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REVIEW

Macrophages in graft-versus-host disease (GVHD): dual roles as therapeutic tools and targets

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Abstract

Graft-versus-host disease remains one of the most formidable barriers to the complete success of hematopoietic stem cell transplantation that has emerged as the curative approach for many hematopoietic malignancies because it affects quality of life and overall survival. Macrophages are among the important members of the immune system, which perform dual roles in GVHD as both therapeutic tools and targets. This review epitomizes the multifunctional role of macrophages in the pathophysiology of both acute and chronic GVHD. Macrophages play an important role in the early phase of GVHD because of their recruitment and infiltration into target organs. Furthermore, they polarize into two functionally different phenotypes, including M1 and M2. In the case of acute GVHD, most macrophages express the M1 phenotype characterized by the production of pro-inflammatory cytokines that contribute to tissue damage. In contrast, in chronic GVHD, macrophages tend toward the M2 phenotype associated with the repair of tissues and fibrosis. A critical balance among these phenotypes is central to the course and severity of GVHD. Further interactions of macrophages with other lymphocytes such as T cells, B cells, and fibroblast further determine the course of GVHD. Macrophage interaction associated with alloreactive T cells promotes inflammation. This is therefore important in inducing injuries of tissues during acute GVHD. Interaction of macrophages, B cell, fibroblast, and CD4+ T cells promotes fibrosis during chronic GVHD and, hence, the subsequent dysfunction of organs. These are some insights, while several challenges remain. First, the impact of the dominant cytokines in GVHD on the polarization of macrophages is incompletely characterized and sometimes controversial. Second, the development of targeted therapies able to modulate macrophage function without systemic side effects remains an area of ongoing investigation. Future directions involve the exploration of macrophage-targeted therapies, including small molecules, antibodies, and nanotechnology, which modulate macrophage behavior and improve patient outcomes. This underlines the fact that a profound understanding of the dual role of macrophages in GVHD is essential for developing new and more effective therapeutic strategies. Targeting macrophages might represent one avenue for decreasing the incidence and severity of GVHD and improving the success and safety of HSCT.

Keywords Macrophages · GVHD · Graft-versus-host disease · Immunotherapy · Inflammation · Therapeutic targets · Transplantation

Introduction

Graft-versus-host disease (GVHD) is an unwanted inflammatory reaction caused by allogeneic bone marrow stem cell transplantation, which greatly reduces the therapeutic effects of transplantation in more than half of patients affected by malignant and nonmalignant blood disorders and causes significant mortality [1, 2]. One of the good features of allogeneic transplantation is that, owing to the incompatibility of HLA polymorphic antigens, donor immune cells have the ability to identify the sick or malignant hematopoietic stem cells of the recipient and destroy them [3, 4]. This beneficial response, called the graft-versus-leukemia (GvL) effect, accelerates the improvement of hematopoiesis. However, sometimes immune responses extend beyond hematopoietic cells to the tissues and other organs of the recipient and cause adverse effects of GVHD [3, 5]. In this disease, two phases, which have their own pathophysiology and cause their specific clinical symptoms, are observed in different patients. Three stages of

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acute GVHD are involved in the pathophysiology of the disease. First, the process of conditioning results in injury to the tissue, thus causing the activation of antigen-presenting cells (APCs), which proceed to preset the antigens specific to the recipient. The subsequent stage, known as the afferent phase, involves the activation of T cells by APCs, initiating a sequence of events in the third phase, where cellular immunity, in conjunction with various inflammatory mediators, contributes to tissue damage [6, 7]. Although the pathophysiology of chronic GVHD has not been fully elucidated, various studies have shown that the acute phase is considered a risk factor for progression to the chronic type [8, 9]. In this way, in the continuation of the acute phase, it occurs approximately 100 days after transplantation, but sometimes it has clinical manifestations as de novo or together with the acute phase [9]. The clinical symptoms depend on the stage of GVHD. Acute GVHD usually involves three organs: the skin, gastrointestinal tract (GI), and liver, while the main organs involved in chronic GVHD include the skin, mouth, eyes, GI, liver, lungs, joints, fascia, and reproductive system [10–13].

Macrophages are essential for immunological responses and perform a wide range of functions, including phagocytosis, antigen presentation, and the ability to activate and recruit other immune cells through the secretion of different cytokines and chemokines, which thus initiates and strengthens immune reactions [14]. In addition, owing to their multiple functions, macrophages maintain balance in the immune system and prevent damage, which can either be caused by the pathogen itself or by a greater inflammatory reaction, in which case macrophages play an important role in wound healing and tissue repair [15]. However, in chronic inflammatory conditions, these macrophages can have adverse effects and lead to fibrosis, scarring, and organ loss of function [16, 17]. Accordingly, there are different categories of macrophages. In one classification, macrophages are grouped into classically activated types, which have inflammatory properties; macrophages with wound healing properties; and the third type, which is called regulatory macrophages. In another common classification, macrophages are categorized into M1 macrophages, which have inflammatory properties, and M2 macrophages, which have anti-inflammatory and wound healing properties (Fig. 1) [18, 19]. Moreover, macrophages, on the basis of their location, perform specific functions in various diseases. Examples of tissue-resident macrophages include microglia (in the central nervous system), Kupffer cells (in the liver), alveolar macrophages (in the lungs), cardiac macrophages, and osteoclasts (involved in bone remodeling) [20–22]. There are also other specialized types of macrophages, such as Langerhans cells (found in the epidermis and mucous membranes), macrophages (located in the peritoneal cavity), and splenic macrophages (red pulp macrophages in the spleen) [23–25].

In this review, an evaluation is conducted of the prevailing understanding of the pathophysiology of GVHD and the distinct correlation of macrophages in various stages of GVHD. Furthermore, an analysis of how diverse classifications of macrophages could be associated with the progression of GVHD-related disorders is provided. Moreover, we aimed to elucidate the importance of macrophages not only as targets for therapeutic interventions but also as modalities for treatment.

Mechanism of GVHD

The exact mechanism of GVHD is not understood. Tissue damage from regimens, chemotherapy, and total body radiation will be essential in the biology of GVHD [26]. Human leukocyte antigen receptor mismatch is a significant risk factor for GVHD [27]. Because it can initiate the response of alloreactive T cells with the help of antigen-presenting cells. Alloreactive T cells recognize the recipient as non-self, attack the recipient's target organs, and initiate GVHD. Antigen presentation, simple T cell differentiation, inducible cytolytic machinery, and a cytokine regulatory network drive the course of acute GVHD [28]. The effect of these mechanisms in acute GVHD is apoptosis caused by cytolytic factors and adaptive and innate immune cells, while end organ fibrosis is one of the important features of chronic GVHD. Chronic GVHD is characterized by thymus damage, pathogenic germinal center (GC) reactivity of B cells and macrophages, imbalanced differentiation of T cells with accumulation of Th17/Tc17 and T follicular helper (Tfh) cells, and T-regulatory suppression. Treg cell status, antibody status and simultaneous cytokine production (eg, increased transforming growth factor (TGF)-β, interleukin (IL)-17 from Th17, and IL-21 produced by Tfh) cells B GC and antibody secretion [29]. Therapies are developed by targeting T cells and B cells, injecting immune regulatory cells, using cytokine antagonists [30]. Macrophages play a crucial role in immune processes and can participate in both acute and chronic phases of GVHD. In the acute phase of GVHD, macrophages act as host cells that are actively involved in identifying and responding to tissue damage and immune stimuli, contributing to the inflammatory process. They can recognize DAMPs (Damage-Associated Molecular Patterns) that are released due to tissue injury and enhance the inflammatory response. DAMPs include molecules such as ATP, free DNA, or specific proteins that the body identifies as damage signals, triggering macrophages and other immune cells. In the chronic phase of GVHD, macrophages may continue to be involved in sustaining inflammation and tissue destruction. Additionally, they can become activated by existing pathogens or in response to PAMPs (Pathogen-Associated Molecular Patterns), which are microbial or

