabe, Y., and Medzhitov, R. (2014). Tissue-specific signals control reversible program of localization and functional polarization of macrophages. *Cell* 157 (4), 832–844. doi:10.1016/j.cell.2014.04.016

Oosterhof, N., Chang, I. J., Karimiani, E. G., Kuil, L. E., Jensen, D. M., Daza, R., et al. (2019). Homozygous mutations in CSF1R cause a pediatric-onset leukoencephalopathy and can result in congenital absence of microglia. *Am. J. Hum. Genet.* 104 (5), 936–947. doi:10.1016/j.ajhg.2019.03.010

Oosterling, S. J., van der Bij, G. J., Bogels, M., van der Sijp, J. R., Beelen, R. H., Meijer, S., et al. (2006). Insufficient ability of omental milky spots to prevent peritoneal tumor outgrowth supports omentectomy in minimal residual disease. *Cancer Immunol. Immunother.* 55 (9), 1043–1051. doi:10.1007/s00262-005-0101-y

Palmieri, E. M., Menga, A., Martin-Perez, R., Quinto, A., Riera-Domingo, C., De Tullio, G., et al. (2017). Pharmacologic or genetic targeting of glutamine synthetase skews macrophages toward an M1-like phenotype and inhibits tumor metastasis. *Cell Rep.* 20 (7), 1654–1666. doi:10.1016/j.celrep.2017.07.054

Pathria, P., Louis, T. L., and Varner, J. A. (2019). Targeting tumor-associated macrophages in cancer. *Trends Immunol.* 40 (4), 310–327. doi:10.1016/j.it.2019.02.003

Peng, D., Kryczek, I., Nagarsheth, N., Zhao, L., Wei, S., Wang, W., et al. (2015). Epigenetic silencing of TH1-type chemokines shapes tumour immunity and immunotherapy. *Nature* 527 (7577), 249–253. doi:10.1038/nature15520

Penn, C. A., Yang, K., Zong, H., Lim, J. Y., Cole, A., Yang, D., et al. (2018). Therapeutic impact of nanoparticle therapy targeting tumor-associated macrophages. *Mol. Cancer Ther.* 17 (1), 96–106. doi:10.1158/1535-7163.MCT-17-0688

Pepe, G., Braga, D., Renzi, T. A., Villa, A., Bolego, C., D'Avila, F., et al. (2017). Self-renewal and phenotypic conversion are the main physiological responses of macrophages to the endogenous estrogen surge. *Sci. Rep.* 7, 44270. doi:10.1038/srep44270

Pittet, M. J., Michielin, O., and Migliorini, D. (2022). Clinical relevance of tumour-associated macrophages. *Nat. Rev. Clin. Oncol.* 19, 402–421. doi:10.1038/s41571-022-00620-6

Qian, B. Z., Li, J., Zhang, H., Kitamura, T., Zhang, J., Campion, L. R., et al. (2011). CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis. *Nature* 475 (7355), 222–225. doi:10.1038/nature10138

Rangel-Moreno, J., Moyron-Quiroz, J. E., Carragher, D. M., Kusser, K., Hartson, L., Moquin, A., et al. (2009). Omental milky spots develop in the absence of lymphoid tissue-inducer cells and support B and T cell responses to peritoneal antigens. *Immunity* 30 (5), 731–743. doi:10.1016/j.immuni.2009.03.014

Rei, M., Goncalves-Sousa, N., Lanca, T., Thompson, R. G., Mensurado, S., Balkwill, F. R., et al. (2014). Murine CD27(-) Vγ6(+) γδ T cells producing IL-17A promote ovarian cancer growth via mobilization of protumor small peritoneal macrophages.. *Proc. Natl. Acad. Sci. U. S. A.* 111 (34), E3562–E3570. doi:10.1073/pnas.1403424111

Reinfeld, B. I., Madden, M. Z., Wolf, M. M., Chytil, A., Bader, J. E., Patterson, A. R., et al. (2021). Cell-programmed nutrient partitioning in the tumour microenvironment. *Nature* 593 (7858), 282–288. doi:10.1038/s41586-021-03442-1

Roberts, A. W., Lee, B. L., Deguine, J., John, S., Shlomchik, M. J., and Barton, G. M. (2017). Tissue-resident macrophages are locally programmed for silent clearance of apoptotic cells. *Immunity* 47 (5), 913–927. doi:10.1016/j.immuni.2017.10.006

Robinson-Smith, T. M., Isaacsohn, I., Mercer, C. A., Zhou, M., Van Rooijen, N., Husseinzadeh, N., et al. (2007). Macrophages mediate inflammation-enhanced metastasis of ovarian tumors in mice. *Cancer Res.* 67 (12), 5708–5716. doi:10.1158/0008-5472.CAN-06-4375

Rodriguez, G. C., Haisley, C., Hurteau, J., Moser, T. L., Whitaker, R., Bast, R. C., Jr., et al. (2001). Regulation of invasion of epithelial ovarian cancer by transforming growth factor-beta. *Gynecol. Oncol.* 80 (2), 245–253. doi:10.1006/gyno.2000.6042

Rodriguez-Garcia, A., Lynn, R. C., Poussin, M., Eiva, M. A., Shaw, L. C., O'Connor, R. S., et al. (2021). CAR-T cell-mediated depletion of immunosuppressive tumor-associated macrophages promotes endogenous antitumor immunity and augments adoptive immunotherapy. *Nat. Commun.* 12 (1), 877. doi:10.1038/s41467-021-20893-2

Rojo, R., Raper, A., Ozdemir, D. D., Lefevre, L., Grabert, K., Wollscheid-Lengeling, E., et al. (2019). Deletion of a Csf1r enhancer selectively impacts CSF1R expression and development of tissue macrophage populations. *Nat. Commun.* 10 (1), 3215. doi:10.1038/s41467-019-11053-8

Rosas, M., Davies, L. C., Giles, P. J., Liao, C. T., Kharfan, B., Stone, T. C., et al. (2014). The transcription factor Gata6 links tissue macrophage phenotype and proliferative renewal. *Science* 344 (6184), 645–648. doi:10.1126/science.1251414

Ruiz-Alcaraz, A. J., Carmona-Martinez, V., Tristan-Manzano, M., Machado-Linde, F., Sanchez-Ferrer, M. L., Garcia-Penarrubia, P., et al. (2018). Characterization of human peritoneal monocyte/macrophage subsets in homeostasis: Phenotype, GATA6, phagocytic/oxidative activities and cytokines expression. *Sci. Rep.* 8 (1), 12794. doi:10.1038/s41598-018-30787-x

Ruiz-Alcaraz, A. J., Tapia-Abellan, A., Fernandez-Fernandez, M. D., Tristan-Manzano, M., Hernandez-Caselles, T., Sanchez-Velasco, E., et al. (2016). A novel CD14(high) CD16(high) subset of peritoneal macrophages from cirrhotic patients is associated to an increased response to LPS. *Mol. Immunol.* 72, 28–36. doi:10.1016/j.molimm.2016.02.012

Sikic, B. I., Lakhani, N., Patnaik, A., Shah, S. A., Chandana, S. R., Rasco, D., et al. (2019). First-in-Human, first-in-class phase I trial of the anti-CD47 antibody Hu5F9-G4 in patients with advanced cancers. *J. Clin. Oncol.* 37 (12), 946–953. doi:10.1200/JCO.18.02018

Song, H., Wang, T., Tian, L., Bai, S., Chen, L., Zuo, Y., et al. (2019). Macrophages on the peritoneum are involved in gastric cancer peritoneal metastasis. *J. Cancer* 10 (22), 5377–5387. doi:10.7150/jca.31787

Song, M., Yeku, O. O., Rafiq, S., Purdon, T., Dong, X., Zhu, L., et al. (2020). Tumor derived UBR5 promotes ovarian cancer growth and metastasis through inducing immunosuppressive macrophages. *Nat. Commun.* 11 (1), 6298. doi:10.1038/s41467-020-20140-0

Steinman, R. M., and Moberg, C. L. (1994). Zanvil alexander Cohn 1926-1993. *J. Exp. Med.* 179 (1), 1–30. doi:10.1084/jem.179.1.1

Thion, M. S., Low, D., Silvin, A., Chen, J., Grisel, P., Schulte-Schrepping, J., et al. (2018). Microbiome influences prenatal and adult microglia in a sex-specific manner. *Cell* 172 (3), 500–516. e516. doi:10.1016/j.cell.2017.11.042

Wang, Y. T., Zaitsev, K., Lu, Q., Li, S., Schaiff, W. T., Kim, K. W., et al. (2020). Select autophagy genes maintain quiescence of tissue-resident macrophages and increase susceptibility to Listeria monocytogenes. *Nat. Microbiol.* 5 (2), 272–281. doi:10.1038/s41564-019-0633-0

Weiss, J. M., Davies, L. C., Karwan, M., Ileva, L., Ozaki, M. K., Cheng, R. Y., et al. (2018). Itaconic acid mediates crosstalk between macrophage metabolism and peritoneal tumors. *J. Clin. Invest.* 128 (9), 3794–3805. doi:10.1172/JCI99169

Wilkinson, N. M., Chen, H. C., Lechner, M. G., and Su, M. A. (2022). Sex differences in immunity. *Annu. Rev. Immunol.* 40, 75–94. doi:10.1146/annurev-immunol-101320-125133

Willingham, S. B., Volkmer, J. P., Gentles, A. J., Sahoo, D., Dalerba, P., Mitra, S. S., et al. (2012). The CD47-signal regulatory protein alpha (SIRPa) interaction is a therapeutic target for human solid tumors. *Proc. Natl. Acad. Sci. U. S. A.* 109 (17), 6662–6667. doi:10.1073/pnas.1121623109

Wu, J. Y., Huang, T. W., Hsieh, Y. T., Wang, Y. F., Yen, C. C., Lee, G. L., et al. (2020). Cancer-derived succinate promotes macrophage polarization and cancer metastasis via succinate receptor. *Mol. Cell* 77 (2), 213–227. e215. doi:10.1016/j.molcel.2019.10.023

Xia, H., Li, S., Li, X., Wang, W., Bian, Y., Wei, S., et al. (2020). Autophagic adaptation to oxidative stress alters peritoneal residential macrophage survival and ovarian cancer metastasis. *JCI Insight* 5 (18), 141115. doi:10.1172/jci.insight.141115

Yin, M., Li, X., Tan, S., Zhou, H. J., Ji, W., Bellone, S., et al. (2016). Tumor-associated macrophages drive spheroid formation during early transcoelomic metastasis of ovarian cancer. *J. Clin. Invest.* 126 (11), 4157–4173. doi:10.1172/JCI87252

Yona, S., Kim, K. W., Wolf, Y., Mildner, A., Varol, D., Breker, M., et al. (2013). Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* 38 (1), 79–91. doi:10.1016/j.immuni.2012.12.001

Zeng, Y., Li, B., Liang, Y., Reeves, P. M., Qu, X., Ran, C., et al. (2019). Dual blockade of CXCL12-CXCR4 and PD-1-PD-L1 pathways prolongs survival of ovarian tumor-bearing mice by prevention of immunosuppression in the tumor microenvironment. *FASEB J.* 33 (5), 6596–6608. doi:10.1096/fj.201802067RR

Zhang, L., Conejo-Garcia, J. R., Katsaros, D., Gimotty, P. A., Massobrio, M., Regnani, G., et al. (2003). Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *N. Engl. J. Med.* 348 (3), 203–213. doi:10.1056/NEJMoa020177

Zhang, N., Kim, S. H., Gainullina, A., Erlich, E. C., Onufer, E. J., Kim, J., et al. (2021). LYVE1(+) macrophages of murine peritoneal mesothelium promote omentum-independent ovarian tumor growth. *J. Exp. Med.* 218 (12), e20210924. doi:10.1084/jem.20210924

Zhao, L. J., Wang, W., Huang, S., Yang, Z. N., Xu, L., Yang, Q. L., et al. (2018). The RNA binding protein SORBS2 suppresses metastatic colonization of ovarian cancer by stabilizing tumor-suppressive immunomodulatory transcripts. *Genome Biol.* 19, 35. doi:10.1186/s13059-018-1412-6

Zhu, Y., Herndon, J. M., Sojka, D. K., Kim, K. W., Knolhoff, B. L., Zuo, C., et al. (2017). Tissue-resident macrophages in pancreatic ductal adenocarcinoma originate from embryonic hematopoiesis and promote tumor progression. *Immunity* 47 (2), 323–338. doi:10.1016/j.immuni.2017.07.014

Zou, W., Wolchok, J. D., and Chen, L. (2016). PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: Mechanisms, response biomarkers, and combinations. *Sci. Transl. Med.* 8 (328), 328rv4. doi:10.1126/scitranslmed.aad7118

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Neuroendocrine regulations in tissue-specific immunity: From mechanism to applications in tumor

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Immune responses in nonlymphoid tissues play a vital role in the maintenance of homeostasis. Lots of evidence supports that tissue-specific immune cells provide defense against tumor through the localization in different tissue throughout the body, and can be regulated by diverse factors. Accordingly, the distribution of nervous tissue is also tissue-specific which is essential in the growth of corresponding organs, and the occurrence and development of tumor. Although there have been many mature perspectives on the neuroendocrine regulation in tumor microenvironment, the neuroendocrine regulation of tissue-specific immune cells has not yet been summarized. In this review, we focus on how tissue immune responses are influenced by autonomic nervous system, sensory nerves, and various neuroendocrine factors and reversely how tissue-specific immune cells communicate with neuroendocrine system through releasing different factors. Furthermore, we pay attention to the potential mechanisms of neuroendocrine-tissue specific immunity axis involved in tumors. This may provide new insights for the immunotherapy of tumors in the future.

KEYWORDS

neuroendocrine regulation, neurotransmitter, neuropeptide, tissue-specific immunity, cancer

1 Introduction

Tumors develop in complicated tissue environments, which they rely on for growth, invasion and metastasis (Quail and Joyce, 2013). The tumor microenvironment (TME) is a heterogeneous ecosystem composed of all the structures at the site and those that are recruited to the area—immune cells, mesenchymal stem cells, vascular vessels, nerves and matrix components (Hanahan and Coussens, 2012). Tumors are derived from the complex interactions that occur between them (Gysler and Drapkin, 2021). With the constant exploration of new ways to treat tumors, therapies targeting the TME have

emerged as a promising approach for cancer treatment for the past few years (Bejarano et al., 2021). Research has progressed, but there are remaining unknowns. The composition of the TME is being elucidated, with the neurological, immunological and microbiological components being identified. However, other characteristics and the dynamic evolution of the TME require further in-depth exploration.