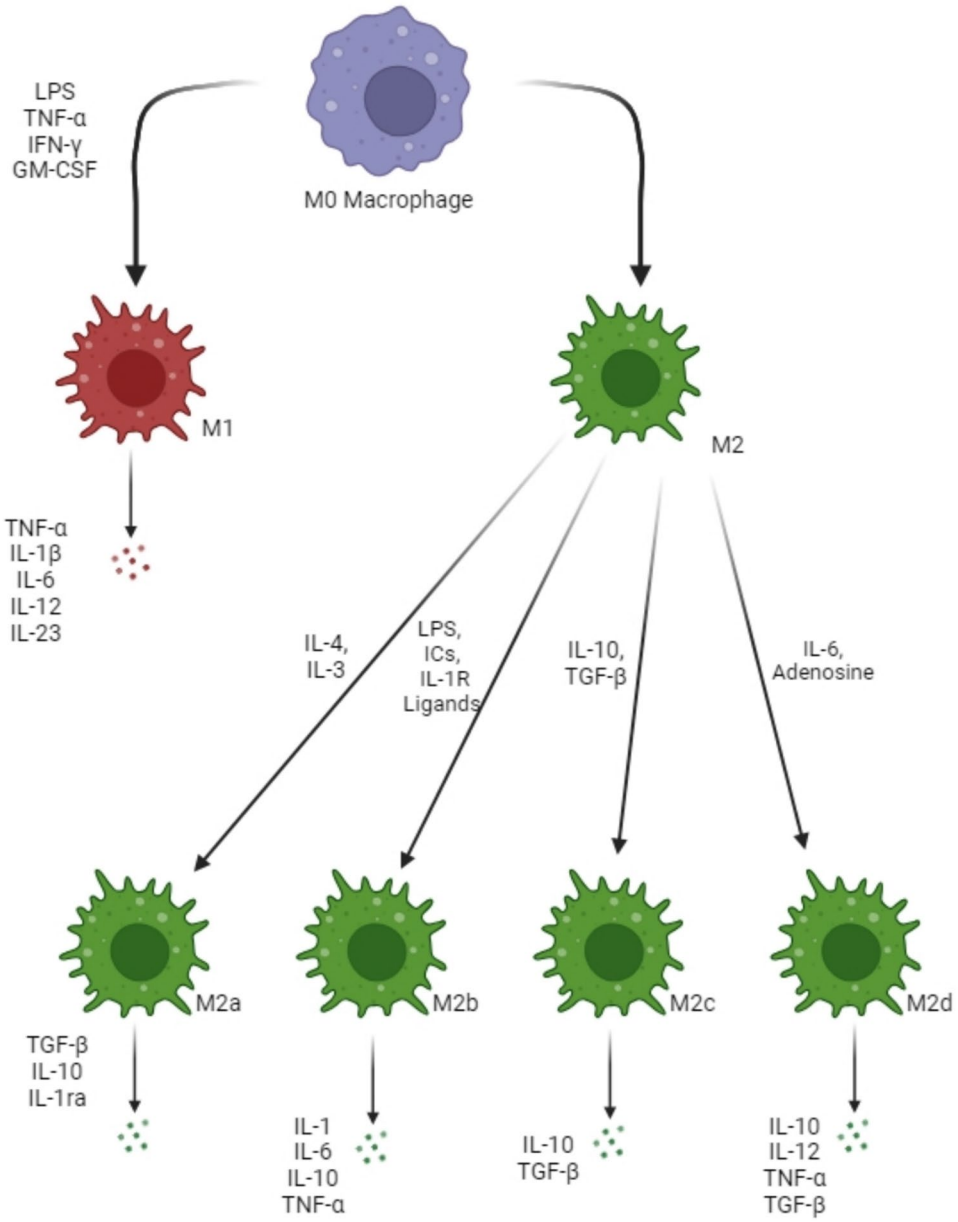

Fig. 1 Illustrative depiction of macrophage polarization. When subjected to various stimuli as outlined in the diagram and the accompanying text, naïve or M0 macrophages undergo differentiation into pro-inflammatory M1 macrophages, also known as classically activated macrophages, and anti-inflammatory macrophages referred to as alternatively activated macrophages or M2 macrophages. The M2 macrophages can be classified into M2a, M2b, M2c, and M2d macrophages based on the specific stimulus, as outlined in the figure and accompanying text

pathogen-derived products that can enter the body during graft transfer or secondary infections. These interactions lead to the amplification of the inflammatory process and may further increase the severity of GVHD [30].

Macrophage heterogeneity and polarization

Macrophages, the vigilant guardians of the immune system, are diverse [31, 32]. Their plasticity allows them to adopt a distinct phenotype and a functional response to the microenvironmental stimuli and signals encountered in each tissue. In 1882, macrophages, as phagocytic cells, were recognized by Élie Metchnikoff [33]. The advancement of information in the previous century has significantly enhanced our comprehension of macrophage heterogeneity and adaptability. Nonpolarized M0 macrophages can be polarized into the two primary categories of the standard M1/M2 categorization scheme developed by Mills et al. in 2000, depending on the signals received [34, 35]. Classically activated macrophages or M1 macrophages exhibit an inflammatory profile, and alternatively activated macrophages, or M2 macrophages, can heal damaged tissues and reduce inflammation [35–39].

M0 macrophages, also known as naive macrophages, are monocytes that become M0 macrophages through the action

of GM-CSF. Notably, these macrophages are not activated by environmental variables or stimuli that either promote or inhibit inflammation [40].

M1 polarized macrophages

M1 polarization is generally induced by bacterial lipopolysaccharide (LPS) and Th1 or inflammatory cytokines, such as IL-2, IL-12, IL-18, IFN-γ, and TNF-β. In this stage of activation, cytokines that promote inflammation are produced, including IL-1α, IL-1β, IL-6, IL-12, IL-23, TNF-α, IFN-γ, and cyclooxygenase-2 (COX-2), whereas IL-10 expression is diminished. The M1 phenotype is characterized by CD80, CD86, major histocompatibility complex (MHC) class II molecules, and Th1 cell-attracting chemokines, including CCL5, CXCL9, and CXCL10. M1 macrophages play crucial roles in different fields, including (a) Pathogen clearance, specifically resistance to intracellular infections, is achieved by effective mechanisms such as the generation of reactive nitrogen species (RNS) and reactive oxygen species (ROS), the activation of inducible nitric oxide synthase (iNOS), and the secretion of inflammatory cytokines [41, 42]. (b) Repair process: Matrix metalloproteinases (MMPs) contribute to the degradation of extracellular matrix components secreted by M1 macrophages, assisting in the restructuring and repair of tissues [43]. (c) Antigen presentation: the expression of MHC II molecules via M1 macrophages allows them to effectively present antigens to immune system cells [39, 40, 44–48]. Therefore, M1 macrophages have robust activity against microbes and tumors and tissue injury, coordinate the body's innate immune response, and begin repairing.

Macrophage polarization is a complex process regulated by a network of transcription factors and epigenetic regulators. During M1 polarization, IFNs and Toll-like receptors (TLRs) activate canonical IRF/STAT signaling pathways that alter macrophage activity toward the M1 phenotype via STAT1 [49, 50]. SOCS family members control the activation of macrophages through STAT. The latter, IFN-γ, upregulates SOCS3, inhibiting the action of STAT3. M1 macrophages increase the expression of IRF5, which is essential for the induction of TNF, IL-12, and IL-23, as well as Th1 and Th17 responses [51]. The TLR pathway plays a critical role in the polarization and activation of M1 macrophages, as previously noted [52, 53]. TLR engagement by ligands triggers the MyD88 and TRIF pathways, activating NF-κB, AP-1, and IRFs and producing inflammatory mediators associated with M1 macrophages [31, 46, 54–57].

M2 polarized macrophages

Unlike the proinflammatory M1 phenotype, M2 are characterized as anti-inflammatory or alternatively activated. However, M2 macrophages can induce allergic inflammation, promote tumor progression, and serve as cellular reservoirs for various pathogens [40, 46, 58]. M2 macrophages promote remodeling of tissues, efferocytosis, wound healing, tissue repair, and as part of their ability to reduce inflammation, they secrete anti-inflammatory compounds such as IL-10 and TGFβ [59–61]. M2 polarization is triggered by Th2 cytokines (including IL-4, IL-5, IL-6, IL-10 and IL-13) and by fungal cells, parasites, immune complexes, complement, and apoptotic cells [31, 40, 46, 57]. M2 can be divided into four subdivisions on the basis of the applied stimuli and resulting transcriptional changes: M2a, M2b, M2c, and M2d [40, 57, 62, 63].

M2a refers to alternatively activated macrophages that develop upon exposure to IL-4 and IL-13. M2a activation dramatically stimulates the expression of mannose receptor (CD206), CD36, CCL17 and several proteases, which are crucial for removing debris and the antiparasitic immune response [40, 57, 58]. M2a macrophages secrete IL-10 and TGF-β via transcription factors such as STAT6 and IRF4 and participate in the processes of proliferation of cells and repair damaged tissues [62].

The activation of M2b macrophages involves the recognition of immune complexes, LPS, and IL-1R ligands, causing the secretion of cytokines that promote inflammation (IL-1β, IL-6, TNF-α, and IL-12) [58]. CD32 (member of Fcγ receptor family) appears to be essential for macrophage type 2 activation [57]. M2b cells, alongside proinflammatory cytokines, secrete substantial amounts of IL-10 and low amounts of IL-12, which functionally counteract M1 cells. Consequently, the regulation of inflammatory reactions is a function of M2b macrophages, which is why they are referred to as regulatory macrophages [62, 64]. Finally, NF-κB is involved in the signaling pathways that are triggered, together with MAPKs and PI3K/Akt [62].

IL-10 and glucocorticoids induce M2c (acquired deactivation macrophages) by activating STAT3 [58, 62]. The word "deactivated" denotes the capacity of macrophages to shift from M1 activation to M2 activation in vitro. IL-10 and TGF-β secreted by M2c present anti-inflammatory and pro-fibrotic activities [57]. Furthermore, M2c macrophages are able to efficiently phagocytose apoptotic cells because they exhibit large quantities of Mer receptor tyrosine kinase (MerTK) [65, 66].

M2d or tumor-associated macrophages (TAMs) can be polarized by IL-6 and adenosines [57, 58, 62]. Co-stimulation of the adenosine A2 receptor and TLR diminishes TLR-mediated synthesis of TNF-α, IL-12, and other inflammatory cytokines, while enhancing the expression of IL-10, TGF-β, and vascular endothelial growth factor (VEGF). M2d significantly contributes to the facilitation of angiogenesis and the spread of cancer [67].

Emerging technologies like mass cytometry and single-cell RNA sequencing (scRNA-seq) have led to the identification of previously unknown macrophage subtypes, each with its own unique set of functions and transcriptional fingerprints [46, 68, 69]. The effective immune response depends on the dynamic interaction of M1 and M2 macrophages, which balances inflammation and tissue repair and maintains homeostasis. Numerous clinical illnesses, including chronic inflammatory diseases, autoimmune disorders, and impaired healing of wounds, may arise and advance as a result of an imbalance in this interaction (Table 1).

Immune cells crosstalk in macrophage polarization

Various mechanisms enable immune cells to interact with one another during the process of macrophage polarization. This communication is essential in defining the functional characteristics of macrophages and their contributions to the immune response. The main product of Th1 cells and the primary cytokine linked to M1 activation is IFN-γ [70]. Various cells, such as natural killer (NK) cells and macrophages, have shown the ability to produce the cytokine [71]. The engagement of IFN-γ with its receptor, known as interferon-gamma receptor (IFNGR), first stimulates the Janus kinase (Jak) adapters, which subsequently initiates the activation of STAT1 [70]. Furthermore, IFN-α induces distinct gene expression profiles, encompassing MHC II, IL-12, and SOCS1 [70]. As the initial line of defense against intracellular infections, M1 macrophages release massive amounts of IL-12, which helps to enhance CD4 + cells' Th1 polarization. Therefore, M1 macrophages are highly effective effector cells that produce a profusion of inflammatory cytokines and engage in cytotoxic and microbicidal actions. M2 macrophages can be stimulated by the interaction with the cytokines IL-4, IL-13, and IL-10 [31, 70]. IL-4 and IL-13, generated by Th2 cells, basophil, and innate lymphoid cell, bind to the IL-4Rα receptor [47, 72]. Typically, the signaling pathways of JAK1 and JAK3 facilitate the activation of STAT6, which subsequently translocates to the nucleus to inhibit the expression of M1 genes [47, 70]. Additional transcription factors that play a role in this process include IRF4 and peroxisome proliferator-activated receptor γ (PPARγ). A diverse range of proteins, including Arg1, Ym1, Fizz1, CCL17, and CD206, are regulated by the actions of STAT6, IRF4, and PPARγ [47, 70]. All leukocyte types, particularly Treg, have the potential to express IL-10, which interacts with the heterodimeric IL-10 receptors (IL10-R1 and IL10-R2) [70, 73]. Upon binding of IL-10 to IL-10R, autophosphorylation of the receptor occurs, resulting in the activation of STAT3. The interaction of STAT3 with its promoter can modulate the production of SOCS3, which in turn inhibits the signaling pathways associated with proinflammatory cytokines [70].

B cells influence the polarization of macrophages toward either M1 or M2 by releasing cytokines or engaging in direct cell-to-cell interactions. B lymphocytes facilitate macrophage polarization toward the M1 phenotype through the secretion of IFN-γ and TNFα [74]. Wu el. studied the functions of B lymphocytes in mesenteric adipose tissue (MAT) regarding inflammation in adipose tissue. Compared to MAT B cells from mice on a standard diet, those from mice on a high-fat diet may promote the polarization of macrophages toward the M1 phenotype through cytokine secretion, resulting in fatty liver disease [75]. B lymphocytes facilitate the polarization of macrophages to the M2 via IL-10 [74]. B cells release more IL-10 during inflammation, which repolarizes M1 macrophages into M2 ones, or during SIV infection, B cells release IL-10, which attracts neighboring macrophages [76, 77]. This interaction activates FOXO1 in macrophages, resulting in an increase in interleukin-10

Table 1 Overview of the phenotypic characteristics of M1 and M2 macrophage subtypes in humans

Phenotype	Stimuli	Cell expression markers	Cytokines, chemokines, and other secreted mediators	Functions
M1	IFN-γ, TNFα, LPS, GM-CSF	CD68, CD86, CD80, MHC II, IL-1R, TLR2, TLR4	TNF-α, IL-1β, IL-6, IL-12, IL-23, IL-27, CXCL9, CXCL10, CXCL11, iNOS (mouse), ROS	Pro-inflammatory, Stimulation of Th1 immune response, Antigen presentation, Tissue damage
M2a	IL-4, IL-13	CD163, MHC II (low), MMR/CD206, CD200R, IL-1R II	IL-10, TGF-β, IL-1RA, CCL17, CCL18, CCL22, CCL24	Anti-inflammatory, Stimulation of Th2 immune response, Tissue remodeling
M2b	Immune complexes, LPS, IL-1R Ligands	CD86, MHC II?	TNF-α, IL-1β, IL-6, IL-10, CCL1, CCL20	Immunoregulation, Tumor progression
M2c	IL-10, TGF-β, glucocorticoids	MMR/CD206, TLR-1, TLR-8	IL-10, TGF-β, CCL16, CCL18, CXCL13, MerTK	Tissue repair/wound healing, phagocytosis of apoptotic cells
M2d	Adenosine receptor ligands, TLR ligands	NA	IL-10, VEGF	Angiogenesis, tumor progression

NA not available

production. As a result, this mechanism promotes the polarization of macrophages toward the M2 phenotype [74]. Additionally, the interaction between B cells and macrophages triggers the activation of macrophage STAT6, leading to their polarization into the M2 phenotype [78].

Role of miRNA in regulating macrophage polarization

The process of macrophage polarization is highly dynamic and complex, and posttranscriptional regulators play pivotal roles in controlling macrophage polarization. The following section briefly overviews how microRNAs contribute to macrophage differentiation, polarization, and plasticity. MicroRNAs (miRNAs) are short single-stranded noncoding RNA molecules containing 18–24 nucleotides (nts) that have been highly conserved throughout evolution. MicroRNAs are essential in modulating gene expression at the posttranscriptional stage by interacting with the 3′-untranslated region (UTR) of target messenger RNAs (mRNAs). This binding can result in either an increase in mRNA degradation or a repression of the translation process. miR-9, miR-125b, and miR-155 promote M1 and proinflammatory responses through the inhibition of several factors. MiR-9 overexpression reportedly increases macrophage-mediated inflammatory responses by downregulating the extracellular signal-regulated kinase 1/2 (ERK1/2) phosphatase Dusp6 through CCL2 [79]. Additionally, miR-9 improves M1 polarization via interacting with the PPARδ, hence reducing PPARδ activity and blocking the anti-inflammatory actions mediated by BCL-6 [80]. The M1 phenotype in macrophages is favorably controlled by miR-125b, which enhances their responsiveness to the M1 stimulant IFN-γ by targeting IRF4. Enhancement of M1 activation and proinflammatory responses in macrophages is achieved by knockdown of IRF4. [81]. Increased levels of miR-155 in M1 macrophages interact with NF-κB signaling inhibitors such SOCS1 and Src homology-2 domain-containing inositol-5-phosphatase 1 (SHIP1), which in turn stimulate the activation of proinflammatory genes [82, 83]. miR-155 suppresses C/EBPβ by interacting with its 3′UTR, which leads to the inhibition of M2 polarization [84]. Several miRNAs such as miR-124 and miR-223 regulate M2 polarization. MiR-124 decreases the release of IL-6 and TNF-α, respectively, by directly targeting STAT3 and TNF-α converting enzyme [85]. Furthermore, it was shown that miR-124 was elevated in macrophages treated with IL-4 and IL-13. Remarkably, knocking down miR-124 downregulated M2 markers (Ym1 and CD206) while upregulating M1 hallmarks (TNF, iNOS, and CD86) [86]. Mir-124 has been reported to target C/EBPα and PU.1 directly, regulating M2 polarization and playing a vital role in the CNS microenvironment. Further investigations indicated that miR-124 has the potential to be a therapeutic agent for alleviating inflammation and clinical symptoms, as well as promoting recovery in the central nervous system [87]. miR-223 has also been demonstrated to trigger M2 polarization in macrophages, and downregulation of miRNA enhances the release of IL-1β and IL-6 induced by LPS [88]. In RAW264.7 macrophages, increased miR-223 levels inhibit LPS-induced IL-6 and IL-1β secretion by targeting STAT3 [89]. MiR-223 attenuates proinflammatory polarization by inhibiting the NFκB/JNK pathway via the suppression of PBX/Knotted 1 homeobox 1 (Pknox1) [90, 91].