Tissue-specific immunity occurs when immune cells establish permanent tissue residency in different organs, thus creating a defensive system (Klose and Artis, 2020). Numerous immune cells reside in nonlymphoid tissues. These tissue-resident populations do not recirculate and adopt a unique phenotype that is distinct from immune cells in the blood or lymphatic system (Mackay and Kallies, 2017). The origin of tissue-resident immune cells is complex and includes tissue innate immune cells and immune cells that have migrated from the peripheral blood. Innate immune cells, including macrophages, dendritic cells (DCs) and innate lymphoid cells (ILCs), exhibit tissue-specific subset compositions in the lung, skin, intestines and comprise the early responders to pathogen encounters. Adaptive immune cells, such as T cells and B cells, also have tissue-resident subsets that help to build immune memory. For example, tissue-resident memory T cells persist as tissue-resident populations in mucosal and exocrine sites, while memory B cells predominate in the intestines. In general, the human immune system is localized in a tissue-specific manner in diverse sites (Weisberg et al., 2021). Functionally, tissue-specific immunity is observed to play a part in tumor development through various regulatory mechanisms (Lei et al., 2020; Liu et al., 2022a).

Recently, the contribution of nerves to the pathogenesis of malignancies has emerged (Zahalka and Frenette, 2020). Several landmark studies have demonstrated that the nervous system plays an active role in tumorigenesis (Reavis et al., 2020). Meanwhile, some studies have suggested that tumor innervation is associated with accelerated tumor progression in multiple cancers (Albo et al., 2011; Magnon et al., 2013; Huang et al., 2014; Kappos et al., 2018; Renz et al., 2018). The nervous system is composed of the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS is the main part of the nervous system and is composed of the spinal cord and brain, which play a part in tumor development. Studies have reported that chronic stress causes disorders in the CNS and promotes tumor initiation and progression (Saul et al., 2005; Goldfarb et al., 2011; Levi et al., 2011; Partecke et al., 2016). β-adrenergic activation by the cAMP–PKA signaling pathway, e.g., β-adrenergic receptors (ARs) stimulated by norepinephrine (NE), is a major mechanism by which stress can enhance tumor development and increase vascularization (Thaker et al., 2006). Furthermore, immune regulation also plays a vital role in stress-induced tumor development (Dhabhar, 2009). Studies have shown that chronic stress causes a large-scale alteration of immune cells in tissues and induces suppression or dysregulation of immune function (Dhabhar et al., 2012; Dhabhar, 2014). The PNS emanates from the CNS and is divided into the autonomic systems and somatosensory, which are responsible for communicating with all parts of the body. Autonomic nerve density was reported to be associated with tumor prognosis and progression (Silverman et al., 2021). In terms of mechanism, infiltrating sympathetic nerves facilitated tumor progression and invasion through β-adrenergic activation (Pon et al., 2016). Adrenergic nerves also facilitate tumor growth by releasing NE to stimulate angiogenesis via VEGF signaling (Zahalka et al., 2017). In light of neural receptors are expressed by many immune cell types, it follows that immune cells may also participate in oncogenesis through adrenergic signaling (Silverman et al., 2021). Neuroendocrine factors contain neurotransmitters, neuropeptides and many other factors secreted by the nervous system mediate stimulatory or inhibitory functions by binding to their respective receptors. In recent decades, many discoveries have elucidated their regulatory roles in tissues and organs (Jiang et al., 2020a). For instance, studies in nerve growth factor (NGF) has reported that this factor acts not only on the PNS and CNS but also on nonneuronal and cancer cells (Aloe et al., 2016). NGF and its receptor TrkA have been implicated in the development of many aggressive cancers. However, NGF pathway has been proved to be critical to inflammation control and the immune response in tumor (Campos et al., 2007; Retamales-Ortega et al., 2017; Wehkamp et al., 2018; Triaca et al., 2019; Jiang et al., 2020b). Nerves regulate multiple components of the TME. Importantly, with the development of high-throughput sequencing techniques including single-cell RNA sequencing and spatial transcriptomics (Liu et al., 2022b), researchers found that immune and neuronal cells are often colocalized to form neuroimmune cell units (Huh and Veiga-Fernandes, 2020). And, both immune cells and neurons express receptors to sense neurotransmitters and cytokines, allowing direct interactions between the two systems (Seillet and Jacquelot, 2019).

Relatively few studies have investigated the functional role of neuroendocrine in carcinogenesis and regulation of the TME. Here, we summarize the mechanisms of neuroendocrine regulation of tissue-specific immunity based on the neuroendocrine signal transmission medium and how tissue-specific immune cells communicate with nerves by releasing different factors. We then focus on the role of neuroendocrine regulation in tumor immunity and how it informs prognosis and treatment.

2 Neuroendocrine regulation in tissue-specific immunity

The nervous system is composed of various components such as the CNS, sympathetic nerves, parasympathetic nerves, and sensory nerve nociceptors, and it mediates immune cells

primarily by secreting neuroendocrine factors such as neurotransmitters and neuropeptides. These molecules can regulate tissue-specific immunity by binding to their corresponding receptors expressed on immune cells in various tissues. Here, we summarize the neuroendocrine regulation of tissue-specific immunity, categorized by signaling mediator.

2.1 Adrenergic regulation

The adrenergic system is composed of NE and epinephrine, which are the primary neurotransmitters secreted by postganglionic sympathetic neurons. They regulate cellular function through ARs, including the α1-, α2-, β1-, β2-, and β3-ARs (Tanner et al., 2021). The immune effects of adrenergic signaling are primarily transmitted by β2-ARs, which are expressed on nearly all major immune cell types (Marino and Cosentino, 2013). NE has been shown to mediate the cell trafficking and effector activities of immune cells *via* β2-AR.

NE affects the function of the CNS. Microglia are the most common resident innate immune cells in the CNS and monitor brain homeostasis by producing ligands that support neuronal survival, pruning non-functional synapses and removing dying neurons (Nayak et al., 2014; Werneburg et al., 2017). Studies have shown that NE upregulates the expression of the amyloid beta peptide (Aβ) receptor mFPR2 through activation of β2-AR in microglia, which helps to maintain the adequate uptake and clearance of Aβ(42). Moreover, increased secretion of NE suppresses the microglial response and reduces the upregulation of both anti- and pro-inflammatory cytokines through β2-adrenergic signaling (Lechtenberg et al., 2019). In general, NE can modulate microglial motility, which can affect the function of microglia in some pathogenic situations (Gyoneva and Traynelis, 2013; Umpierre and Wu, 2020).

Tissue-resident macrophages, ILCs, tissue-resident T cells and NK cells are common tissue-resident immune cells in the periphery. They act in a tissue-specific manner in adipose tissue, liver, lung and gut, where NE has been shown to modulate these cells via β2-adrenergic signaling. In adipose tissue, adipose tissue macrophages (ATMs) and ILC2s are common resident immune cells that act as dominant initiators of type 2 inflammation and tissue repair (Cording et al., 2016). Some studies have shown that sympathetic nerves maintain an anti-inflammatory state in mice by inhibiting TNF-α level in macrophages (Tang et al., 2015). Additionally, NE can promote extracellular fatty acid uptake and storage as triglycerides and reduce free fatty acid release from triglyceride-laden macrophages (Petkevicius et al., 2021a). However, another study found that the sympathetic nervous system exerts an indirect effect on ATMs through the modulation of adipocyte function instead of modulating the phenotype of ATMs directly (Petkevicius et al., 2021b). Similar findings were also found for ILC2s, in which PDGFRA + adipose stem cell (ASC) could serve as a messenger between the immune cells and nervous system. The sympathetic nerve can act on ASCs *via* β2-AR to control the level of glial-derived neurotrophic factor and indirectly regulate the activity of adipose tissue ILC2s via the neurotrophic factor receptor RET (50). Likewise, *β*-adrenergic signaling was also reported to regulate the production of IL-33 by a DPP4+PDGFRB + ASC subpopulation and enhance ILC2 accumulation indirectly (Shan et al., 2021).

In the intestine, tissue-specific immune cells include muscularis macrophages (MMs) and ILC2s. By studying the transcriptional profile, gut-innervating sympathetic neurons have been found to polarize MMs towards a protectively M2 phenotype through β2-AR (52). In murine models of enteric infections, MMs upregulate a neuroprotective program via β2-AR and constrain neuronal death through an arginase 1-polyamine axis (Matheis et al., 2020). ILC2s participate in multiple intestinal physiological processes, including tissue repair, metabolic homeostasis, allergic inflammation and host defense against infections (Cardoso et al., 2017). Research has shown that β2-AR deficiency results in exaggerated ILC2 responses and type 2 inflammation in the intestine (Moriyama et al., 2018). Another study also demonstrated that sympathetic innervation constrains the effects of innate immune responses on microbes in the gut through the β2-AR pathway (Willemze et al., 2019). β2-AR has been proven to mediate negative regulation of ILC2s through the inhibition of cell proliferation and effector function (Moriyama et al., 2018). A recent study reported that colonic sympathetic nerves can also exert an indirect effect on immune cells through endothelial MAdCAM-1. In murine models, activation of local sympathetic nerves decelerated colitis and reduced the abundance of immune cell (Schiller et al., 2021). Hepatic invariant NKT (iNKT) cells are tissue-specific immune cells that primarily reside in the liver (Bendelac et al., 2007). One study showed that the immunosuppressive function of iNKT cells was mediated through NE (59).

In summary, adrenergic signaling regulates a variety of tissue-resident immune cells in the CNS and PNS in which plays an anti-inflammatory and immunosuppressive role (Figure 1, Table 1). Thus, adrenergic regulation is an important neuroendocrine modulating method for tissue-specific immunity.

2.2 Cholinergic regulation

The cholinergic system, which is found in both neuronal and nonneuronal cells, is a network that performs various complex functions in the body. It is composed of acetylcholine (ACh), cholinergic receptors (AChRs), acetylcholinesterase enzyme and choline acetyltransferase enzyme (Halder and Lal, 2021). ACh is the classical neurotransmitter in the cholinergic system. The receptor of ACh is divided into ionotropic nicotinic ACh

receptors (nAChRs) and metabotropic muscarinic ACh receptors (mAChRs).

ACh affects the function of the CNS. AChRs are expressed on microglia in the CNS. mAChR stimulation modulates microglial chemotaxis and phagocytic activity via IFN-γ activation (Pannell et al., 2016). Regarding nAChRs, α7nAChR is an essential regulator of inflammation (Wang et al., 2003). The anti-inflammatory ACh response in microglia is mediated through α7nAChR. This response helps to lower inflammatory cytokine levels and microglial activation (Li et al., 2019). Similarly, ACh inhibits LPS-induced IL-1β and IL-6 elevation and promote IL-4 and IL-10 production through α7nAChRs to promote the M2 phenotype (Zhang et al., 2017). In addition, ACh stimulation of α7nAChR in microglia also enhances Aβ clearance (Hoskin et al., 2019).

Tissue-resident macrophages and ILCs are common tissue-resident immune cells in the periphery. They act in a tissue-specific manner in the liver, lung, gut, where ACh has been shown to modulate these cells *via* ACh-AChR signaling. In the lung, ILC2s are common resident immune cells. The ACh-α7nAChR axis in ILC2s decreases the synthesis of TNF-α, IL-1, and IL-6 (66). However, ILC2 transcription factor GATA-3 and the inflammatory modulator NF-κB is diminished. In general, ACh-α7nAChR signaling in ILC2s helps to promote anti-inflammatory function (Galle-Treger et al., 2016). In the liver, hepatic macrophages are the common resident immune cells. The vagus nerve regulates the secretory and phagocytic activity of resident macrophages *via* cholinergic signaling (Fonseca et al., 2019). ACh-AChR signal-mediated IL-6 production in hepatic macrophages upregulates the expression of FoxM1 in hepatocytes, leading to liver regeneration (Izumi et al., 2018). In the intestine, tissue-specific immune cells include MMs, ILC2s and ILC3s. The vagus nerve regulates the anti-inflammatory effect of intestinal MMs via α7nAChR-mediated JAK2/STAT3 signaling pathway (Matteoli et al., 2014; Yang et al., 2021). The ACh-AChR axis in ILC2s also causes anti-inflammatory effects by promoting ILC2 cytokine production (Chu et al., 2021). Moreover, AChs upregulate the PCTR biosynthetic pathway in ILC3s(Dalli et al., 2017). Besides, the vague nerve is also reported to modulate anti-inflammatory effect through the α7nAChR on macrophages in spleen and pancreas (Zhang et al., 2020).

In summary, cholinergic signaling facilitates the behavior of tissue-resident macrophages and promotes the anti-inflammatory function of ILC2s in the nervous system (Figure 1, Table 1). Thus, cholinergic regulation is another important neuroendocrine modulating method for tissue-specific immunity.

2.3 Regulation *via* other neurotransmitters

In addition to NE, epinephrine and ACh, neurotransmitters also include dopamine (DA), serotonin (5-HT) and gamma-aminobutyric acid (GABA). Research on the neuroendocrine regulation of immune cells by these other neurotransmitters has mainly focused on microglia. Microglia express several receptors for DA, 5-HT and GABA (Younger et al., 2019; Stolero and Frenkel, 2021).

DA is a neurotransmitter synthesized in both the CNS and PNS. It is mainly distributed in the CNS and has been detected in the liver, spleen, pancreas and gut (Peters et al., 2014; Klein et al., 2019). Dopamine receptors (DRs), including D1R, D2R, D3R, D4R, and D5R, are present on microglia and some other macrophages (Thomas Broome et al., 2020). DA promotes microglial migration, increases NO and ROS production through D1R, and the secretion of proinflammatory cytokines, such as IL-1β and IL-6 through D2R (75). Also, DA can induce the formation of DNA-based extracellular traps in microglia which participate in immunosurveillance and pathogen clearance (Agrawal et al., 2021) (Figure 1, Table 1).

5-HT, a biogenic amine synthesized from tryptophan, is a well-known neurotransmitter in the CNS which plays a critical roles in mood stability, sleep patterns and pain tolerance. The effects of 5-HT are regulated through 5-HT receptors. It also exist as an important signaling molecule in the periphery, especially in the gastrointestinal tract (Ye et al., 2021). 5-HT plays a role in CNS inflammation and repair. Administering 5-HT to microglia induces inflammatory initiation and IL-6 production (Quintero-Villegas and Valdés-Ferrer, 2019) (Figure 1, Table 1).

GABA is known as a main inhibitory amino acid neurotransmitter in the CNS. The physiological roles of GABA are related to the modulation of synaptic transmission. Two distinct classes of GABA receptors have been identified, including ionotropic receptor $GABA_A$ and metabotropic receptor $GABA_B$ (Ngo and Vo, 2019). Microglia are known to express both $GABA_A$ and $GABA_B$. GABA has been shown to decrease microglial neurotoxicity and exert an anti-inflammatory effect (Lee, 2013; Crowley et al., 2016) (Figure 1, Table 1).

There are few studies on the role of these neurotransmitters in peripheral tissue-specific immunity. However, there is evidence for their presence in the periphery; for instance, the airway epithelium contains pulmonary neuroendocrine cells filled with 5-HT and GABA neuropeptides (Magnon, 2021). Some peripheral tissue-specific immune cells have been reported to express corresponding neurotransmitter receptors, providing the possibility for neuroendocrine interactions between them.