Macrophage polarization in GVHD

Macrophage infiltration serves as a biomarker of GVHD occurrence and development. A study by Nissen et al. revealed that in 11 out of 30 patients, a greater number of macrophages were found. Among these eleven patients, ten developed GVHD [92]. Terakura et al. also found that cutaneous GVHD is more severe when macrophage infiltration is higher [93]. As noted previously, macrophages play a role in GVHD; however, macrophage populations differ depending on the phase, tissue, and condition of GVHD. The macrophages that infiltrate acute GVHD usually display proinflammatory characteristics such as M1 macrophages, whereas chronic and refractory acute GVHD mainly comprises M2 macrophages.

Seno et al. reported that the presence of active M1 macrophages in the oral mucosa was associated with the severity of aGVHD [94]. Grafts with an increased M1/M2 ratio are more likely to experience Grade 2–4 aGVHD [95]. Liu et al. indicated an increase in M1 macrophages and a reduction in M2 macrophages during aGVHD [96]. Moreover, switching from M1 to M2 macrophages may serve as a therapeutic target in aGVHD [97]. Furthermore, CD163, an immune-regulating scavenger receptor, is found primarily in M2 macrophages. Biopsy analysis from patients with cutaneous GVHD revealed that an increased presence of CD163+ M2 macrophages is a significant predictor of refractory GVHD and worse prognosis [98]. The level of plasma sCD163 in allo-HSCT patients is a strong indicator of a high risk for cGVHD, suggesting the involvement of M2 macrophage activation and oxidative stress in the development of cGVHD [99]. Additionally, the interaction of M2 macrophages with CD4+ cells and fibroblasts may contribute to tissue fibrosis in cGVHD [100]. It can be concluded that acute GVHD is characterized by prevalent M1 macrophage activation, whereas refractory acute GVHD and chronic GVHD exhibit prominent M2 macrophage activation.

Macrophages in GVHD pathogenesis

Recruitment and activation of macrophages in GVHD

Chemokines are a wide class of immune cell movement coordinators that consist mostly of 8–12 kDa polypeptide subunits [101]. Chemokines also regulate the development and activation of myeloid cell subsets and effector lymphocyte subsets (Th1, Th2, Th17) [102]. More than 50 chemokines exist, and they are categorized into four main groups on the basis of the arrangement of cysteine residues near the NH2 terminus. These groups include CC, CXC, C, and CX3C [102]. M1 macrophages can undergo polarization by being activated through CXCR3, whereas CCR2 and CCR4 seem to preferentially polarize M2 macrophages [101, 103, 104]. The infiltration of macrophages in GVHD is regulated by chemokines [18]. One of the names for CXCL2 is macrophage inflammatory protein-2. CXCL2 is essential for recruiting T lymphocytes and macrophages to certain tissues in GVHD, and the inhibition of CXCL2 and CXCR2 leads to diminished severity of GVHD [101, 105]. Macrophages react to the epithelium through MCP-1, a vital chemokine [106]. The production of MCP-1 in the gastrointestinal tract, liver, skin, and lungs correlates with elevated inflammatory cytokines throughout the progression of aGVHD. CCR2 and MCP-1 regulate leukocyte migration and adhesion during inflammatory responses [106]. A research by Seno et al. demonstrated a substantial reduction in macrophage adherence to the oral mucosal epithelium following the treatment of macrophages from the tongues of aGVHD rats with an anti-CCR2 antibody and tissue sections with an anti-MCP-1 antibody [94]. Du et al. [107] revealed that CCL9 in cGVHD plays a significant role by enhancing macrophage infiltration, promoting lung immunoglobulin accumulation, elevating the ratio of splenic germinal center B cells to Tfh cells, and increasing the Tfh to T follicular regulatory cell ratio. Moreover, studies have noted that CCL15, the mouse counterpart of human CCL15, has the potential to serve as a diagnostic and prognostic biomarker for cGVHD [107]. In a study by Du et al. [108], pirfenidone significantly reduced the number of macrophages in the skin. In vitro chemotaxis assays revealed that pirfenidone impaired macrophage migration to MCP-1/CCL2 and IL-17A, which is linked to cGVHD generation [108]. Thus, previous studies have demonstrated that chemokines can control the recruitment of macrophages and that inhibiting them leads to an adjustment in GVHD severity.

Role of macrophages in tissue damage

The majority of myeloid inflammatory cells (monocytes and neutrophils) and antigen-presenting cells (macrophages and dendritic cells) are activated by DAMPs and PAMPs [109]. Moreover, the synthesis of proinflammatory cytokines by monocytes and inflammatory macrophages in reaction to DAMPs and PAMPs facilitates the progression of GVHD and enhances the activation of donor T cells [110]. The proliferated alloreactive T lymphocytes assault host tissues, including as the skin, liver, gastrointestinal tract, and hematopoietic system, resulting in organ damage and malfunction [111, 112]. Jardine et al. demonstrated the accumulation of allo-stimulatory donor-derived calprotectin + macrophages in acute skin GVHD [113]. Similarly, Aasebo et al. reported the accumulation of donor-derived calprotectin + macrophages in acute colonic GVHD [114]. Macrophages were identified in proximity to T cells, indicating that donor-derived macrophages engage with host T cells during acute colonic GVHD. The host T cells can be activated by the arrival of donor macrophages, which can present allogeneic peptides or produce proinflammatory cytokines [114]. Macrophage polarization and fibroblast activation will exaggerate collagen production, fibrosis, and tissue remodeling. Axatilimab inhibits CSF1-R + macrophages secreting profibrotic cytokines; -TGF-β production is inhibited by Belumosudil, Nintedanib, Pirfenidone or by Imatinib; and these compounds also inhibit the fibrotic process via PDGF-R. Fibrotic manifestations of cGVHD are often refractory to several treatments and, in some cases, are judged irreversible. Profibrotic cytokines such as TGF-beta and PDGF are upregulated and play key roles in fibrogenesis, stimulating the aberrant fibroblast activation with exaggerated collagen matrix production [114]. Activated macrophages derived from donors may cause tissue fibrosis by producing the profibrotic cytokine TGF-β [18]. This cytokine triggers the conversion of fibroblasts into myofibroblasts, which synthesize collagen, hence facilitating collagen production and accumulation in cGVHD [115]. As previously stated, pirfenidone can improve cGVHD by preventing macrophage infiltration and reducing TGF-β production [108]. During the development of cGVHD, macrophages may play a part in this process by interacting with T cells [115]. TLR signaling in APCs such as dendritic cells enhances antigen endocytosis and autophagy and augments the assembly of key antigen transport and processing systems [108]. In turn, activated host and donor APCs stimulate donor T cells either directly through donor T-cell receptors that recognize minor histocompatibility antigens, foreign MHC molecules and allogeneic peptides or indirectly through the release of pro-inflammatory cytokines and chemokines such as IL-1β, IL-6, IL-8, IL-10, IL-12, IL-21, IL-23, TGFβ and TNFα. Compared

with non-GVHD patients after HSCT and healthy donor controls, TLR4-mediated NF-κB signaling-related genes including TLR4, NF-κB, IL-6 and intercellular adhesion molecules 1 (ICAM-1) were significantly increased in patients with cutaneous cGVHD [111]. Alloreactive T cells in cGVHD become activated and undergo differentiation into different cell types, such as Th1/Tc1, Th17/Tc17, and Tfh cells, due to the influence of inflammatory cytokines (IL-6 and IL-12) [116, 117]. Th17/Tc17 cells are crucial in the pathogenesis of cGVHD [118]. In cGVHD, IL-17 is pivotal in disease pathophysiology by modulating the infiltration of F4/80 + macrophages into the skin, therefore facilitating the advancement of scleroderma [119]. IL-6 is a cytokine with multiple functions in inflammation. In addition to activating macrophages, IL-6 can promote the differentiation of Th17 cells [120]. Rintaro et al. conducted a study using a humanized cGVHD mouse model in which they engrafted human hematopoietic stem/progenitor cells into recipient mice transgenic for hIL-6. The findings indicated the coactivation of T cell and macrophages in the liver and lung, facilitating the progression of cGVHD [120]. The acute inflammation of the first phase of cGVHD creates an environment that favors excessive pro-inflammatory Th17 cells over regulatory T cells that suppress inflammation. The development of cGVHD has been shown to be associated with a dynamic imbalance that favors the production, expansion, and persistence of effector T cells, in particular Th17 cells driven by BCL2 expression over CD4 regulatory T cells [52]. Patients with active cGVHD had a significantly lower frequency of circulating T follicular helper cells (cTFH) compared with patients without cGVHD. This was associated with higher CXCL13 plasma levels suggesting increased homing of TFH to secondary lymphoid organs. The cTFH phenotype was skewed toward a highly activated profile with predominance of Th2/Th17 subsets and demonstrated increased functional ability to promote B cell immunoglobulin secretion and maturation [121].