2.4 Regulation *via* neuropeptides

Neuropeptides are peptide substances produced by neurons that play key roles in modulating cell function by binding to specific receptors. Examples of neuropeptides include neuromedin U (NMU), neuropeptide calcitonin gene-related peptide (CGRP), substance P (SP), neuropeptide TAFA4,

pituitary adenylate cyclase-activating polypeptide (PACAP), vasoactive intestinal peptide (VIP), neuropeptide FF (NPFF), neuropeptide Y (NPY) and somatostatin (SST). Neuropeptides and their receptors have regionally restricted distributions in the nervous system (Hoyer and Bartfai, 2012). Transcriptomic analyses have indicated that immune cells express numerous neuropeptide receptors (Seillet and Jacquelot, 2019). Thus, neuropeptides may play a vital role in mediating tissue-specific immunity.

2.4.1 Neuropeptides secreted by the central nervous system and autonomic nerves

NPFF is a CNS octapeptide that plays a part in pain modulation and opiate tolerance. NPFF has two receptors, NPFF receptor 1 and 2 (NPFFR1, NPFFR2) (Bonini et al., 2000). In peripheral tissues, only NPFFR2 is expressed in ATMs (Waqas et al., 2017). NPFF promotes M2 phenotype and increase the proliferation of ATMs. Specifically, NPFF suppressed the expression of the E3 ubiquitin ligase RNF128, which promote the stability of phosphorylated STAT6 and increased expression of gene related to M2 macrophage. NPFF induced ATM proliferation accompanied with the increase of NDRG2 expression and suppression of MAFB expression (Waqas et al., 2017) (Figure 1, Table 1).

NMU is a neuropeptide widely distributed in the human body and has two receptors, NMU receptor 1 and 2 (NMUR1, NMUR2). NMU mainly functions on ILC2s through NMUR1 (Martinez and O'Driscoll, 2015). NMU stimulates ILC2 activation and promotes type 2 cytokine responses that can induce antimicrobial and inflammatory responses (Klose et al., 2017). In the intestine, NMU increases IL-10 production in intestinal ILC2s. However, the function of ILC2-derived IL-10 in the intestine remains unknown (Bando et al., 2020). NMU can also amplify lung inflammation driven by ILC2s. IL-9 derived from ILC2 plays an important role in increasing the abundance of γδ T cell and IL-17A production (Wallrapp et al., 2017; Chen et al., 2021).

PACAP and VIP are two highly related neuropeptides widely distributed in organisms and have immunomodulatory actions. The receptors of PACAP and VIP are expressed by numerous immune cell types (Abad and Tan, 2018). The neuropeptide PACAP is an antimicrobial peptide induced in the brain in response to bacterial and fungal infection (Lee et al., 2021). PACAPR1 is a main receptor of PACAP expressed on DC. PACAP induces cutaneous DC functions and promotes the development of contact hypersensitivity through PACAPR1. The expression of CCR7 and CXCR4 of DCs was enhanced by PACAP *in vitro* (Yamamoto et al., 2021).

NPY is a neuropeptide widely distributed in the human body and is secreted mainly by the CNS and sympathetic nerves, where it is co-released with NE. NPY exerts its effects through interacting with NPY receptors (NPYRs), among which NPY1R is the most abundantly expressed NPYR receptor in immune cells (Dimitrijevic and Stanojevic, 2013). NPY inhibits microglial activation and phagocytosis and affects the direction of migration and phagocytosis. NPY also inhibits cytokine secretion of microglia, especially the secretion of proinflammatory factors (Gonçalves et al., 2012; Pain et al., 2019; Chen et al., 2020). Besides, NPY was reported to attenuate the splenic immune response as well (Yu et al., 2022) (Figure 1, Table 1).

Regarding VIP, intestinal ILC3s and pulmonary ILC2s highly express VIP receptor 2 (VIPR2). In the intestine, VIP activates ILC3s, enhancing the production of IL-22 and the barrier function of the epithelium (Seillet et al., 2020). This dynamic neuroimmune circuit in the intestine is regulated by feeding and circadian mechanisms (Talbot et al., 2020) (Figure 1, Table 1).

SST is a neuropeptide with generally inhibitory function which commonly produced by endocrine cells and the CNS(Weckbecker et al., 2003). SST mediates its biological functions via SST receptors (subtype 1–5), which was found expressing in multiple immune cells (Møller et al., 2003). However, existing studies have shown that SST only affects immune cells in peripheral blood of normal tissues. For instance, SST reduced the secretion of INF-γ from peripheral blood mononuclear cell and inhibit the production of immunoglobulin by B lymphocytes. Also, the chemotaxis of peripheral blood monocytes is inhibited by this peptide (Pintér et al., 2006).

2.4.2 Neuropeptides secreted by sensory nerves

Nociceptor sensory neurons are responsible for the detection of potentially damaging stimuli and elicit defensive behaviors (Chiu et al., 2013). The neuropeptides CGRP and SP are nociceptor mediators that act in the skin, lung and intestine. TAFA4 is a neuropeptide secreted by sensory neurons in skin. VIP mentioned above can also be secreted by nociceptors. Tissue-resident macrophages, ILC2s, NK cells, DCs and γδ T cells are common tissue-resident immune cells reside within epithelial layers of the lung, skin, and gut (Zheng et al., 2013).

CGRP regulates a variety of immune cells which express the receptor, calcitonin receptor-like receptor (CRLR). Broadly speaking, CGRP is a negative regulator of ILC2 responses, which is necessary for suppressing ILC2 expansion and maintaining homeostasis of the type 2 immune machinery (Wallrapp et al., 2019; Xu et al., 2019). Additionally, CGRP in concert with NMU is reported to promote IL-5 but constrain IL-13 expression (Nagashima et al., 2019). In the lung, nociceptors suppress protective immunity through the release of CGRP. Specifically, CGRP suppressed the recruitment and surveillance of neutrophils (Pinho-Ribeiro et al., 2018), and reduced lung γδ T cell numbers, which act as first responders to infection (Baral et al., 2018). On the contrary, pulmonary neuroendocrine cells (PNECs) are a kind of rare airway epithelial cells that reside near airway branches. CGRP secreted by PNECs enhances ILC2 activity and the production of cytokines (Sui et al., 2018).

In the intestine, precursor and immature NK cells exhibit tissue-resident signatures (Dogra et al., 2020). SP is released from nociceptors after stimulation through the neurokinin receptors NK1R, NK2R and NK3R and has been shown to regulate cell migration and proliferation to generate neurogenic inflammation (Seillet and Jacquelot, 2019). In the airways, lung nociceptor neurons release SP, and amplify T helper 2 cell influx and polarization via NK1R (Crosson et al., 2021). In skin, SP interacts with DCs and mast cells through the Mas-related G-protein coupled receptor member (MRGPR). Sensory neurons release SP, which acts through MRGPRA1 to induce CD301b+ DC migration to the draining lymph node (dLN). Migrated DCs initiate T helper-2 cell differentiation in the dLNs(Perner et al., 2020). One study reported that nociceptors induce the recruitment of neutrophils and monocytes, driving skin inflammation. Nociceptors promote the production of IL-23 by dermal DCs. IL-23 then acts on IL-23R+ γδT17 cells to promote the secretion of IL-17F and IL-22 (119). Nociceptors can also amplify skin inflammation by inducing the degranulation of mast cells that are contiguous to the nociceptors and activating MRGPRB2 on the mast cells (Serhan et al., 2019).

TAFA4 is a neuropeptide secreted by sensory neurons in skin. TAFA4 reduce inflammation and cell infiltration by inducing IL-10 production from Tim4+ dermal macrophages (Flayer and Sokol, 2021; Soler Palacios and Gutiérrez-González, 2022). TAFA4 can also affect the inflammatory mechanisms of other macrophage subsets, including CD206+ dermal macrophages and peritoneal macrophages (Hoeffel et al., 2021). In the lung, nociceptors can induce the production of VIP which in turn stimulates ILC2s to promote the occurrence of inflammation (Talbot et al., 2015; Seillet and Jacquelot, 2019).

In a word, sensory nerves function primarily in their widely distributed areas including skin, lung and gut. Neuropeptides secreted by sensory neurons play diverse roles in immune responses against various pathogens (Figure 1, Table 1).

3 Tissue-specific immune effects on nerves

Tissue-specific immune cells regulate nerves by releasing various factors such as cytokines, chemokines, small-molecule peptides and neurotrophic factors.

Among the tissue-specific immune cells, tissue-resident macrophages exhibit a rich regulatory role in different tissues. In adipose tissue, ATM expresses neurotrophic factors to promote white adipose tissue innervation (Xie et al., 2022). Also, ATM reportedly controls brown adipose tissue innervation (Wolf et al., 2017). In subcutaneous fat, a new subset of immune cells called cholinergic adipose macrophages (ChAMs) has been identified. ChAMs secrete ACh to regulate thermogenic activation via β2-AR (Knights et al., 2021). ATM can also mediate sympathetic neurons through ROBO1 receptor. Slit3 is a macrophage cytokine secreted by ATM. It binds to the ROBO1 receptor to stimulate Ca2+/calmodulin-dependent protein kinase II signaling and NE release, which enhances adipocyte thermogenesis (Wang et al., 2021). Additionally, MMs regulate peristaltic activity of the colon by secreting bone morphogenetic protein 2 (BMP2), which activates the expression of BMP receptor on enteric neurons. Enteric neurons, in turn, secrete a growth factor required for macrophage development called colony stimulatory factor 1 (CSF1), (Muller et al., 2014). Sympathetic neuron-associated macrophages are another previously undescribed population of resident macrophages that mediate NE clearance via SLC6A2 and MAOA expression (Pirzgalska et al., 2017). A nerve- and airway-associated macrophage (NAM) subset has been identified in the lung. Their closely associated nerves have sympathetic fibers. However, the relationship between NAMs and their associated nerves is unclear (Ural et al., 2020).

In the CNS, microglia perform negative feedback control of neuronal activity. The suppression of neuronal activation depends on the ability to sense and catabolize extracellular ATP of microglia (Badimon et al., 2020). ILC2s also play a part. In the lung, nociceptors can sense IL-5 released by activated immune cells, and IL-5 further induces VIP production. VIP then stimulates ILC2s, creating an inflammatory signaling loop that promotes allergic inflammation (Talbot et al., 2015; Seillet and Jacquelot, 2019). ACh is a broadly distributed signaling molecule that is not only produced by neurons, but also by numerous immune cells. Several immune cell types respond to ACh signaling and can also produce ACh directly (Cox et al., 2020).

4 Neuroendocrine tissue-specific immunity axis in cancer

It is generally accepted that tumor innervation correlates with tumor progression across multiple solid tumor types (Albo et al., 2011; Huang et al., 2014; Kappos et al., 2018; Renz et al., 2018). Specifically, autonomic nervous infiltration was discovered to affect the development and dissemination of breast cancer, head and neck carcinoma, prostate cancer, liver cancer, lung cancer, pancreatic cancer, cholangiocarcinoma, colorectal cancer, ovarian cancer, glioma and gastric cancer (Magnon et al., 2013; Zhao et al., 2014; Partecke et al., 2016; Pon et al., 2016; Zahalka et al., 2017; Faulkner et al., 2019; Sha et al., 2019). While sensory neurons were found to play a part in tumor development of melanoma and pancreatic cancer (Saloman et al., 2016; Prazeres et al., 2020). Meanwhile, high intra-tumoral nerve density is associated with poor prognosis and high recurrence in multiple cancers (Silverman et al., 2021). Studies have shown that the mechanism by which tumor

innervation affects cancer can be divided into three parts: direct stimulation of tumor proliferation, promotion of vascularization, and indirect modulation of tumors through immune changes. In this context, we discuss the effect of neuroendocrine regulation on tissue-specific immunity in the TME.

In breast cancer, sympathetic innervation accelerates tumor progression, while parasympathetic innervation decelerates tumor progression in the TME (26). The sympathetic nervous system affects stress-induced cancer behaviors, including initiation, progression and metastasis, by modulating tumor-associated immune cells via the NE-βAR signaling pathway in four ways. First, *β*-adrenergic signaling represses an effector phenotype of $CD8^+$ T cells in the TME. Reducing *β*-adrenergic signaling induced an immunologically active TME in tumor-bearing animal models (Bucsek et al., 2017). Second, parasympathetic innervation constrains the function of immune checkpoints. Sympathetic nerve denervation and parasympathetic neurostimulation reduced the level of PD-1, PD-L1, and FOXP3 on $CD4^+$ or $CD8^+$ T cells in murine models of breast cancer. This effect was also proved in human breast cancer samples (Kamiya et al., 2019). Next, the NE-βAR signaling pathway increased the infiltration of macrophages in the TME and induced the differentiation to a prometastatic M2 macrophage phenotype. Stress-induced NE-βAR signaling activation induced a metastasis to distant tissues in mouse breast cancer models (Sloan et al., 2010). Finally, NE-βAR signaling pathway promotes breast cancer metastasis by recruiting myeloid-derived suppressor cells (MDSCs). MDSCs are a population of cells with immunosuppressive phenotype. In murine models of breast cancer, chronic stress leaded to the elevation of MDSCs, accelerated breast cancer metastasis, and upregulated IL-6 expression and JAK/STAT3 signaling pathways (an et al., 2021). Another study reveals that the expression level of β2-AR on MDSCs increases with tumor growth. β2-adrenergic signaling increases oxidative phosphorylation, increase fatty acid oxidation, decreases glycolysis, and also increases autophagy and activates the arachidonic acid cycle (Mohammadpour et al., 2021). A recent study revealed that in brain metastases from breast cancer, neuronal exposure induces synaptic mediators and neurotransmitter signaling in tumors (Deshpande et al., 2022). This research suggests that neuroendocrine regulation in brain metastases seems to be more direct, and whether immune cells in brain metastases TME are regulated by neuroendocrine factors might be the direction of future research.

Likewise, in hepatocellular carcinoma, *β*-adrenergic signaling promotes tumor growth by mobilizing MDSCs to tumor tissues. In the hepatocellular carcinoma mouse models, stress enhanced tumor progression. Specifically, *β*-adrenergic signaling changed the spleen structure, and caused a redistribution of MDSCs to tumors. Also, the author found that splenectomy could constrain tumor growth and prevent increasement of macrophages in tumor tissues in stressed mice (Jiang et al., 2019). Another study pointed out the possible mechanism of this mobilization. The recruitment of MDSCs was modulated through *β*-adrenergic-activated CXCL5-CXCR2-Erk signaling cascades. Chronic stress upregulated the expression of CXCR2 and pErk1/2 in MDSCs, and the expression of CXCL5. *In vitro*, T-cell proliferation was obviously constrained in NE treated medium. *In vivo*, *β*-adrenergic blockade reversed the acceleration of tumor growth induced by chronic stress and CXCL5-CXCR2-Erk signaling pathway was suppressed (Cao et al., 2021).

Moreover, in prostate cancer, the density of sympathetic fibers in tumors is associated with poor clinical outcomes (Magnon et al., 2013). It was observed that prostate cancer patients with depression showed higher tumor-associated macrophage infiltration. NPY is co-released with NE in sympathetic nerves. Animal experiments revealed that NPY released from NE-treated prostate cancer cells promotes macrophage trafficking and IL-6 release, which subsequently activates the STAT3 signaling pathway (Cheng et al., 2019). Analyses of clinical prostate cancer samples have also suggested that elevated NPY promotes prostate cancer development and is associated with poor prognosis and therapy resistance (Rasiah et al., 2006; Ding et al., 2021). Additionally, NK cells are mobilized by epinephrine through the *β*-adrenergic signaling pathway, which can reduce the tumor initiation and recurrence. In mouse tumor models, epinephrine induced selective mobilization of IL-6-sensitive NK cells while IL-6 inhibited the infiltration and activation of NK cells in TME (Pedersen et al., 2016).

Yet, different perspectives have been raised in pancreatic cancer. Chronic stress is observed to increase pancreatic cancer progression and can be antagonized by *β*-AR blockade in some studies (Partecke et al., 2016; Renz et al., 2018). However, a recently published study demonstrated that sympathetic nerves ablation increases tumor growth and spread by increasing intra-tumoral CD163+ macrophage numbers. In the mouse model of pancreatic cancer, the sympathetic nerves exert a protective function during the early stage of tumor. The author mentioned that the converse response to sympathectomy in pancreatic cancer may be shaped by the particular TME (Guillot et al., 2022). Otherwise, ablation of sensory neurons of pancreatic cancer reportedly slows initiation and progression of cancer. However, the study did not mention its impact on tumor immunity (Saloman et al., 2016).

Sensory innervation was reported to constrain melanoma progression. Ablation of sensory nerves lead to worse outcomes in melanoma-bearing mice (Prazeres et al., 2020). In a recent study, Costa et al. (2021) informs that sensory neuron activity may slow the melanoma progression by inducing tumor immunosurveillance. Specifically, in sensory neuron-overactivated melanoma mice, the number of MDSCs and neutrophils significantly decreased, while tumor-infiltrating DCs, CD8 + T cells, CD4 + T cells, γδ T cells and NK cell was detected to increase. The expression of immune checkpoint

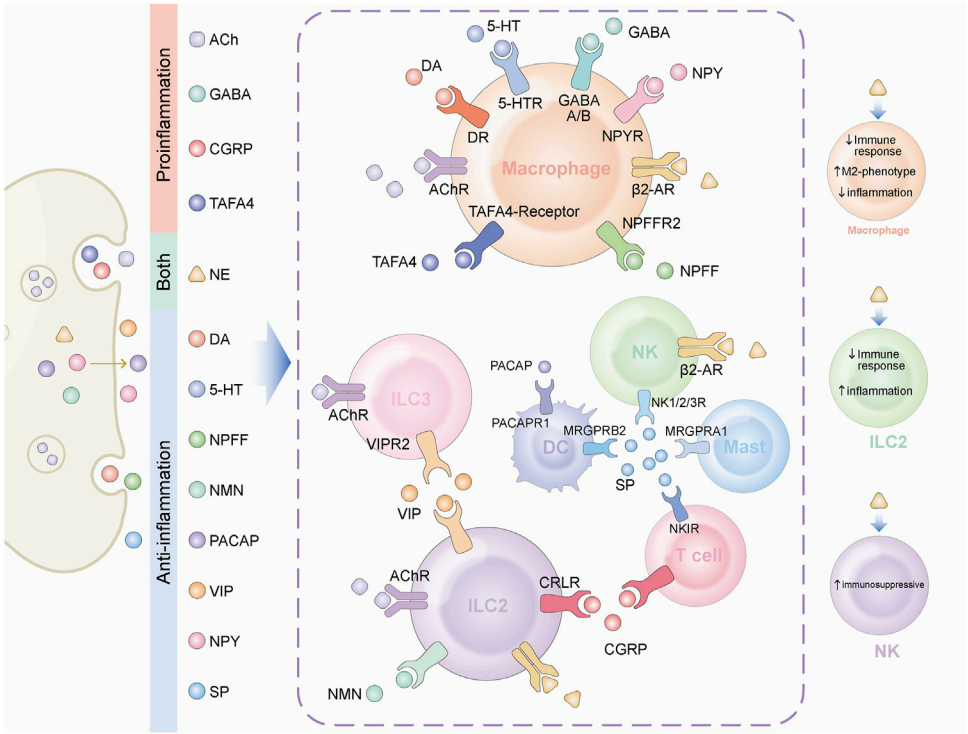

FIGURE 1
Neuroendocrine factors play various role in tissue immune regulation. A variety of neuroendocrine factors play regulatory roles in tissue-resident immune cells. The target cells and their receptors for different factors are displayed in the figure. On the left, neuroendocrine factors are classified according to their function. ACh, GABA, CGRP and TAFA4 mainly play a pro-inflammatory function. DA, 5-HT, NPFF, NMU, PACAP, VIP, NPY, and SP mainly play an anti-inflammatory function. NE plays different roles in different target cells. On the right side of the figure, we marked the functions of NE.

molecules such as PD-1 and CTLA-4 decreased. And it induces a Th17-immune response in the melanoma microenvironment. Furthermore, the author validated these results in a large human melanoma cohort. They found that SCN10A (encoding Nav1.8), a key gene of sensory neurons, has higher expression in patients with better prognosis. The enrichment of tumor-infiltrating immune cells showed an increase of DCs, CD8 + T cells, CD4 + T cells and NK cell in patients with better prognosis. Conversely, an earlier published study by Keskinov et al. (2016) reported that the dorsal root ganglia (DRG) of sensory nerves contribute to melanoma progression by recruiting MDSCs which help to create an oncogenic TME. Experimental results suggested that, *in vitro*, melanoma cells can activate DRG neurons and increase the expression of chemokines that attract MDSC. *In vivo*, in the presence of DRG cells, tumor growth was accelerated and the number of MDSC increased. To sum up, for sensory neuron modulation, Costa et al. (2021) used a designer drug to selectively activate or inhibit sensory neurons within the tumor *in vivo*, whereas Keskinov et al. (2016) used DRG cells injection in mice. Besides, Costa et al. (2021) focused on multiple kinds of cells involved in tumor immunity including T cells, DCs, and MDSCs, while Keskinov et al. (2016) focused only on the alteration of MDSCs. Furthermore, Costa et al. (2021) proved their findings through *in vivo* assays and bioinformatic validations, while Keskinov et al. (2016) validated through *in vitro* and *in vivo* assays. In our opinion, there are several possible reasons for the opposite results produced by the two studies. First, the nerve fibers at work are different. It speculates that it is inappropriate to choose DRG cells as a representation of sensory neurons. The DRG is an enlargement of the dorsal root that houses somata of sensory neurons. But DRGs can also receive signals from the autonomic nervous system by connecting to the sympathetic nerve via rami communicantes nerves (Esposito et al., 2019). Consequently, the tumor-promoting effects of DRGs mentioned in the study of Keskinov et al. (2016) is likely to be partially mediated by the autonomic nerves. The oncogenic role of the autonomic nervous system in multiple cancers has been mentioned above. Apart from that, the neuroendocrine factors at work are different. Signals from sensory neurons in normal tissues can be transmitted to immune cells through various factors such as CGRP, SP, and VIP. But neither study involved exploration on factors that signal between neurons and immune cells. Although Costa et al. (2021) observed an elevation of CGRP level after

TABLE 1 Summary of central and peripheral neuroendocrine factors regulating tissue specific immunity.

Ligand	Target tissue	Target cell	Receptor	Effect
NE	CNS	Microglia	β2-AR	↑ Aβ clearance (Kong et al. (2010)
				↓ Microglia response (Lechtenberg et al. (2019)
	Adipose tissue	ATM	β2-AR	↓ Inflammation (Tang et al. (2015)
				↓ Extracellular fatty acid (Petkevicius et al. (2021a)
				indirect effect through adipocyte (Petkevicius et al. (2021b)
		ILC2	β2-AR	Indirect effect through ASC (Cardoso et al. (2021); Shan et al. (2021)
	Intestine	MM	β2-AR	↑ M2 phenotype (Gabanyi et al. (2016)
				↑ Neuroprotection (Matheis et al. (2020)
		ILC2	β2-AR	↑ Type 2 inflammation (Moriyama et al. (2018)
				↓ Innate immune response (Willemze et al. (2019)
		Immune	β2-AR	Indirect effect through endothelium (Schiller et al. (2021)
	liver	iNKT	β2-AR	↑ Immunosuppressive function (Bendelac et al. (2007); Wong et al. (2011)
ACh	CNS	Microglia	mAChR	↑Chemotaxis and phagocytosis (Pannell et al. (2016)
				↓ Inflammation (Wang et al. (2003); Li et al. (2019)
			α7nAChR	↑ M2 phenotype (Zhang et al. (2017)
				↑ Aβ clearance (Hoskin et al. (2019)
	Llung	ilc2	α7nAChR	↓Type 2 inflammation (Galle-Treger et al. (2016); Pavón-Romero et al. (2021)
	Liver	Macrophage	AChR	↑Phagocytosis and secretion (Fonseca et al. (2019)
				↑ FoxM1, ↑ liver regeneration (Izumi et al. (2018)
	Intestine	MM	α7nAChR	↓ Inflammation (Matteoli et al. (2014); Yang et al. (2021)
		ILC2	AChR	↓Type 2 inflammation (Chu et al. (2021)
		ILC3	AChR	↑ PCTR biosynthetic pathway (Dalli et al. (2017)
	Spleen, pancreas	Macrophage	α7nAChR	↓ Inflammation (Zhang et al. (2020)
DA	CNS	Microglia	D1R	↑ Microglial migration (Stolero and Frenkel (2021)
			D2R	↑ ROS and NO (Stolero and Frenkel (2021)
				↑Inflammation (Stolero and Frenkel (2021)
			DR	↑ Extracellular trap in microglia (Agrawal et al. (2021)
5-HT	CNS	Microglia	5-HTR	↑Inflammation ↑IL-6 (Quintero-Villegas and Valdes-Ferrer, 2019)
GABA	CNS	Microglia	$GABA_A$/$GABA_B$	↓ Microglial neurotoxicity (Lee (2013); Crowley et al. (2016)
				↓ Inflammation (Lee (2013); Crowley et al. (2016)
NPFF	Adipose tissue	ATM	NPFFR2	↑ M2 phenotype (Waqas et al. (2017)
				↑ATM proliferation (Waqas et al. (2017)
NMU	Lung	ILC2	NMUR1	↑Type 2 inflammation (Wallrapp et al. (2017); Chen et al. (2021)
				↑γδ T cell, ↑IL-17A (Wallrapp et al. (2017); Chen et al. (2021)
	Intestine	ILC2	NMUR1	↑Type 2 inflammation (Klose et al. (2017)
				↑ IL-10 (Bando et al., 2020)
PACAP	Skin	DC	PACAPR1	↑ Contact hypersensitivity (Yamamoto et al., 2021)
				↑ CCR7, ↑ CXCR4 (Yamamoto et al., 2021)
VIP	Lung	ILC2	VIPR2	↑Type 2 inflammation (Talbot et al. (2015); Seillet and Jacquelot (2019))
	Intestine	ILC3	VIPR2	↑ IL-22, ↑ barrier function of the epithelium (Seillet et al. (2020); Talbot et al. (2020))

(Continued on following page)

TABLE 1 (*Continued*) Summary of central and peripheral neuroendocrine factors regulating tissue specific immunity.

Ligand	Target tissue	Target cell	Receptor	Effect
NPY	CNS	Microglia	NPYR	↑ Inflammation, ↓ microglial activation and phagocytosis (Gonçalves et al. (2012); Pain et al. (2019); Chen et al. (2020)
	Spleen	Immune	NPYR	↓ Immune responses (Yu et al. (2022)
CGRP	Skin, lung and intestine	ILC2	CRLR	↓ Type 2 inflammation (Wallrapp et al. (2019); Xu et al. (2019)
				↑ IL-5, ↓IL-13 (Nagashima et al. (2019)
	Lung	T cell	CRLR	↓ protective immunity (Baral et al. (2018); Pinho-Ribeiro et al. (2018)
				↓ γδ T cell number (Baral et al. (2018); Pinho-Ribeiro et al. (2018)
				↓ Neutrophil (Baral et al. (2018); Pinho-Ribeiro et al. (2018)
CGRP (PNEC)	Lung	ILC2	CRLR	↑Type 2 inflammation (Sui et al. (2018)
				↑ ILC2 activity (Sui et al. (2018)
SP	Intestine	NK cell	NK1R/NK2R/ NK3R	↑ Inflammation (Talbot et al. (2015); Seillet and Jacquelot (2019)
	Lung	T cell	NK1R	↑ T helper 2 cell influx and polarization (Crosson et al. (2021)
	Skin	DC	MRGPRA1	↑ DC migration, ↑ T helper 2 cell differentiation (Perner et al. (2020)
				↑ IL-23, ↑neutrophil, ↑monocytes (Riol-Blanco et al. (2014)
				↑ Inflammation (Riol-Blanco et al. (2014)
		Mast cell	MRGPRB2	↑ Mast cells degranulation (Serhan et al. (2019)
				↑ Inflammation (Serhan et al. (2019)
TAFA4	Skin	Macrophage	unknown	↑ IL-10, ↓ inflammation (Hoeffel et al. (2021)

DC, dendritic cell; ILC, innate lymphoid cell; CNS, central nervous system; NE, norepinephrine; AR, adrenergic receptor; ATM, adipose tissue macrophage; ASC, adipose stem cell; MM, muscularis macrophage; iNKT, invariant natural killer T; ACh, acetylcholine; mAChR: metabotropic muscarinic ACh, receptor; nAChR: ionotropic nicotinic ACh, receptor; 5-HT: serotonin; GABA, gamma-aminobutyric acid; DA, dopamine; DR, dopamine receptor; 5-HTR: 5-HT, receptor; NMU, neuromedin U; SP, substance P; CGRP, calcitonin gene-related peptide; PNEC, pulmonary neuroendocrine cell; PACAP, pituitary adenylate cyclase-activating polypeptide; VIP, vasoactive intestinal peptide; NPFF, neuropeptide FF; NPY, neuropeptide Y; CRLR, receptor calcitonin receptor like receptor; MRGPR, Mas-related G-protein coupled receptor member.

sensory nerve activation, this mechanism has not been confirmed. Whether sensory nerves also interact with immune cells through these substances in the melanoma microenvironment might be a direction of future research. We believe that further explorations of neuroendocrine factors involved in immune regulation in melanoma may answer the reasons for the opposite conclusions of the two studies above.

An analysis of clinical samples reveals that GABA is associated with poor prognosis in lung cancer and colon adenocarcinoma (Huang et al., 2022). GABA is reported to have an anti-tumor immunity function through two different pathways. One study informed GABA promotes the differentiation from monocyte to anti-inflammatory macrophages that secrete IL-10. GABA also inhibit $CD8^+$ T cell killer function (Zhang et al., 2021). Another study suggested GABA activates the $GABA_B$ receptor to stimulate tumor cell proliferation and suppress $CD8^+$ T cell infiltration in TME. In mouse models, targeting $GABA_B$ overcomed resistance to anti-PD-1 immune checkpoint blockade therapy (Huang et al., 2022).