Macrophages and cytokines

The role of antigen presentation in the development of aGVHD and cGVHD is well established. Donor and recipient APC activation plays a crucial role in initiating GVHD. Host APC activation occurs in the initial stage of GVHD and before donor cell infusion [121, 122]. Pretransplantation triggers inflammation, translocating PAMP molecules from the intestinal microbiota and releasing DAMP molecules from dying host cells [123]. PAMPs activate TLRs on innate cells, such as monocytes/macrophages and DCs, triggering an inflammatory cytokine cascade consisting of IL-12, IL-23, and IL-6 [110, 124]. Additionally, macrophages can engage with intestinal epithelial cells and control the expression of MHC II on these cells through the IL-12-IFN-γ cytokine axis. Lastly, deadly gut aGVHD is initiated by APC function and donor T cell priming [110]. Heurinen et al. investigated the gene expression associated with GVHD. They discovered that genes controlling IL-1β, interferon (IFN)-γ, and IL-6 responses were associated with GVHD. In addition, genes such as IL-1, IL-23R, TLR9, TNF and NOD2 are associated with the immune response in monocytes/macrophages, which can precede GVHD in intestinal lesions [125]. Kunpeng et al. indicated that trimethylamine N-oxide (TMAO), derived from choline, facilitates the advancement of GVHD by inducing M1 polarization, which in turn activates Th1 and Th17 responses. Furthermore, there was an elevation in the expression of the IL-1β, IL-6, and TNF-α genes in splenic F4/80 + macrophages within TMAO-induced GVHD tissues and in bone marrow-derived macrophages grown with TMAO [112]. The secretion of IL-12 by monocytes and macrophages enhances antigen presentation by nonhematopoietic cells in the host and worsens GVHD following irradiation [110]. In a study including a human IL-6 transgenic mouse model, Ono et al. demonstrated that increased quantities of human IL-12p40, IL-18, M-CSF, and IFN-α2 released by monocytes and macrophages might play a role in the onset of cGVHD in these animals [120]. Macrophage activation induced by M-CSF has been demonstrated to play a role in the advancement and onset of cGVHD through the generation of TGF-β, stimulation of fibroblasts, excessive creation of the extracellular matrix and collagen, and consequent tissue fibrosis [29, 119]. In line with these findings, pirfenidone improves murine chronic GVHD by hindering macrophage infiltration and reducing TGF-β production [108]. Strobel's et al. [126] work is the initial demonstration that the transcriptional statuses of macrophages are distinct in acute and chronic GVHD. In his investigation of macrophage subgroups in acute and chronic cutaneous GVHD, he employed scRNA-seq in conjunction with tissue immunofluorescence (IF) in GVHD skin lesions from adult HSCT patients. The cutaneous acute GVHD was shown to involve expanded CD163 + host and donor macrophages that produced less IFN-α and had a tissue-remodeling cytokine signature (TGF-β, IL-10). In contrast, the cutaneous chronic GVHD was associated with macrophages that exhibited a proinflammatory profile and decreased IL-10 production [126]. In aGVHD, there is an increase in TNF-α, IL-12, and IL-6, while cGVHD is characterized by upregulation of TGF-β.

Communication between macrophages and other immune cells

Macrophages can affect the activation and function of T cells through polarization, cytokine release and antigen

presentation [115–117, 127, 128]. Activated macrophages can induce donor T cells to become Th17 polarized cells by increasing the levels of IL-6, IL-1β, and IL-23. Ultimately, the accumulation of CCR6 + /CCR4 + Th17 cells that produce IL-17 exacerbates lung GVHD [129]. These findings suggest that macrophages play a role in T cell differentiation and influence the balance of the immune response. Using a human model and in vitro experiments, the researchers observed that the infiltration of F4/80 + macrophages and human effector memory T helper cells in lymphoid tissues and skin was affected by GVHD [130]. The interaction between these cells showed that in human mice, macrophages are stimulated by human T helper type 2 inflammatory cytokines. In a study by Haniffa et al., persistent receptor CD1a-/CD14 + /FXIIIa + skin macrophages were observed in GVHD lesions [131]. These macrophages impact the proliferation, cytokine secretion, and activation of antigen expression of allogeneic CD8 + T cells [131]. Additionally, diminished macrophage infiltration results in a decrease in the proportion of Th1 and Tc1 lineages but an increase in the proportions of Th2, Tc2, and Treg lineages, which are capable of suppressing effector T-cell infiltration and ultimately mitigating acute GVHD [95, 120, 132, 133]. Macrophages may facilitate immunological tolerance by diminishing effector T cell activation and fostering regulatory T cell growth through the secretion of an immune checkpoint molecule, namely the complement receptor of the immunoglobulin family [134]. A striking reciprocity was seen in the relationship between B cells and macrophages. In the early stages of chronic sclerodermatous GVHD, CD19-/-donor B cells escalated the severity of the disease and fibrosis by encouraging the growth of splenic IL-6-producing monocytes and macrophages. In the later stages of the disease, these cells encouraged the infiltration of TGF-β-producing monocytes and macrophages [135]. Alternatively, antibody production can be influenced by macrophages, and the output of antigraft antibodies is hindered by depleting macrophages [136, 137]. The depletion of macrophages resulted in decreased levels of TGF-β and exacerbated GVHD but led to increased B-cell infiltration. Conversely, depleting B cells results in increased levels of TGF-β and less severe GVHD, particularly liver fibrosis [138]. One possible reason for this might be that controlling B cells, the main players in the immune system, can have immediate and obvious effects, whereas reducing macrophages can throw off the equilibrium of the immune response [138].

Macrophage metabolism in GVHD

Glycolysis is essential in the metabolic processes of M1 macrophages [139]. Inhibiting glycolysis impacts various functions associated with inflammatory characteristics, such as phagocytosis, the generation of ROS and the release of proinflammatory cytokines [140]. M1 macrophages promote glycolysis by two stops in the tricarboxylic acid (TCA) cycle to generate citrate and succinate [141]. The collected citrate produces itaconate, a significant antimicrobial substance [142]. Succinate supports HIF1α, which maintains the glycolytic metabolism of M1 cells [143]. The activation of two enzymes—lactate dehydrogenase and pyruvate dehydrogenase kinase—by HIF1α enhances the process of pyruvate to lactate conversion and inhibits pyruvate's entrance into the TCA cycle, respectively [140, 144]. The pentose phosphate pathway is fuelled by glycolysis to produce NADPH, which is a co-factor for iNOS in NO production [145]. The acetyl-CoA produced during glycolysis synthesizes fatty acids (FASs) [140, 146]. An key part of M1 cell energy generation and prostaglandin biosynthesis is FAS [140]. The buildup of malonyl-CoA, which results from the first phase of the FAS process, can regulate the proinflammatory responses of macrophages [140]. M2 cells, on the other hand, rely mostly on oxidative phosphorylation (OXPHOS) and do not exhibit any interruption in the TCA cycle, which supplies materials for electron transport chain (ETC) complexes [141, 146]. In M2, there is an increase in fatty acid oxidation (FAO) and glutamine metabolism to produce the compounds needed for the TCA cycle [140]. For M2 OXPHOS and FAO, the α-ketoglutarate that is produced by glutaminolysis is crucial. Additionally, α-ketoglutarate promotes the activity of phosphogluconate dehydrogenase (PHD) and, as a result, suppresses the expression of HIF1α [147]. Therefore, glycolysis and the PPP are downregulated in M2 macrophages.