Regarding neuropeptides, a systematic review found evidence that the elevation of SP and NK-1R are oncogenic events in head and neck carcinogenesis and probably act in the early stages of tumorigenesis (Gonzalez-Moles et al., 2021). Proline rich polypeptide 1, (PRP-1) is a neuropeptide secreted by the brain neurosecretory cells which causes inhibition of chondrosarcoma cell growth (Galoyan, 2000; Galoian et al., 2009). A study proves that PRP-1 functions via toll like receptor family TLR1/2, TLR6 and mucin MUC5B which are responsible for innate immunity pattern recognition. This result suggests that the anti-tumor effect of PRP-1 might be related to immune cells in TME. But the author also suggested that the receptors predominantly located in the tumor nucleus (Galoian et al., 2018). Another research demonstrated that PRP-1 can also inhibit cancer stem cell proliferation in chondrosarcoma (Granger et al., 2020). In addition, somatostatin analog (SSA) is the analog of SST which is now widely used in the treatment of neuroendocrine neoplasms. Long-acting repeatable of SSA decrease the level of total regulatory T cells and MDSCs, as well as the expression of PD1, CTLA4 and ENTPD1 in neuroendocrine neoplasms which exert an anti-tumor immunosurveillance function (von Arx et al., 2020). SSA was also reported to decrease cell proliferation and tumor growth in multiple cancers, such as lung cancer, breast cancer, colon carcinoma and endometrial carcinomas (Kahán et al., 1999; Engel et al., 2005; Treszl et al., 2009; Hohla et al., 2010). However, if these oncogenic effects are associated with tumor-

associated immune cells has not yet been discussed. The function of neuropeptides in tumor-specific immunity requires further research.

In brief, under the regulation of neuroendocrine factors, some components of TME play the role of immunosurveillance, such as effective T cells, NK cells, and the immune checkpoint molecules produced by them. Yet some other components play the opposite pro-tumor effects including MDSCs, regulatory T cells and tumor-associated macrophages. Immunosurveillance effects are negatively regulated by β-adrenergic signaling in breast, prostate and liver cancers. In lung and colon cancer, immunosurveillance is negative regulated by sensory nerves. Pancreatic cancer immunosurveillance is positive regulated by β-adrenergic signaling, however negative regulated by sensory nerves. Melanoma is mainly mediated by sensory nerves, but the direction of regulation is controversial. Lastly, immunosurveillance in neuroendocrine neoplasm is positive regulated by SST.

From the perspective of therapy, there are also many researches emerged in recent years. Since tumor innervation plays an active role in cancer initiation and progression, neuron ablation emerged as a treatment strategy mentioned a lot. Some studies demonstrate that surgical or pharmacological denervation attenuate tumor growth in a tissue-specific manner. For instance, sensory neuron ablation is proved to favor melanoma progression, but slows the initiation and progression of pancreatic cancer (Saloman et al., 2016; Prazeres et al., 2020). And denervation suppresses gastric and breast tumorigenesis (Zhao et al., 2014). Although method to manoeuvre intra-tumoral innervations is not yet mature for clinical treatment, it provides a promising new avenue for anti-cancer therapy (Prazeres et al., 2020).

Apart from neuron ablation, antagonize β2-AR seems to be a more mature approach to block the neuroendocrine signal. Propranolol is a nonselective beta blocker used for cardiovascular indications for decades (Srinivasan, 2019). However, in recent years, propranolol has been applicated in the treatment of some kinds of tumors successfully, such as angiosarcoma (Wagner et al., 2018). Retrospective studies have observed a correlation between β blocker usage and increased overall survival among cancer patients (Nagaraja et al., 2013). Blockade of β-AR reduces tumor progression and upregulates the response to anti-CTLA4 therapy contributes to the formation of an immunosuppressive TME. In mouse fibrosarcoma models, propranolol increased the number of T cells, reduced the number of intra-tumoral MDSCs and altered the gene expression profile of tumor-associated macrophages significantly in the TME. Similar phenomenon was observed in murine models of colon cancer (Fjæstad et al., 2022). Specifically, one study demonstrates that NE-βAR pathway helps to maintain the level and the suppressive function of MDSCs. In murine breast cancer models, β2-adrenergic signaling modulated the expression of immunosuppressive molecules such as arginase-I and PD-L1 and suppressed the proliferation of T cells. Also, the author pointed out that the regulatory functions of β2-AR signaling in MDSCs is activated by STAT3 phosphorylation. The β2-AR-mediated increase in MDSC is dependent on Fas-FasL interactions. Besides, the immunosuppressive function of MDSCs can be decelerated by β-AR antagonists (Mohammadpour et al., 2019). Propranolol was also reported to reduce stress-induced elevation of regulatory T cells in breast cancer patients (Zhou et al., 2016). Another study report that β2-adrenergic signaling contributes to an exhausted phenotype in T cells and induce metabolic dysfunction in the TME. In mouse melanoma and colon cancer models, it is observed that by using propranolol, tumor growth rate was slowed accompanied by a significantly decrease of tumor-infiltrating T cells that express exhaustion related genes and an increase in progenitor exhausted T cells. Also, β-AR blockade in mice increases oxidative phosphorylation and glycolysis in tumor-infiltrating lymphocyte (Qiao et al., 2021). Therefore, β-AR antagonists such as propranolol could be a potentially efficacious therapy approach of tumors in the future.

In traditional Chinese medicine, moxibustion, a treatment modality with a long history, has been used to treat cancer-related symptoms in clinical practice for years. However, few people have explored its mechanism of action. Interestingly, a recent study informed that grain-sized moxibustion significantly reduced tumor growth in lung cancer. Grain-sized moxibustion performed at the acupoint of Zusanli promotes anti-tumor immunity of NK cell by inhibiting adrenergic signaling. The acupoint of Zusanli is an important acupoint involved in the interaction between neuroendocrine systems and immune cells (Zhang et al., 2018). *In vitro*, grain-sized moxibustion increased the proportion, infiltration and activation of NK cells, yet it didn't affect T cells. Additionally, the grain-sized moxibustion mediated NK cells activation can be reduced by β-blocker treatment (Hu et al., 2021). To sum up, we describe the role of moxibustion in neuroendocrine regulation of lung cancer, which indicated that traditional Chinese medicine is still an ancient but promising therapeutic regimen for cancers.

5 Conclusion

Many studies have shown that tumor innervation is associated with tumor initiation, progression and a worse prognosis, and neuroendocrine regulation is the main mechanism by which nerves modulate tumor cells and other non-nerve cells. Several studies have focused on the neuroendocrine regulation of tumor cells; however, immune cells are also a vital part of the TME. Various tumor-associated immune cells have complex functions that can greatly impact tumor development, prognosis and treatment. Therefore, this review discusses the existing research on the neuroendocrine regulation of tissue-specific immunity. The

regulatory mechanisms of different immune cell types in various tissues were summarized according to the classification of regulatory factors. Adrenergic and cholinergic signaling are two common regulatory pathways. In central and peripheral tissues, sympathetic nerves mainly modulate tissue-resident immune cells through the NE-β2A pathway, exerting anti-inflammatory and immunosuppressive roles. The parasympathetic nervous system regulates resident macrophages and ILCs mainly by secreting ACh from the vagus nerve to promote macrophage activity and anti-inflammatory effects. Other neurotransmitters are secreted primarily in the CNS and regulate microglia activity. Neuropeptides are another type of neuroendocrine factor that is secreted by central, autonomic and sensory neurons. These neuropeptides have various functions in tissues. Furthermore, many tissue-resident immune cells secrete cytokines and other factors to regulate nerves. We also summarized the available studies on neuroendocrine immunomodulatory regulation in different kinds of tumors. Some studies focused on autonomic nervous regulation in cancers and have helped to explain why chronic stress promotes tumor development from an immune aspect. Yet, many tumors are also regulated by sensory nerves and other factors. After that we discuss the neuroendocrine regulation of tumor immunity from the perspective of immune cell function and tumor therapy.

There are still knowledge gaps in the field of neuroendocrine regulation of immune cells in the TME. Many receptors of neuroendocrine factors are expressed on macrophages, T cells, and other cells that play important roles in tumor immunity. Some regulatory pathways have been studied in nontumor tissues, while their roles in tumors have not been studied. Moreover, a variety of cancers have been reported to be affected by tumor innervation, but the neuron-immune interactions in most cancers remain unclear. Thus, we also covered some articles that only discussed neuroendocrine regulation from the perspective of TME. Although they did not mention the neuroendocrine regulation of immune cells, we believe that some of them can provide ideas for the following research.

Due to the tissue specificity of immune cells, individualized immunotherapy in tumors may bring greater benefits to patients. For example, some studies have found that β2-AR blockade can inhibit NE-mediated tumor progression, but it has not been applied clinically in most kinds of tumors. Therefore, enriching the research focusing on these aspects may provide new insights for personalized tumor immunotherapy.

Author contributions

QW, BL, and S-QL are responsible for collecting and collating documents. S-QL and BL are responsible for writing this review, while QW and S-RS are responsible for the revision, and J-JL and SS are responsible for editing and submission. All authors read and approved the final manuscript.

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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

Abad, C., and Tan, Y. V. Immunomodulatory roles of PACAP and VIP: Lessons from knockout mice. *J. Mol. Neurosci.* 2018;66(1):102–113.doi:10.1007/s12031-018-1150-y

Agrawal, I., Sharma, N., Saxena, S., Arvind, S., Chakraborty, D., Chakraborty, D. B., et al. Dopamine induces functional extracellular traps in microglia. *iScience.* 2021;24(1):101968.doi:10.1016/j.isci.2020.101968

Albo, D., Akay, C. L., Marshall, C. L., Wilks, J. A., Verstovsek, G., Liu, H., et al. Neurogenesis in colorectal cancer is a marker of aggressive tumor behavior and poor outcomes. *Cancer.* 2011;117(21):4834–4845.doi:10.1002/cncr.26117

Aloe, L., Rocco, M. L., Balzamino, B. O., and Micera, A. Nerve growth factor: Role in growth, differentiation and controlling cancer cell development. *J. Exp. Clin. Cancer Res.* 2016;35(1):116.doi:10.1186/s13046-016-0395-y

an, J., Feng, L., Ren, J., Li, Y., Li, G., Liu, C., et al. Chronic stress promotes breast carcinoma metastasis by accumulating myeloid-derived suppressor cells through

activating β-adrenergic signaling. *Oncoimmunology*. 2021;10(1):2004659.doi:10.1080/2162402x.2021.2004659

Badimon, A., Strasburger, H. J., Ayata, P., Chen, X., Nair, A., Ikegami, A., et al. Negative feedback control of neuronal activity by microglia. *Nature*. 2020;586(7829):417–423.doi:10.1038/s41586-020-2777-8

Bando, J. K., Gilfillan, S., Di Luccia, B., Fachi, J. L., Sécca, C., Cella, M., et al. ILC2s are the predominant source of intestinal ILC-derived IL-10. *J. Exp. Med.* 2020;217(2).doi:10.1084/jem.20191520

Baral, P., Umans, B. D., Li, L., Wallrapp, A., Bist, M., Kirschbaum, T., et al. Nociceptor sensory neurons suppress neutrophil and γδ T cell responses in bacterial lung infections and lethal pneumonia. *Nat. Med.* 2018;24(4):417–426.doi:10.1038/nm.4501

Bejarano, L., Jordão, M. J. C., and Joyce, J. A. Therapeutic targeting of the tumor microenvironment. *Cancer Discov*. 2021;11(4):933–959.doi:10.1158/2159-8290.cd-20-1808

Bendelac, A., Savage, P. B., and Teyton, L. The biology of NKT cells. *Annu. Rev. Immunol*. 2007;25:297–336.doi:10.1146/annurev.immunol.25.022106.141711

Bonini, J. A., Jones, K. A., Adham, N., Forray, C., Artymyshyn, R., Durkin, M. M., et al. Identification and characterization of two G protein-coupled receptors for neuropeptide FF. *J. Biol. Chem*. 2000;275(50):39324–39331.doi:10.1074/jbc.m004385200

Bucsek, M. J., Qiao, G., MacDonald, C. R., Giridharan, T., Evans, L., Niedzwecki, B., et al. β-Adrenergic signaling in mice housed at standard temperatures suppresses an effector phenotype in CD8+ T cells and undermines checkpoint inhibitor therapy. *Cancer Res*. 2017;77(20):5639–5651.doi:10.1158/0008-5472.can-17-0546

Campos, X., Muñoz, Y., Selman, A., Yazigi, R., Moyano, L., Weinstein-Oppenheimer, C., et al. Nerve growth factor and its high-affinity receptor trkA participate in the control of vascular endothelial growth factor expression in epithelial ovarian cancer. *Gynecol. Oncol*. 2007;104(1):168–175.doi:10.1016/j.ygyno.2006.07.007

Cao, M., Huang, W., Chen, Y., Li, G., Liu, N., Wu, Y., et al. Chronic restraint stress promotes the mobilization and recruitment of myeloid-derived suppressor cells through β-adrenergic-activated CXCL5-CXCR2-Erk signaling cascades. *Intl J. Cancer*. 2021;149(2):460–472.doi:10.1002/ijc.33552

Cardoso, F., Klein Wolterink, R. G. J., Godinho-Silva, C., Domingues, R. G., Ribeiro, H., da Silva, J. A., et al. Neuro-mesenchymal units control ILC2 and obesity via a brain-adipose circuit. *Nature*. 2021;597(7876):410–414.doi:10.1038/s41586-021-03830-7

Cardoso, V., Chesné, J., Ribeiro, H., García-Cassani, B., Carvalho, T., Bouchery, T., et al. Neuronal regulation of type 2 innate lymphoid cells via neuromedin U. *Nature*. 2017;549(7671):277–281.doi:10.1038/nature23469

Chen, W., Lai, D., Li, Y., Wang, X., Pan, Y., Fang, X., et al. Neuronal-activated ILC2s promote IL-17a production in lung γδ T cells during sepsis. *Front. Immunol*. 2021;12:670676.doi:10.3389/fimmu.2021.670676

Chen, W. C., Liu, Y. B., Liu, W. F., Zhou, Y. Y., He, H. F., and Lin, S. Neuropeptide Y is an immunomodulatory factor: Direct and indirect. *Front. Immunol*. 2020;11:580378.doi:10.3389/fimmu.2020.580378

Cheng, Y., Tang, X. Y., Li, Y. X., Zhao, D. D., Cao, Q. H., Wu, H. X., et al. Depression-induced neuropeptide Y secretion promotes prostate cancer growth by recruiting myeloid cells. *Clin. Cancer Res*. 2019;25(8):2621–2632.doi:10.1158/1078-0432.ccr-18-2912

Chiu, I. M., Heesters, B. A., Ghasemlou, N., Von Hehn, C. A., Zhao, F., Tran, J., et al. Bacteria activate sensory neurons that modulate pain and inflammation. *Nature*. 2013;501(7465):52–57.doi:10.1038/nature12479

Chu, C., Parkhurst, C. N., Zhang, W., Zhou, L., Yano, H., Arifuzzaman, M., et al. The ChAT-acetylcholine pathway promotes group 2 innate lymphoid cell responses and anti-helminth immunity. *Sci. Immunol*. 2021;6(57).doi:10.1126/sciimmunol.abe3218

Cording, S., Medvedovic, J., Aychek, T., and Eberl, G. Innate lymphoid cells in defense, immunopathology and immunotherapy. *Nat. Immunol*. 2016;17(7):755–757.doi:10.1038/ni.3448

Costa, P. A. C., Silva, W. N., Prazeres, P., Picoli, C. C., Guardia, G. D. A., Costa, A. C., et al. Chemogenetic modulation of sensory neurons reveals their regulating role in melanoma progression. *Acta Neuropathol. Commun*. 2021;9(1):183.doi:10.1186/s40478-021-01273-9

Cox, M. A., Bassi, C., Saunders, M. E., Nechanitzky, R., Morgado-Palacin, I., Zheng, C., et al. Beyond neurotransmission: Acetylcholine in immunity and inflammation. *J. Intern Med*. 2020;287(2):120–133.doi:10.1111/joim.13006

Crosson, T., Wang, J. C., Doyle, B., Merrison, H., Balood, M., Parrin, A., et al. FcεR1-expressing nociceptors trigger allergic airway inflammation. *J. Allergy Clin. Immunol*. 2021;147(6):2330–2342.doi:10.1016/j.jaci.2020.12.644

Crowley, T., Cryan, J. F., Downer, E. J., and O'Leary. Inhibiting neuroinflammation: The role and therapeutic potential of GABA in neuro-immune interactions. *Brain, Behav. Immun*. 2016;54:260–277.doi:10.1016/j.bbi.2016.02.001

Dalli, J., Colas, R. A., Arnardottir, H., and Serhan, C. N. Vagal regulation of group 3 innate lymphoid cells and the immunoresolvent PCTR1 controls infection resolution. *Immunity*. 2017;46(1):92–105.doi:10.1016/j.immuni.2016.12.009

Deshpande, K., Martirosian, V., Nakamura, B. N., Iyer, M., Julian, A., Eisenbarth, R., et al. Neuronal exposure induces neurotransmitter signaling and synaptic mediators in tumors early in brain metastasis. *Neuro Oncol*. 2022;24(6):914–924.doi:10.1093/neuonc/noab290

Dhabhar, F. S. Effects of stress on immune function: The good, the bad, and the beautiful. *Immunol. Res*. 2014;58(2-3):193–210.doi:10.1007/s12026-014-8517-0

Dhabhar, F. S. Enhancing versus suppressive effects of stress on immune function: Implications for immunoprotection and immunopathology. *Neuroimmunomodulation*. 2009;16(5):300–317.doi:10.1159/000216188

Dhabhar, F. S., Malarkey, W. B., Neri, E., and McEwen, B. S. Stress-induced redistribution of immune cells-from barracks to boulevards to battlefields: A tale of three hormones - curt richter award winner. *Psychoneuroendocrinology*. 2012;37(9):1345–1368.doi:10.1016/j.psyneuen.2012.05.008

Dimitrijevic, M., and Stanojevic, S. The intriguing mission of neuropeptide Y in the immune system. *Amino Acids*. 2013;45(1):41–53.

Ding, Y., Lee, M., Gao, Y., Bu, P., Coarfa, C., Miles, B., et al. Neuropeptide Y nerve paracrine regulation of prostate cancer oncogenesis and therapy resistance. *Prostate*. 2021;81(1):58–71.doi:10.1002/pros.24081

Dogra, P., Rancan, C., Ma, W., Toth, M., Senda, T., Carpenter, D. J., et al. Tissue determinants of human NK cell development, function, and residence. *Cell*. 2020;180(4):749–763.doi:10.1016/j.cell.2020.01.022

Engel, J. B., Schally, A. V., Halmos, G., Baker, B., Nagy, A., and Keller, G. Targeted therapy with a cytotoxic somatostatin analog, AN-238, inhibits growth of human experimental endometrial carcinomas expressing multidrug resistance protein MDR-1. *Cancer*. 2005;104(6):1312–1321.doi:10.1002/cncr.21327

Esposito, M. F., Malayil, R., Hanes, M., and Deer, T. Unique characteristics of the dorsal root ganglion as a target for neuromodulation. *Pain Med*. 2019;20(1):S23–S30.doi:10.1093/pm/pnz012

Faulkner, S., Jobling, P., March, B., Jiang, C. C., and Hondermarck, H. Tumor neurobiology and the war of nerves in cancer. *Cancer Discov*. 2019;9(6):702–710.doi:10.1158/2159-8290.cd-18-1398

Fjæstad, K. Y., Rømer, A. M. A., Goitea, V., Johansen, A. Z., Thorseth, M. L., Carretta, M., et al. Blockade of beta-adrenergic receptors reduces cancer growth and enhances the response to anti-CTLA4 therapy by modulating the tumor microenvironment. *Oncogene*. 2022;41(9):1364–1375.doi:10.1038/s41388-021-02170-0

Flayer, C. H., and Sokol, C. L. No pain, no gain: Sensory neurons heal a sunburn. *Immunity*. 2021;54(7):1374–1376.doi:10.1016/j.immuni.2021.06.011

Fonseca, R. C., Bassi, G. S., Brito, C. C., Rosa, L. B., David, B. A., Araújo, A. M., et al. Vagus nerve regulates the phagocytic and secretory activity of resident macrophages in the liver. *Brain, Behav. Immun*. 2019;81:444–454.doi:10.1016/j.bbi.2019.06.041

Gabanyi, I., Muller, P. A., Feighery, L., Oliveira, T. Y., Costa-Pinto, F. A., and Mucida, D. Neuro-immune interactions drive tissue programming in intestinal macrophages. *Cell*. 2016;164(3):378–391.doi:10.1016/j.cell.2015.12.023

Galle-Treger, L., Suzuki, Y., Patel, N., Sankaranarayanan, I., Aron, J. L., Maazi, H., et al. Nicotinic acetylcholine receptor agonist attenuates ILC2-dependent airway hyperreactivity. *Nat. Commun*. 2016;7:13202.doi:10.1038/ncomms13202

Galoian, K., Abrahamyan, S., Chailyan, G., Qureshi, A., Patel, P., Metser, G., et al. Toll like receptors TLR1/2, TLR6 and MUC5B as binding interaction partners with cytostatic proline rich polypeptide 1 in human chondrosarcoma. *Int. J. Oncol*. 2018;52(1):139–154.doi:10.3892/ijo.2017.4199

Galoian, K., Scully, S., McNamara, G., Flynn, P., and Galoyan, A. Antitumorigenic effect of brain proline rich polypeptide-1 in human chondrosarcoma. *Neurochem. Res*. 2009;34(12):2117–2121.doi:10.1007/s11064-009-0009-6

Galoyan, A. Neurochemistry of brain neuroendocrine immune system: Signal molecules. *Neurochem. Res*. 2000;25(9-10):1343–1355.doi:10.1023/a:1007656431612

Goldfarb, Y., Sorski, L., Benish, M., Levi, B., Melamed, R., and Ben-Eliyahu, S. Improving postoperative immune status and resistance to cancer metastasis. *Ann. Surg*. 2011;253(4):798–810.doi:10.1097/sla.0b013e318211d7b5

Gonçalves, J., Ribeiro, C. F., Malva, J. O., and Silva, A. P. Protective role of neuropeptide Y Y2receptors in cell death and microglial response following methamphetamine injury. *Eur. J. Neurosci*. 2012;36(9):3173–3183.doi:10.1111/j.1460-9568.2012.08232.x

Gonzalez-Moles, M. A., Ramos-Garcia, P., and Esteban, F. Significance of the overexpression of substance P and its receptor NK-1R in head and neck carcinogenesis: A systematic review and meta-analysis. *Cancers (Basel)*. 2021; 13(6).doi:10.3390/cancers13061349

Granger, C. J., Hoyt, A. K., Moran, A., Becker, B., Sedani, A., Saigh, S., et al. Cancer stem cells as a therapeutic target in 3D tumor models of human chondrosarcoma: An encouraging future for proline rich polypeptide-1. *Mol. Med. Rep*. 2020;22(5):3747–3758.doi:10.3892/mmr.2020.11480

Guillot, J., Dominici, C., Lucchesi, A., Nguyen, H. T. T., Puget, A., Hocine, M., et al. Sympathetic axonal sprouting induces changes in macrophage populations and protects against pancreatic cancer. *Nat. Commun*. 2022;13(1):1985.doi:10.1038/s41467-022-29659-w

Gyoneva, S., and Traynelis, S. F. Norepinephrine modulates the motility of resting and activated microglia via different adrenergic receptors. *J. Biol. Chem*. 2013; 288(21):15291–15302.doi:10.1074/jbc.m113.458901

Gysler, S. M., and Drapkin, R. Tumor innervation: Peripheral nerves take control of the tumor microenvironment. *J. Clin. Invest*. 2021;131(11).doi:10.1172/JCI147276

Halder, N., and Lal, G. Cholinergic system and its therapeutic importance in inflammation and autoimmunity. *Front. Immunol*. 2021;12:660342.doi:10.3389/fimmu.2021.660342

Hanahan, D., and Coussens, L. M. Accessories to the crime: Functions of cells recruited to the tumor microenvironment. *Cancer Cell*. 2012;21(3):309–322.doi:10.1016/j.ccr.2012.02.022

Hoeffel, G., Debroas, G., Roger, A., Rossignol, R., Gouilly, J., Laprie, C., et al. Sensory neuron-derived TAFA4 promotes macrophage tissue repair functions. *Nature*. 2021;594(7861):94–99.doi:10.1038/s41586-021-03563-7

Hohla, F., Buchholz, S., Schally, A. V., Krishan, A., Rick, F. G., Szalontay, L., et al. Targeted cytotoxic somatostatin analog AN-162 inhibits growth of human colon carcinomas and increases sensitivity of doxorubicin resistant murine leukemia cells. *Cancer Lett*. 2010;294(1):35–42.doi:10.1016/j.canlet.2010.01.018

Hoskin, J. L., Al-Hasan, Y., and Sabbagh, M. N. Nicotinic acetylcholine receptor agonists for the treatment of alzheimer's dementia: An update. *Nicotine Tob. Res*. 2019;21(3):370–376.doi:10.1093/ntr/nty116

Hoyer, D., and Bartfai, T. Neuropeptides and neuropeptide receptors: Drug targets, and peptide and non-peptide ligands: A tribute to Prof.Dieter seebach. *Chem. Biodivers*. 2012;9(11):2367–2387.doi:10.1002/cbdv.201200288

Hu, D., Shen, W., Gong, C., Fang, C., Yao, C., Zhu, X., et al. Grain-sized moxibustion promotes NK cell antitumour immunity by inhibiting adrenergic signalling in non-small cell lung cancer. *J. Cell Mol. Med*. 2021;25(6): 2900–2908.doi:10.1111/jcmm.16320

Huang, D., Su, S., Cui, X., Shen, X., Zeng, Y., Wu, W., et al. Nerve fibers in breast cancer tissues indicate aggressive tumor progression. *Med. Baltim*. 2014;93(27): e172.doi:10.1097/md.0000000000000172

Huang, Wang Y., Wang, J. W., Thompson, T., Yin, P. B., Alexander, D., Qin, et al. Cancer-cell-derived GABA promotes β-catenin-mediated tumour growth and immunosuppression. *Nat. Cell Biol*. 2022;24(2):230–241.doi:10.1038/s41556-021-00820-9

Huh, J. R., and Veiga-Fernandes, H. Neuroimmune circuits in inter-organ communication. *Nat. Rev. Immunol*. 2020;20(4):217–228.doi:10.1038/s41577-019-0247-z

Izumi, T., Imai, J., Yamamoto, J., Kawana, Y., Endo, A., Sugawara, H., et al. Vagus-macrophage-hepatocyte link promotes post-injury liver regeneration and whole-body survival through hepatic FoxM1 activation. *Nat. Commun*. 2018;9(1): 5300.doi:10.1038/s41467-018-07747-0

Jiang, J., Bai, J., Qin, T., Wang, Z., and 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(10):5901–5910.doi:10.1111/jcmm.15265

Jiang, S. H., Hu, L. P., Wang, X., Li, J., and Zhang, Z. G. Neurotransmitters: Emerging targets in cancer. *Oncogene*. 2020;39(3):503–515.doi:10.1038/s41388-019-1006-0

Jiang, W., Li, Y., Li, Z. Z., Sun, J., Li, J. W., Wei, W., et al. Chronic restraint stress promotes hepatocellular carcinoma growth by mobilizing splenic myeloid cells through activating β-adrenergic signaling. *Brain, Behav. Immun*. 2019;80: 825–838.doi:10.1016/j.bbi.2019.05.031

Kahán, Z., Nagy, A., Schally, A. V., Hebert, F., Sun, B., Groot, K., et al. Inhibition of growth of MX-1, MCF-7-MIII and MDA-MB-231 human breast cancer xenografts after administration of a targeted cytotoxic analog of somatostatin, AN-238. *Int. J. Cancer*. 1999;82(4):592–598.

Kamiya, A., Hayama, Y., Kato, S., Shimomura, A., Shimomura, T., Irie, K., et al. Genetic manipulation of autonomic nerve fiber innervation and activity and its effect on breast cancer progression. *Nat. Neurosci*. 2019;22(8):1289–1305.doi:10.1038/s41593-019-0430-3

Kappos, E. A., Engels, P. E., Tremp, M., Sieber, P. K., von Felten, S., Madduri, S., et al. Denervation leads to volume regression in breast cancer. *J. Plastic, Reconstr. Aesthetic Surg*. 2018;71(6):833–839.doi:10.1016/j.bjps.2018.03.012

Keskinov, A. A., Tapias, V., Watkins, S. C., Ma, Y., Shurin, M. R., and Shurin, G. V. Impact of the sensory neurons on melanoma growth *in vivo*. *PLoS One*. 2016; 11(5):e0156095.doi:10.1371/journal.pone.0156095

Klein, M. O., Battagello, D. S., Cardoso, A. R., Hauser, D. N., Bittencourt, J. C., and Correa, R. G. Dopamine: Functions, signaling, and association with neurological diseases. *Cell Mol. Neurobiol*. 2019;39(1):31–59.doi:10.1007/s10571-018-0632-3

Klose, C. S. N., and Artis, D. Innate lymphoid cells control signaling circuits to regulate tissue-specific immunity. *Cell Res*. 2020;30(6):475–491.doi:10.1038/s41422-020-0323-8

Klose, C. S. N., Mahlakõiv, T., Moeller, J. B., Rankin, L. C., Flamar, A. L., Kabata, H., et al. The neuropeptide neuromedin U stimulates innate lymphoid cells and type 2 inflammation. *Nature*. 2017;549(7671):282–286.doi:10.1038/nature23676

Knights, A. J., Liu, S., Ma, Y., Nudell, V. S., Perkey, E., Sorensen, M. J., et al. Acetylcholine-synthesizing macrophages in subcutaneous fat are regulated by β2-adrenergic signaling. *EMBO J*. 2021;40(24):e106061.doi:10.15252/embj.2020106061

Kong, Y., Ruan, L., Qian, L., Liu, X., and Le, Y. Norepinephrine promotes microglia to uptake and degrade Amyloid peptide through upregulation of mouse formyl peptide receptor 2 and induction of insulin-degrading enzyme. *J. Neurosci*. 2010;30(35):11848–11857.doi:10.1523/jneurosci.2985-10.2010