Macrophages and GVHD-related diseases

Although the mechanism of GVHD is not fully understood and there are controversial results in this field, various studies indicate the importance of macrophages in the pathogenesis of GVHD [94, 148, 149]. Data indicate that macrophages play dual roles in the immunopathogenesis of various diseases; for example, following irradiation, macrophages specifically engage with damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), thus activating T cells and subsequently contributing to the manifestation of GVHD. Furthermore, these proinflammatory macrophages secrete interleukin-12 (IL-12) and, through the stimulation of antigen presentation by nonhematopoietic host cells, exacerbate GVHD, ultimately leading to disease-related complications (Fig. 2) [150]. Given that macrophages interact with alloreactive T cells, the infiltration of macrophages can serve as a biomarker for GVHD. Specifically, the presence of M1 macrophages is more prominent in the acute phase, which

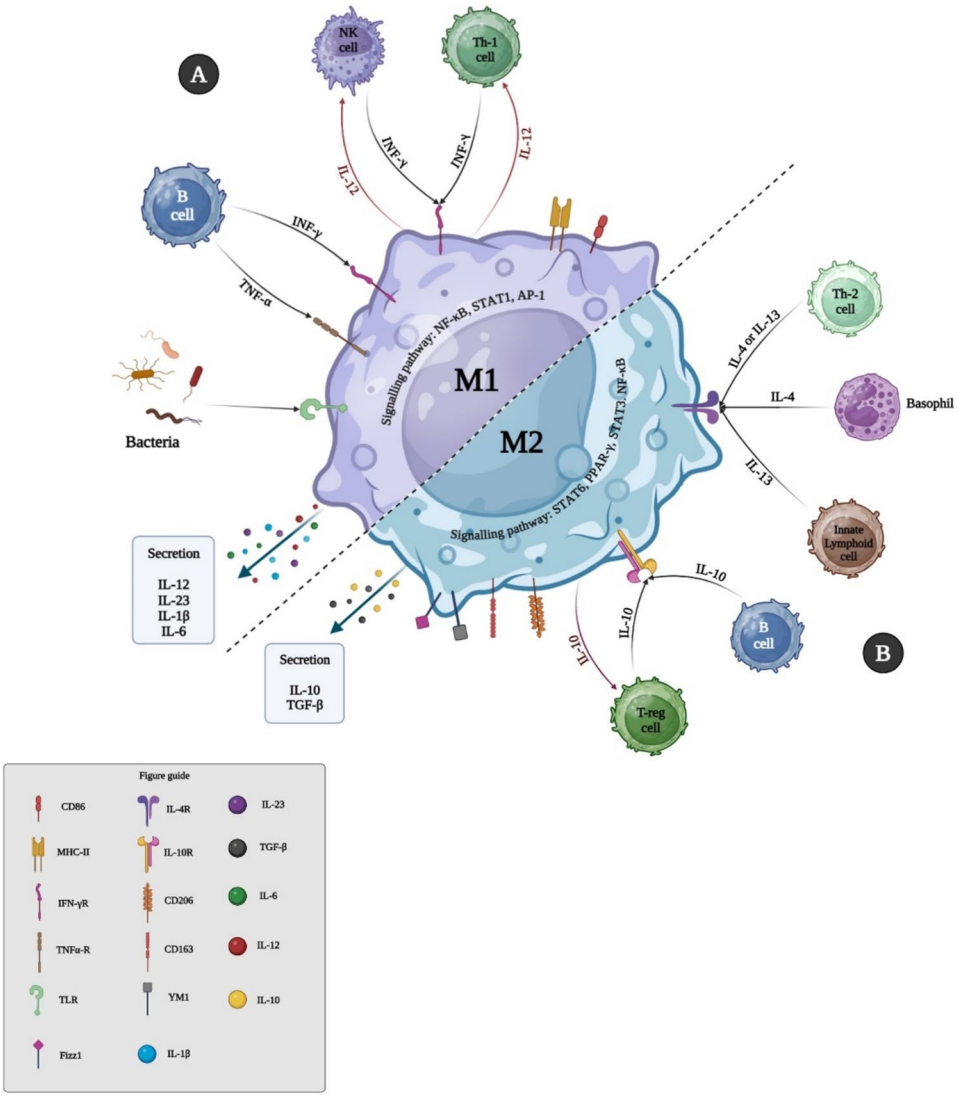

Fig. 2 The orchestration of macrophage activation and polarization by immune cells. **A** Macrophages with an M1 phenotype and the communication between TH1, NK and B cells. M1 stimuli, including LPS, IFN-γ, and TNF, activate the transcription factors NF-κB, STAT1, and AP1 through the TLR4, IFN-γ receptor, and TNFR pathways, resulting in the transcription of M1 genes. **B** TH2 cells, basophils, and innate lymphoid cells cause M2 polarization of macrophages by secreting IL-4 and IL-13 or by interacting with Treg cells and B cells via IL-10. M2 stimuli such as IL-4, IL-13, and IL-10 signal through IL-4Rα and IL-10R activate STAT6 and STAT3, which regulate the production of M2 genes. Additionally, PPAR-γ and NF-κB are involved in the control of these genes

is correlated with the cytokine storm and cell lysis events. Studies indicate that the ratio of M1 to M2 macrophages is directly correlated with the incidence of aGVHD within severity grades ranging from II to IV [18, 151]. This trend is reversed in the chronic phase, where it is associated with transforming growth factor beta (TGF-β) production by M2 cells and fibrosis [18], as evidence indicates that the ongoing substitution of tissue-resident monocytes with bone marrow-derived macrophages subsequent to allo-HSCT results in complications related to chronic GVHD across various tissues [152]. Furthermore, a research investigation revealed that the macrophages implicated in the pathophysiological mechanisms of both ulcerative colitis and acute GI-GVHD exhibit distinct gene expression profiles, indicating that a particular subtype of macrophages is specifically involved in the pathogenesis of graft-versus-host disease. The genes that are implicated are related to cellular adhesion, signaling pathways, the immune response to infectious agents, and the metabolic processes of macrophages [153]. In a separate investigation that conducted a retrospective analysis of the macrophage demographics among patients who had HSCT, it was observed that while the population of donor macrophages present in the mucosal secretions of both cohorts—those exhibiting GVHD and those who did not—demonstrated an increase, the subset of macrophages responsible for the secretion of calprotectin, classified as antimicrobial peptides, was notably elevated in individuals experiencing acute GVHD [114]. Moreover, the gut microbiota plays a significant role in the differentiation of TH1 and TH17 cells, which is accompanied by the activation of macrophages within the NLRP3 inflammasome pathway, ultimately resulting in the exacerbation of GI-GVHD [112].

In skin lesions caused by chronic GVHD, proinflammatory CCR7 + macrophages secrete IFN-γ, but in the acute state, the anti-inflammatory type of the population is predominant, which features the occurrence

of the CD163 marker and the secretion of the cytokines IL-10 and TGF-β [149]. In one study, $CD11c^+$ $CD14^+$ macrophages were abundantly found within the dermis of skin lesions in acute GVHD patients. Although there is a pronounced increase in the activity of Tregs, this increase remains inadequate to prevent a decrease in immune tolerance. This group of macrophages becomes the dominant population even after disease recovery, indicating the long-term effects of T-cell pathology on the dermal immune environment and the subsequent probability of autoimmune disease onset and progression [154]. The targeting of proinflammatory macrophages presents potential therapeutic applications for GVHD, as numerous studies indicate that the suppression of GVHD can be achieved through certain pharmacological agents that are prescribed, which possess the capacity to impede macrophage polarization in acute and chronic GVHD or facilitate the transition toward M2 macrophages in acute GVHD [96, 97, 152]. It has been demonstrated that macrophages play a role in the pathogenesis of bone marrow fibrosis, a rare manifestation of chronic GVHD. These cells accomplish this through the secretion of TGF-β, which facilitates the migration of Nestin + mesenchymal stem cells (MSCs) and initiates their differentiation into fibroblasts [155]. In the context of lung and joint chronic graft-versus-host disease (cGVHD), there are discernible alterations in the surface markers of both classical and nonclassical macrophages, indicating their potential involvement in organ-specific cGVHD progression. For example, the expression level of CCR5 on the surface of classical macrophages has decreased, whereas nonclassical macrophages exhibit increased expression of CD204 and decreased expression of CX3CR1 [156]. Furthermore, the progression of cGVHD affecting the liver and lungs may involve the coactivation of both macrophages and T cells, which could play a significant role. Additionally, alveolar macrophages, which are derived from monocytes, exhibit distinct characteristics from those of tissue-resident macrophages, particularly their increased capacity to secrete TGF-β and their manifestation of fibrotic features [157].