Lechtenberg, K. J., Meyer, S. T., Doyle, J. B., Peterson, T. C., and Buckwalter, M. S. Augmented β2-adrenergic signaling dampens the neuroinflammatory response following ischemic stroke and increases stroke size. *J. Neuroinflammation*. 2019; 16(1):112.doi:10.1186/s12974-019-1506-4

Lee, E. Y., Chan, L. C., Wang, H., Lieng, J., Hung, M., Srinivasan, Y., et al. PACAP is a pathogen-inducible resident antimicrobial neuropeptide affording rapid and contextual molecular host defense of the brain. *Proc. Natl. Acad. Sci. U. S. A*. 2021; 118(1).doi:10.1073/pnas.1917623117

Lee, M. Neurotransmitters and microglial-mediated neuroinflammation. *Cpps*. 2013;14(1):21–32.doi:10.2174/1389203711314010005

Lei, X., Lei, Y., Li, J. K., Du, W. X., Li, R. G., Yang, J., et al. Immune cells within the tumor microenvironment: Biological functions and roles in cancer immunotherapy. *Cancer Lett*. 2020;470:126–133.doi:10.1016/j.canlet.2019.11.009

Levi, B., Benish, M., Goldfarb, Y., Sorski, L., Melamed, R., Rosenne, E., et al. Continuous stress disrupts immunostimulatory effects of IL-12. *Brain, Behav. Immun*. 2011;25(4):727–735.doi:10.1016/j.bbi.2011.01.014

Li, L., Liu, Z., Jiang, Y. Y., Shen, W. X., Peng, Y. P., and Qiu, Y. H. Acetylcholine suppresses microglial inflammatory response via α7nAChR to protect hippocampal neurons. *J. Integr. Neurosci*. 2019;18(1):51–56.doi:10.31083/j.jin.2019.01.114

Liu, S. Q., Gao, Z. J., Wu, J., Zheng, H. M., Li, B., Sun, S., et al. Single-cell and spatially resolved analysis uncovers cell heterogeneity of breast cancer. *J. Hematol. Oncol*. 2022;15(1):19.doi:10.1186/s13045-022-01236-0

Liu, Z., Gao, Z., Li, B., Li, J., Ou, Y., Yu, X., et al. Lipid-associated macrophages in the tumor-adipose microenvironment facilitate breast cancer progression. *OncoImmunology*. 2022;11(1):2085432.doi:10.1080/2162402x.2022.2085432

Mackay, L. K., and Kallies, A. Transcriptional regulation of tissue-resident lymphocytes. *Trends Immunol*. 2017;38(2):94–103.doi:10.1016/j.it.2016.11.004

Magnon, C., Hall, S. J., Lin, J., Xue, X., Gerber, L., Freedland, S. J., et al. Autonomic nerve development contributes to prostate cancer progression. *Science*. 2013; 341(6142):1236361.doi:10.1126/science.1236361

Magnon, C. The adrenergic nerve network in cancer. *Adv. Exp. Med. Biol*. 2021; 1329:271–294.doi:10.1007/978-3-030-73119-9_15

Marino, F., and Cosentino, M. Adrenergic modulation of immune cells: An update. *Amino Acids*. 2013;45(1):55–71.doi:10.1007/s00726-011-1186-6

Martinez, V. G., and O'Driscoll, L. Neuromedin U: A multifunctional neuropeptide with pleiotropic roles. *Clin. Chem*. 2015;61(3):471–482.doi:10.1373/clinchem.2014.231753

Matheis, F., Muller, P. A., Graves, C. L., Gabanyi, I., Kerner, Z. J., Costa-Borges, D., et al. Adrenergic signaling in muscularis macrophages limits infection-induced neuronal loss. *Cell*. 2020;180(1):64–78. e16.doi:10.1016/j.cell.2019.12.002

Matteoli, G., Gomez-Pinilla, P. J., Nemethova, A., Di Giovangiulio, M., Cailotto, C., van Bree, S. H., et al. A distinct vagal anti-inflammatory pathway modulates intestinal muscularis resident macrophages independent of the spleen. *Gut*. 2014; 63(6):938–948.doi:10.1136/gutjnl-2013-304676

Mohammadpour, H., MacDonald, C. R., McCarthy, P. L., Abrams, S. I., and Repasky, E. A. β2-adrenergic receptor signaling regulates metabolic pathways critical to myeloid-derived suppressor cell function within the TME. *Cell Rep.* 2021;37(4):109883.doi:10.1016/j.celrep.2021.109883

Mohammadpour, H., MacDonald, C. R., Qiao, G., Chen, M., Dong, B., Hylander, B. L., et al. β2 adrenergic receptor-mediated signaling regulates the immunosuppressive potential of myeloid-derived suppressor cells. *J. Clin. Invest.* 2019;129(12):5537–5552.doi:10.1172/jci129502

Møller, L. N., Stidsen, C. E., Hartmann, B., and Holst, J. J. Somatostatin receptors. *Biochim. Biophys. Acta.* 2003;1616(1):1–84.doi:10.1016/s0005-2736(03)00235-9

Moriyama, S., Brestoff, J. R., Flamar, A. L., Moeller, J. B., Klose, C. S. N., Rankin, L. C., et al. β 2 -adrenergic receptor-mediated negative regulation of group 2 innate lymphoid cell responses. *Science.* 2018;359(6379):1056–1061.doi:10.1126/science.aan4829

Muller, P. A., Koscsó, B., Rajani, G. M., Stevanovic, K., Berres, M. L., Hashimoto, D., et al. Crosstalk between muscularis macrophages and enteric neurons regulates gastrointestinal motility. *Cell.* 2014;158(2):300–313.doi:10.1016/j.cell.2014.04.050

Nagaraja, A. S., Armaiz-Pena, G. N., Lutgendorf, S. K., and Sood, A. K. Why stress is BAD for cancer patients. *J. Clin. Invest.* 2013;123(2):558–560.doi:10.1172/JCI67887

Nagashima, H., Mahlakõiv, T., Shih, H. Y., Davis, F. P., Meylan, F., Huang, Y., et al. Neuropeptide CGRP limits group 2 innate lymphoid cell responses and constrains type 2 inflammation. *Immunity.* 2019;51(4):682–695.doi:10.1016/j.immuni.2019.06.009

Nayak, D., Roth, T. L., and McGavern, D. B. Microglia development and function. *Annu. Rev. Immunol.* 2014;32:367–402.doi:10.1146/annurev-immunol-032713-120240

Ngo, D. H., and Vo, T. S. An updated review on pharmaceutical properties of gamma-aminobutyric acid. *Molecules.* 2019;24(15).doi:10.3390/molecules24152678

Pain, S., Vergote, J., Gulhan, Z., Bodard, S., Chalon, S., and Gaillard, A. Inflammatory process in Parkinson disease: Neuroprotection by neuropeptide Y. *Fundam. Clin. Pharmacol.* 2019;33(5):544–548.doi:10.1111/fcp.12464

Pannell, M., Meier, M. A., Szulzewsky, F., Matyash, V., Endres, M., Kronenberg, G., et al. The subpopulation of microglia expressing functional muscarinic acetylcholine receptors expands in stroke and Alzheimer's disease. *Brain Struct. Funct.* 2016;221(2):1157–1172.doi:10.1007/s00429-014-0962-y

Partecke, L. I., Speerforck, S., Käding, A., Seubert, F., Kühn, S., Lorenz, E., et al. Chronic stress increases experimental pancreatic cancer growth, reduces survival and can be antagonised by beta-adrenergic receptor blockade. *Pancreatology.* 2016;16(3):423–433.doi:10.1016/j.pan.2016.03.005

Pavón-Romero, G. F., Serrano-Pérez, N. H., García-Sánchez, L., Ramírez-Jiménez, F., and Terán, L. M. Neuroimmune pathophysiology in asthma. *Front. Cell Dev. Biol.* 2021;9:663535.doi:10.3389/fcell.2021.663535

Pedersen, L., Idorn, M., Olofsson, G. H., Lauenborg, B., Nookaew, I., Hansen, R. H., et al. Voluntary running suppresses tumor growth through epinephrine- and IL-6-dependent NK cell mobilization and redistribution. *Cell Metab.* 2016;23(3):554–562.doi:10.1016/j.cmet.2016.01.011

Perner, C., Flayer, C. H., Zhu, X., Aderhold, P. A., Dewan, Z. N. A., Voisin, T., et al. Substance P release by sensory neurons triggers dendritic cell migration and initiates the type-2 immune response to allergens. *Immunity.* 2020;53(5):1063–1077.doi:10.1016/j.immuni.2020.10.001

Peters, M. A., Walenkamp, A. M., Kema, I. P., Meijer, C., de Vries, E. G., and Oosting, S. F. Dopamine and serotonin regulate tumor behavior by affecting angiogenesis. *Drug Resist Updat.* 2014;17(4-6):96–104.doi:10.1016/j.drup.2014.09.001

Petkevicius, K., Bidault, G., Virtue, S., Jenkins, B., van Dierendonck, X., Dugourd, A., et al. Norepinephrine promotes triglyceride storage in macrophages via beta2-adrenergic receptor activation. *FASEB J.* 2021;35(2):e21266.doi:10.1096/fj.202001101R

Petkevicius, K., Bidault, G., Virtue, S., Newland, S. A., Dale, M., Dugourd, A., et al. Macrophage beta2-adrenergic receptor is dispensable for the adipose tissue inflammation and function. *Mol. Metab.* 2021;48:101220.doi:10.1016/j.molmet.2021.101220

Pinho-Ribeiro, F. A., Baddal, B., Haarsma, R., O'Seaghdha, M., Yang, N. J., Blake, K. J., et al. Blocking neuronal signaling to immune cells treats streptococcal invasive infection. *Cell.* 2018;173(5):1083–1097.doi:10.1016/j.cell.2018.04.006

Pintér, E., Helyes, Z., and Szolcsányi, J. Inhibitory effect of somatostatin on inflammation and nociception. *Pharmacol. Ther.* 2006;112(2):440–456.doi:10.1016/j.pharmthera.2006.04.010

Pirzgalska, R. M., Seixas, E., Seidman, J. S., Link, V. M., Sánchez, N. M., Mahú, I., et al. Sympathetic neuron-associated macrophages contribute to obesity by importing and metabolizing norepinephrine. *Nat. Med.* 2017;23(11):1309–1318.doi:10.1038/nm.4422

Pon, C. K., Lane, J. R., Sloan, E. K., and Halls, M. L. The β 2 -adrenoceptor activates a positive cAMP-calcium feedforward loop to drive breast cancer cell invasion. *FASEB J.* 2016;30(3):1144–1154.doi:10.1096/fj.15-277798

Prazeres, P., Leonel, C., Silva, W. N., Rocha, B. G. S., Santos, G. S. P., Costa, A. C., et al. Ablation of sensory nerves favours melanoma progression. *J. Cell Mol. Med.* 2020;24(17):9574–9589.doi:10.1111/jcmm.15381

Qiao, G., Chen, M., Mohammadpour, H., MacDonald, C. R., Bucsek, M. J., Hylander, B. L., et al. Chronic adrenergic stress contributes to metabolic dysfunction and an exhausted phenotype in T cells in the tumor microenvironment. *Cancer Immunol. Res.* 2021;9(6):651–664.doi:10.1158/2326-6066.cir-20-0445

Quail, D. F., and Joyce, J. A. Microenvironmental regulation of tumor progression and metastasis. *Nat. Med.* 2013;19(11):1423–1437.doi:10.1038/nm.3394

Quintero-Villegas, A., and Valdés-Ferrer, S. I. Role of 5-HT7 receptors in the immune system in health and disease. *Mol. Med.* 2019;26(1):2.doi:10.1186/s10020-019-0126-x

Rasiah, K. K., Kench, J. G., Gardiner-Garden, M., Biankin, A. V., Golovsky, D., Brenner, P. C., et al. Aberrant neuropeptide Y and macrophage inhibitory cytokine-1 expression are early events in prostate cancer development and are associated with poor prognosis. *Cancer Epidemiol. Biomarkers Prev.* 2006;15(4):711–716.doi:10.1158/1055-9965.epi-05-0752

Reavis, H. D., Chen, H. I., and Drapkin, R. Tumor innervation: Cancer has some nerve. *Trends Cancer.* 2020;6(12):1059–1067.doi:10.1016/j.trecan.2020.07.005

Renz, B. W., Takahashi, R., Tanaka, T., Macchini, M., Hayakawa, Y., Dantes, Z., et al. β2 adrenergic-neurotrophin feedforward loop promotes pancreatic cancer. *Cancer Cell.* 2018;33(1):75–90.doi:10.1016/j.ccell.2017.11.007

Retamales-Ortega, R., Oróstica, L., Vera, C., Cuevas, P., Hernández, A., Hurtado, I., et al. Role of nerve growth factor (NGF) and miRNAs in epithelial ovarian cancer. *Int. J. Mol. Sci.* 2017;18(3).doi:10.3390/ijms18030507

Riol-Blanco, L., Ordovas-Montanes, J., Perro, M., Naval, E., Thiriot, A., Alvarez, D., et al. Nociceptive sensory neurons drive interleukin-23-mediated psoriasiform skin inflammation. *Nature.* 2014;510(7503):157–161.doi:10.1038/nature13199

Saloman, J. L., Albers, K. M., Li, D., Hartman, D. J., Crawford, H. C., Muha, E. A., et al. Ablation of sensory neurons in a genetic model of pancreatic ductal adenocarcinoma slows initiation and progression of cancer. *Proc. Natl. Acad. Sci. U.S.A.* 2016;113(11):3078–3083.doi:10.1073/pnas.1512603113

Saul, A. N., Oberyszyn, T. M., Daugherty, C., Kusewitt, D., Jones, S., Jewell, S., et al. Chronic stress and susceptibility to skin cancer. *J. Natl. Cancer Inst.* 2005;97(23):1760–1767.doi:10.1093/jnci/dji401

Schiller, M., Azulay-Debby, H., Boshnak, N., Elyahu, Y., Korin, B., Ben-Shaanan, T. L., et al. Optogenetic activation of local colonic sympathetic innervations attenuates colitis by limiting immune cell extravasation. *Immunity.* 2021;54(5):1022–1036.doi:10.1016/j.immuni.2021.04.007

Seillet, C., and Jacquelot, N. Sensing of physiological regulators by innate lymphoid cells. *Cell Mol. Immunol.* 2019;16(5):442–451.doi:10.1038/s41423-019-0217-1

Seillet, C., Luong, K., Tellier, J., Jacquelot, N., Shen, R. D., Hickey, P., et al. The neuropeptide VIP confers anticipatory mucosal immunity by regulating ILC3 activity. *Nat. Immunol.* 2020;21(2):168–177.doi:10.1038/s41590-019-0567-y

Serhan, N., Basso, L., Sibilano, R., Petitfils, C., Meixiong, J., Bonnart, C., et al. House dust mites activate nociceptor-mast cell clusters to drive type 2 skin inflammation. *Nat. Immunol.* 2019;20(11):1435–1443.doi:10.1038/s41590-019-0493-z