Macrophages in GVHD resolution

Functions of macrophages in the immune response

Macrophages show great heterogeneity and plasticity and can be activated and polarized into different phenotypes through the stimulation of multiple signaling molecules in the same or different microenvironments [14]. Tissue-resident macrophages participate in many pathologies, such as neurodegeneration in microglia, osteoclasts and macrophages in osteoporosis, cardiac or vascular macrophages in atherosclerosis, Kupffer cells in liver disease, and alveolar macrophages in lung diseases. Macrophages can be classified as classical activated macrophages with germicidal activity, wound-repairing macrophages with tissue repair functions, and regulatory macrophages with anti-inflammatory activity. Another traditional classification divides macrophages into M1 macrophages and M2 macrophages. Remarkably, it is possible to induce a cross-switch between M1 macrophages and M2 macrophages. Macrophage-targeted therapies based on macrophage functions, such as self-renewal, phagocytosis, chemotaxis, the inflammatory response, the protumor response, and therapeutic protein secretion, have been used in clinical trials [158].

Macrophage infiltration in GVHD

Macrophage infiltration is a biomarker for the occurrence and development of GVHD. Both free and clustered macrophages are important in the pathogenesis of GVHD. In Nissen et al.'s study, an increase in the number of macrophages was detected in 11 of 30 patients. Ten of these eleven patients developed GVHD. A significant difference was observed between 10 of the 14 patients who presented with a macrophage before bone marrow transplantation and only one of 19 patients without GVHD. Also, heavier macrophage infiltration will be associated with higher severity of cutaneous GVHD [93]. It also showed that biopsies from liver, intestine and skin of patients with fatal GVHD showed a significant predominance of CD68 + macrophages in the inflammatory infiltrate. These findings show that the infiltration of macrophages is positively correlated with the occurrence and development of GVHD. In addition, macrophages are polarized into different populations and infiltrate different target organs [159].

Infiltration of M1 and M2 macrophages in GVHD

Although an association between M1 macrophages and acute GVHD is reported, CD4 + memory T cells and M0 macrophages have been observed at the onset of acute GI GV, increased M1 macrophages at the onset and steroid-resistant acute GVHD, with increased M2 macrophages [148]. In acute steroid-resistant gastrointestinal GVHD, the difference between macrophage polarization in acute GVHD and refractory acute GI may be due to the complex stages and mechanisms of steroid-resistant GVHD, where resistance is more related to the thrombotic system. The difference between macrophage polarization in acute GVHD and refractory acute GI may be due to the complex stages and mechanism of steroid-resistant GVHD, where

resistant GVHD is more related to the thrombotic system [160]. In addition, CD163, a scavenger receptor, is mostly expressed in M2 macrophages. It was also reported that macrophage CD163 infiltration was the only predictive factor for resistant acute GVHD when the number of CD163(+) macrophages, CD8(+) T cells, and CD1a(+) dendritic cells were considered. [98]. Furthermore, high concentrations of soluble plasma CD163 at day 80 are associated with the incidence of new chronic GVHD [99]. The M2-derived macrophage phenotype contributes to chronic GVHD, resulting in not only a CD163 + macrophage population but also a CD11b + monocyte/macrophage population and an F4/80 + CSF-1R + CD206 + iNOS- population [161]. Thus, M1 macrophage polarization predominates in acute GVHD, while M2 macrophage polarization predominates in refractory acute GVHD and chronic GVHD [161].

Tissue-resident macrophages (Macrophages) are a major antigen-presenting cell subset in adult human barrier organs with a wide spectrum of pro- and anti-inflammatory properties, depending on their polarization state. Macrophages can rapidly change their gene expression in response to environmental stimuli along a continuum of what has formerly been simplified as M1 and M2 polarization. Skin-resident macrophages are induced by a T helper (Th)-2 cytokine environment [7] and express surface receptors, including CD11b, CD68 and CD206 [8]. Their detrimental role in inflammatory skin diseases has been shown for atopic dermatitis, psoriasis and discoid lupus skin lesions, where macrophages are characterized by the expression of F13A1 or CD163 and genes related to chemotaxis and transforming growth factor beta (TGF-β) signaling [9, 10]. In a cytokine-rich environment, proinflammatory macrophages may produce interferon gamma (IFN-γ), which is a potent autocrine mediator that induces the MΦ phenotype found in psoriatic skin. However, skin-resident macrophages also have anti-inflammatory properties and can promote tissue repair via local production of interleukin (IL)-10 [12]. Recently, a role for macrophages in GVHD pathology has been identified in studies of tissues obtained from human HSCT recipients. Aasebo et al. detected a fivefold increase in the number of donor-derived proinflammatory macrophages in colon biopsies of patients with gastrointestinal aGVHD [13]. In cutaneous aGVHD, Jardine et al. reported a population of monocyte-derived macrophages that mediate keratinocyte cytopathicity ex vivo [98, 99, 161].

Regulatory functions of macrophages in GVHD

The elimination of Treg cells as the source of Th17 cell-inducing TGF-β left activated T cells as likely suspects but which members of this heterogeneous population are key? Using a TGF-β reporter mouse line, Gutcher et al. determined that in vitro polarized Th1, Th2 and Th17 cells all can express TGF-β but it is most highly Rag1 −/− reconstituted (Rag1-deficient (Rag1-/-) mice are an immunodeficient stock strain that lack functional B and T lymphocytes) expressed in Th17 cells. Further, induction of EAE (experimental autoimmune encephalomyelitis) in Rag1 −/− reconstituted with an equal mix of wild-type and *Tgfb1*$^{f/n}$ *Cd4-cre* bone marrow(mice with floxed/null alleles (*Tgfb1*f/n mice) that express a *Tgfb1* floxed allele and a TGFβ1-GFP knockin allele [162] resulted in wild-type derived Th17 and Th1 cells in the CNS whereas substantially fewer IL-17$^+$ cells originating from the CD4$^+$ *Tgfb1* deficient bone marrow were detected, elegantly establishing that TGFβ acts in an autocrine manner to promote Th17 cell differentiation [163]. The role of macrophages in GVHD can be regulated by cytokines. Th17-produced IL-17 participates in GVHD by modulating the interaction between macrophages and CD4 + T cells. IL-17 can reduce macrophage infiltration, reduce IL-12 and IFN-γ production, suppress Th1 responses, and reduce acute GVHD [163]. ROCK2 (The rho-associated coiled-coil-containing protein kinase-2 (ROCK2) signaling pathway regulates the Th17/regulatory T cells balance and controls profibrotic pathways) inhibition significantly diminished STAT3 phosphorylation and binding to IL-17 and IL-21 promoters and reduced interferon regulatory factor 4 and nuclear hormone RORyt protein levels in T cells derived from healthy subjects or rheumatoid arthritis patients. Simultaneously, selective ROCK2 inhibition with Belumosudil (KD025) KD025 also promoted the suppressive function of regulatory T cells through up-regulation of STAT5 phosphorylation [164]. KD025 has been shown to ameliorate cGVHD in multiple murine models and inhibit the secretion of IL-21, IL-17 and interferon y along with decreasing phosphorylated STAT3 and reduced protein expression of interferon regulatory factor 4 and B-cell lymphoma (BCL6) in human peripheral blood mononuclear cells purified from active cGVHD patients [165]. Reducing the infiltration of macrophages that have migrated to MCP-1 and IL-17A and increasing TGF-β production could be therapeutic strategies for GVHD. Additionally, M1 macrophage polarization and effector T-cell infiltration can be suppressed via the use of IL-33-expanded Tregs in acute GVHD. IL-33 also has a paradoxical effect because the administration of IL-33 after allogeneic hematopoietic stem cell transplantation exacerbates acute GVHD by engaging in donor T-cell augmentation. The opposite effect may be due to a complex cytokine network that balances synergistic and antagonistic effects [166]. M2 macrophage polarization can mediate immunosuppression through tumor-associated macrophages, which predominantly display an M2-like phenotype. These macrophages suppress CD8 + T-cell recruitment by inhibiting macrophage-produced CD8 + T-cell chemokines such as CXCL9 and CXCL10. Notably, the cytokine efficacy differed between these two studies, which can be explained

by the different subsets of macrophages that interact with T cells [58]. In addition, M2 macrophages may transform into M1 macrophages and contribute to T-cell function. M2-like macrophages stimulated by low-dose radiation can differentiate into iNOS + /M1-type macrophages, produce NO, and enhance the infiltration of intratumoral CD3 + , CD8 + , and CD4 + T cells by increasing the expression of Th1 cytokines. However, it is still incompletely adapted in the interaction between macrophages and T cells. Increased macrophage-derived nitrite production can suppress T cells in the peritoneal cavity [167].