Sha, M., Cao, J., Sun, H. Y., Tong, Y., and Xia, Q. Neuroendocrine regulation of cholangiocarcinoma: A status quo review. *Biochimica Biophysica Acta (BBA) - Rev. Cancer.* 2019;1872(1):66–73.doi:10.1016/j.bbcan.2019.05.005

Shan, B., Shao, M., Zhang, Q., An, Y. A., Vishvanath, L., and Gupta, R. K. Cold-responsive adipocyte progenitors couple adrenergic signaling to immune cell activation to promote beige adipocyte accrual. *Genes Dev.* 2021;35(19-20):1333–1338.doi:10.1101/gad.348762.121

Silverman, D. A., Martinez, V. K., Dougherty, P. M., Myers, J. N., Calin, G. A., and Amit, M. Cancer-associated neurogenesis and nerve-cancer cross-talk. *Cancer Res.* 2021;81(6):1431–1440.doi:10.1158/0008-5472.can-20-2793

Sloan, E. K., Priceman, S. J., Cox, B. F., Yu, S., Pimentel, M. A., Tangkanangnukul, V., et al. The sympathetic nervous system induces a metastatic switch in primary breast cancer. *Cancer Res.* 2010;70(18):7042–7052.doi:10.1158/0008-5472.can-10-0522

Soler Palacios, B., and Gutiérrez-González, A. Neuron–macrophage interaction in the healing process of the skin. *Allergy.* 2022;77(3):1064–1066.doi:10.1111/all.15122

Srinivasan, A. V. Propranolol: A 50-year historical perspective. *Ann. Indian Acad. Neurol.* 2019;22(1):21–26.doi:10.4103/aian.aian_201_18

Stolero, N., and Frenkel, D. The dialog between neurons and microglia in Alzheimer's disease: The neurotransmitters view. *J. Neurochem*. 2021;158(6): 1412–1424.doi:10.1111/jnc.15262

Sui, P., Wiesner, D. L., Xu, J., Zhang, Y., Lee, J., Van Dyken, S., et al. Pulmonary neuroendocrine cells amplify allergic asthma responses. *Science*. 2018; 360(6393).doi:10.1126/science.aan8546

Talbot, J., Hahn, P., Kroehling, L., Nguyen, H., Li, D., and Littman, D. R. Feeding-dependent VIP neuron-ILC3 circuit regulates the intestinal barrier. *Nature*. 2020; 579(7800):575–580.doi:10.1038/s41586-020-2039-9

Talbot, S., Abdulnour, R. E., Burkett, P. R., Lee, S., Cronin, S. J., Pascal, M. A., et al. Silencing nociceptor neurons reduces allergic airway inflammation. *Neuron*. 2015; 87(2):341–354.doi:10.1016/j.neuron.2015.06.007

Tang, L., Okamoto, S., Shiuchi, T., Toda, C., Takagi, K., Sato, T., et al. Sympathetic nerve activity maintains an anti-inflammatory state in adipose tissue in male mice by inhibiting TNF-α gene expression in macrophages. *Endocrinology*. 2015;156(10): 3680–3694.doi:10.1210/en.2015-1096

Tanner, M. A., Maitz, C. A., and Grisanti, L. A. Immune cell β2-adrenergic receptors contribute to the development of heart failure. *Am. J. Physiology-Heart Circulatory Physiology*. 2021;321(4):H633–H649.doi:10.1152/ajpheart.00243.2021

Thaker, P. H., Han, L. Y., Kamat, A. A., Arevalo, J. M., Takahashi, R., Lu, C., et al. Chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma. *Nat. Med*. 2006;12(8):939–944.doi:10.1038/nm1447

Thomas Broome, S., Louangaphay, K., Keay, K. A., Leggio, G. M., Musumeci, G., and Castorina, A. Dopamine: An immune transmitter. *Neural Regen. Res*. 2020; 15(12):2173–2185.doi:10.4103/1673-5374.284976

Treszl, A., Schally, A. V., Seitz, S., Szalontay, L., Rick, F. G., Szepeshazi, K., et al. Inhibition of human non-small cell lung cancers with a targeted cytotoxic somatostatin analog, AN-162. *Peptides*. 2009;30(9):1643–1650.doi:10.1016/j.peptides.2009.06.007

Triaca, V., Carito, V., Fico, E., Rosso, P., Fiore, M., Ralli, M., et al. Cancer stem cells-driven tumor growth and immune escape: The janus face of neurotrophins. *Aging*. 2019;11(23):11770–11792.doi:10.18632/aging.102499

Umpierre, A. D., and Wu, L. J. Microglia research in the 100th year since its discovery. *Neurosci. Bull*. 2020;36(3):303–306.doi:10.1007/s12264-020-00477-8

Ural, B. B., Yeung, S. T., Damani-Yokota, P., Devlin, J. C., de Vries, M., Vera-Licona, P., et al. Identification of a nerve-associated, lung-resident interstitial macrophage subset with distinct localization and immunoregulatory properties. *Sci. Immunol*. 2020;5(45).doi:10.1126/sciimmunol.aax8756

von Arx, C., Rea, G., Napolitano, M., Ottaiano, A., Tatangelo, F., Izzo, F., et al. Effect of octreotide long-acting release on tregs and MDSC cells in neuroendocrine tumour patients: A pivotal prospective study. *Cancers (Basel)*. 2020;12(9).doi:10.3390/cancers12092422

Wagner, M. J., Cranmer, L. D., Loggers, E. T., and Pollack, S. M. Propranolol for the treatment of vascular sarcomas. *Jep*. 2018;Vol. 10:51–58.doi:10.2147/jep.s146211

Wallrapp, A., Burkett, P. R., Riesenfeld, S. J., Kim, S. J., Christian, E., Abdulnour, R. E., et al. Calcitonin gene-related peptide negatively regulates alarmin-driven type 2 innate lymphoid cell responses. *Immunity*. 2019;51(4):709–723.doi:10.1016/j.immuni.2019.09.005

Wallrapp, A., Riesenfeld, S. J., Burkett, P. R., Abdulnour, R. E., Nyman, J., Dionne, D., et al. The neuropeptide NMU amplifies ILC2-driven allergic lung inflammation. *Nature*. 2017;549(7672):351–356.doi:10.1038/nature24029

Wang, H., Yu, M., Ochani, M., Amella, C. A., Tanovic, M., Susarla, S., et al. Nicotinic acetylcholine receptor α7 subunit is an essential regulator of inflammation. *Nature*. 2003;421(6921):384–388.doi:10.1038/nature01339

Wang, Y. N., Tang, Y., He, Z., Ma, H., Wang, L., Liu, Y., et al. Slit3 secreted from M2-like macrophages increases sympathetic activity and thermogenesis in adipose tissue. *Nat. Metab*. 2021;3(11):1536–1551.doi:10.1038/s42255-021-00482-9

Waqas, S. F. H., Hoang, A. C., Lin, Y. T., Ampem, G., Azegrouz, H., Balogh, L., et al. Neuropeptide FF increases M2 activation and self-renewal of adipose tissue macrophages. *J. Clin. Invest*. 2017;127(9):3559.doi:10.1172/jci95841

Weckbecker, G., Lewis, I., Albert, R., Schmid, H. A., Hoyer, D., and Bruns, C. Opportunities in somatostatin research: Biological, chemical and therapeutic aspects. *Nat. Rev. Drug Discov*. 2003;2(12):999–1017.doi:10.1038/nrd1255

Wehkamp, U., Stern, S., Krüger, S., Weichenthal, M., Hauschild, A., Röcken, C., et al. Co-expression of NGF and PD-L1 on tumor-associated immune cells in the microenvironment of Merkel cell carcinoma. *J. Cancer Res. Clin. Oncol*. 2018; 144(7):1301–1308.doi:10.1007/s00432-018-2657-x

Weisberg, S. P., Ural, B. B., and Farber, D. L. Tissue-specific immunity for a changing world. *Cell*. 2021;184(6):1517–1529.doi:10.1016/j.cell.2021.01.042

Werneburg, S., Feinberg, P. A., Johnson, K. M., and Schafer, D. P. A microglia-cytokine axis to modulate synaptic connectivity and function. *Curr. Opin. Neurobiol*. 2017;47:138–145.doi:10.1016/j.conb.2017.10.002

Willemze, R. A., Welting, O., van Hamersveld, P., Verseijden, C., Nijhuis, L. E., Hilbers, F. W., et al. Loss of intestinal sympathetic innervation elicits an innate immune driven colitis. *Mol. Med*. 2019;25(1):1.doi:10.1186/s10020-018-0068-8

Wolf, Y., Boura-Halfon, S., Cortese, N., Haimon, Z., Sar Shalom, H., Kuperman, Y., et al. Brown-adipose-tissue macrophages control tissue innervation and homeostatic energy expenditure. *Nat. Immunol*. 2017;18(6):665–674.doi:10.1038/ni.3746

Wong, C. H., Jenne, C. N., Lee, W. Y., Léger, C., and Kubes, P. Functional innervation of hepatic iNKT cells is immunosuppressive following stroke. *Science*. 2011;334(6052):101–105.doi:10.1126/science.1210301

Xie, H., Heier, C., Meng, X., Bakiri, L., Pototschnig, I., Tang, Z., et al. An immune-sympathetic neuron communication axis guides adipose tissue browning in cancer-associated cachexia. *Proc. Natl. Acad. Sci. U. S. A*. 2022;119(9).doi:10.1073/pnas.2112840119

Xu, H., Ding, J., Porter, C. B. M., Wallrapp, A., Tabaka, M., Ma, S., et al. Transcriptional atlas of intestinal immune cells reveals that neuropeptide α-CGRP modulates group 2 innate lymphoid cell responses. *Immunity*. 2019;51(4): 696–708.doi:10.1016/j.immuni.2019.09.004

Yamamoto, Y., Otsuka, A., Ishida, Y., Wong, L. S., Seidel, J. A., Nonomura, Y., et al. Pituitary adenylate cyclase-activating polypeptide promotes cutaneous dendritic cell functions in contact hypersensitivity. *J. Allergy Clin. Immunol*. 2021;148(3):858–866.doi:10.1016/j.jaci.2021.02.005

Yang, N. N., Yang, J. W., Ye, Y., Huang, J., Wang, L., Wang, Y., et al. Electroacupuncture ameliorates intestinal inflammation by activating α7nAChR-mediated JAK2/STAT3 signaling pathway in postoperative ileus. *Theranostics*. 2021;11(9):4078–4089.doi:10.7150/thno.52574

Ye, D., Xu, H., Tang, Q., Xia, H., Zhang, C., and Bi, F. The role of 5-HT metabolism in cancer. *Biochimica Biophysica Acta (BBA) - Rev. Cancer*. 2021; 1876(2):188618.doi:10.1016/j.bbcan.2021.188618

Younger, D., Murugan, M., Rama Rao, K. V., Wu, L. J., and Chandra, N. Microglia receptors in animal models of traumatic brain injury. *Mol. Neurobiol*. 2019;56(7): 5202–5228.doi:10.1007/s12035-018-1428-7

Yu, J., Xiao, K., Chen, X., Deng, L., Zhang, L., Li, Y., et al. Neuron-derived neuropeptide Y fine-tunes the splenic immune responses. *Neuron*. 2022;110(8): 1327–1339.doi:10.1016/j.neuron.2022.01.010

Zahalka, A. H., Arnal-Estapé, A., Maryanovich, M., Nakahara, F., Cruz, C. D., Finley, L. W. S., et al. Adrenergic nerves activate an angio-metabolic switch in prostate cancer. *Science*. 2017;358(6361):321–326.doi:10.1126/science.aah5072

Zahalka, A. H., and Frenette, P. S. Nerves in cancer. *Nat. Rev. Cancer*. 2020;20(3): 143–157.doi:10.1038/s41568-019-0237-2

Zhang, B., Vogelzang, A., Miyajima, M., Sugiura, Y., Wu, Y., Chamoto, K., et al. B cell-derived GABA elicits IL-10+ macrophages to limit anti-tumour immunity. *Nature*. 2021;599(7885):471–476.doi:10.1038/s41586-021-04082-1

Zhang, K., Guo, X. M., Yan, Y. W., Liu, Y. Y., Xu, Z. F., Zhao, X., et al. Applying statistical and complex network methods to explore the key signaling molecules of acupuncture regulating neuroendocrine-immune network. *Evid. Based Complement. Altern. Med*. 2018;2018:9260630.doi:10.1155/2018/9260630

Zhang, L., Wu, Z., Tong, Z., Yao, Q., Wang, Z., and Li, W. Vagus nerve stimulation decreases pancreatitis severity in mice. *Front. Immunol*. 2020;11: 595957.doi:10.3389/fimmu.2020.595957

Zhang, Q., Lu, Y., Bian, H., Guo, L., and Zhu, H. Activation of the α7 nicotinic receptor promotes lipopolysaccharide-induced conversion of M1 microglia to M2. *Am. J. Transl. Res*. 2017;9(3):971–985.

Zhao, C. M., Hayakawa, Y., Kodama, Y., Muthupalani, S., Westphalen, C. B., Andersen, G. T., et al. Denervation suppresses gastric tumorigenesis. *Sci. Transl. Med*. 2014;6(250):250ra115.doi:10.1126/scitranslmed.3009569

Zheng, J., Liu, Y., Lau, Y. L., and Tu, W. γδ-T cells: An unpolished sword in human anti-infection immunity. *Cell Mol. Immunol*. 2013;10(1):50–57.doi:10.1038/cmi.2012.43

Zhou, L., Li, Y., Li, X., Chen, G., Liang, H., Wu, Y., et al. Propranolol attenuates surgical stress-induced elevation of the regulatory T cell response in patients undergoing radical mastectomy. *J. I.* 2016;196(8):3460–3469.doi:10.4049/jimmunol.1501677

Glossary

TME tumor microenvironment
DC dendritic cell
ILC innate lymphoid cell
TRM tissue-resident memory T cell
CNS central nervous system
PNS peripheral nervous system
NE norepinephrine
AR adrenergic receptor
NGF nerve growth factor
TEM effective memory T cell
ATM adipose tissue macrophage
ASC adipose stem cell
MM muscularis macrophage
iNKT invariant natural killer T
ACh acetylcholine
mAChR metabotropic muscarinic ACh receptor
nAChR ionotropic nicotinic ACh receptor
5-HT serotonin
GABA gamma-aminobutyric acid
DA dopamine
DR dopamine receptor
5-HTR 5-HT receptor
NMU neuromedin U
SP substance P
CGRP calcitonin gene-related peptide
PACAP pituitary adenylate cyclase-activating polypeptide
VIP vasoactive intestinal peptide
NPFF neuropeptide FF
NPY neuropeptide Y
CRLR receptor calcitonin receptor like receptor
PNEC pulmonary neuroendocrine cell
MRGPR Mas-related G-protein coupled receptor member
dLN draining lymph node
ChAM cholinergic adipose macrophage
BMP2 bone morphogenetic protein 2
CSF1 colony stimulatory factor
SAM Sympathetic neuron-associated macrophage
NAM nerve- and airway-associated macrophage
MDSC myeloid-derived suppressor cell
DRG dorsal root ganglia
PRP-1 proline rich polypeptide 1
SST somatostatin
SSA somatostatin analog

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