Macrophages and fibrosis in GVHD

Autoantibody production, immunoglobulin deposition, and fibrosis are characteristic features of chronic GVHD [1]. CD4 + T cells, fibroblasts and B cells interact with macrophages, and this interaction plays an important role in chronic GVHD. Decreased infiltration of CD4 + T cells and CD11b + monocytes/macrophages and suppression of fibroblast proliferation reduce the severity and degree of fibrosis in chronic cutaneous scleroderma GVHD [168]. Additionally, pirfenidone treatment can reduce macrophage infiltration and TGF-β production, impair GC reactivity, and inhibit B-cell antibody production and fibrosis, thus reducing chronic GVHD [108]. In summary, macrophage infiltration, macrophage TGF-β production, B-cell reactivity, fibroblast proliferation, and CD4 + T-cell infiltration contribute to the development of chronic GVHD.

Another attempt to improve fibrosis in chronic GVHD patients is the use of 4-phenylbutyric acid. Markers of endoplasmic reticulum stress are present in chronic GVHD. GVHD-induced chronic endoplasmic reticulum stress in macrophages can be reduced by the administration of 4-phenylbutyric acid, which leads to the differentiation of M1 macrophages and dysfunctional fibroblasts [169]. In other words, macrophages and M2 fibroblasts contribute to fibrosis in GVHD. As previously mentioned, CCL9 functions in chronic GVHD by regulating macrophage infiltration, immunoglobulin deposition, splenic GC B-cell reactivity, and CD4 + T-cell polarization [170]. In other words, the reactivity and interaction of macrophages, B cells and T cells are regulated by cytokines. Therefore, we conclude that macrophage infiltration, interactions between macrophages and other cells, and the cytokine network should be considered in chronic GVHD.

Macrophage infiltration has been observed in many fibrotic diseases. Moreover, the apoptosis and autophagy of macrophages weaken fibrosis [171]. Membrane molecules expressed on macrophages and cytokines produced by macrophages participate in this process of fibrogenesis. CD14, a coreceptor of Toll-like receptor 4 expressed on macrophages, may be stimulated by Toll-like receptor exposure and activate macrophages by inducing TGF-β production, leading to a beneficial effect through myeloid differentiation. The role of MyD88-dependent pathways in systemic sclerosis: Macrophage receptors with a collagen structure containing arginine residues are another type of scavenger receptor expressed on macrophages, which can induce macrophage polarization to the profibrotic M2 subtype and contribute to fibrosis. In terms of cytokines, increased TGF-β signaling further promotes fibrosis [172]. Macrophage receptors with a collagen structure containing arginine residues are another scavenger receptor expressed on macrophages that can induce macrophage polarization to the profibrotic M2 subtype and contribute to fibrosis. In terms of cytokines, increased TGF-β signaling increases fibrosis [173].

Role of macrophage-derived extracellular vesicles in GVHD

Macrophage-derived extracellular vesicles (EVs) are small, membrane-enclosed entities secreted by macrophages that are integral to intercellular signaling and the regulation of numerous biological phenomena. These vesicles exhibit heterogeneity in their composition and possess the capacity to transport a wide variety of molecular constituents, including proteins, lipids, and nucleic acids, which can modulate the activities of recipient cells [174]. Research has demonstrated that extracellular vesicles originating from M1 macrophages have the capacity to promote direct intercellular interactions that subsequently enhance T-cell activation, thereby augmenting the inflammatory environment typical of GVHD [175]. Furthermore, macrophage-derived EVs have the capacity to transport inflammatory cytokines, including TNF-α and IL-6, which are recognized for their role in inducing tissue damage and aggravating the manifestations of GVHD. The release of these cytokines can initiate a feedback loop of inflammation that exacerbates tissue injury and facilitates additional immune activation [176].

Macrophage-derived extracellular vesicles have the capacity to modify the M1/M2 macrophage ratio within the microenvironment of GVHD, which directly impacts the prognosis of this condition. Extracellular vesicles originating from M1 macrophages have the ability to convey miR-155, a microRNA identified to augment inflammatory responses while concurrently inhibiting M2 polarization, thereby disturbing the equilibrium in favor of M1 macrophages [172]. In addition to miR-155, extracellular vesicles derived from macrophages possess the capacity to convey miR-223. miR-223 is implicated in the modulation of macrophage polarization and the orchestration of inflammatory responses. It has the potential to inhibit the activation of the NLRP3 inflammasome, consequently mitigating inflammation, which may prove advantageous in regulating

the progression of GVHD [177]. In addition, macrophages have the potential to disseminate distinct types of miRNAs that help diminish inflammation and contribute to a more favorable prognosis in GVHD. For example, miR-124 exhibits anti-inflammatory characteristics and is recognized for its capacity to modulate macrophage polarization toward an anti-inflammatory phenotype. This particular miRNA may assume a protective function within the GVHD microenvironment by mitigating tissue damage and inflammatory responses [178].

The role of the microbiome in shaping macrophage function in GVHD

Microbiota

A total of 10^{13} to 10^{14} types of microbiota, including bacteria, fungi and viruses, live in a healthy human body. Most of the microbiota colonize the gut, where it is known as the gut microbiota. Under physiological conditions, the gut microbiota is highly diverse and consists mainly of Firmicutes, Bacteroidetes, Actinomycetes, and Proteobacteria. It is involved in several important physiological processes, including host nutrient absorption, substance metabolism, and immune defense [179]. The intestinal microbiota plays an important role in the production of bioactive metabolites. Specifically, the microbiota consumes undigested food eaten by the host and excretes IECs as substrates. They then undergo complex and active metabolic reactions in the intestine to produce a variety of small-molecule metabolites, including short-chain fatty acids (SCFAs), bile acids (BAs), and tryptophan and its derivatives (such as indole and indole derivatives) [180]. These bioactive metabolites can directly or indirectly affect host physiological functions, including host body growth, digestion and metabolism, and immune regulation [181].

Microbiota and GVHD

The delicate balance between the human host and the gut microbiota must be actively maintained by both parties to reach a healthy steady state. Disturbance of the intestinal microbial balance can lead to loss of host functions, including intestinal barrier dysfunction, immune system dysfunction, and inflammation, which are associated with various diseases [181, 182]. In allo-HSCT patients, a pretransplant pretreatment regimen including chemotherapy, radiotherapy, immunotherapy, and broad-spectrum antibiotics followed by posttransplant activation of allogeneic T cells can damage and alter the gut composition of IECs. Microbiota leads to a decrease in intestinal commensal bacteria [183, 184]. The interaction between the gut microbiota and GVHD has been studied for decades. GVHD most commonly affects tissues that have a microbiota, such as the gut, mouth, and skin, or the liver (which is seeded with microbial products from the portal circulation). What is the evidence that the microbiota may influence GVHD? In the early 1970s, rearing mice in a sterile environment or antibiotic-mediated intestinal cleansing was shown to reduce the symptoms of aGVHD. However, several subsequent clinical sample studies confirmed that reduced gut microbial diversity in post-HSCT allo-HSCT patients was associated with a significantly increased risk of GVHD. Therefore, changes in gut microbial diversity and composition play important roles in the incidence and development of GVHD and can therefore be used as prognostic indicators for GVHD patients [180].

Microbiome and GVHD with Th

Changes in the gut microbial composition may contribute to the imbalance of specific immune cell subsets that contribute to the pathogenesis of GVHD. Changes in gut microbial diversity are significantly associated with the occurrence, development, and prognosis of GVHD and have confirmed that decreased gut microbial diversity after allo-HSCT was significantly associated with shortened overall survival (OS), increased GVHD-related mortality, and increased risk of developing GVHD. Dysbiosis of the gut microbiota is manifested by altered composition of the microbiota and is significantly associated with GVHD. 37 Studies have indicated that GVHD is associated with decreased abundance of specific bacteria, including Firmicutes (Clostridium, Faecalibacterium, Lachnospiraceae, Ruminococcaceae, Eubacteriaceae and Peptostreptococcaceae), Bacteroidetes (Bacteroides and Parabacteroides), and Actinobacteria, as well as an increase in the abundance of Proteobacteria (Gammaproteobacteria and Enterobacteriales), Verrucomicrobia (Akkermansia) and opportunistic pathogens belonging to the Firmicutes (Lactobacillus, Staphylococcaceae, and Enterococcus). Gut microbiota may contribute to aGVHD by affecting the Treg/Th17 balance. 38 In addition, using a cGVHD mouse model, bacterial extracts of gut microbiota from cGVHD patients were found to induce the murine splenic T cells to differentiate into Th1 cells and inhibit their differentiation to Treg cells, resulting in Th1/Treg imbalance, which was significantly correlated with the onset of cGVHD [53]. Therefore, these studies have shown that gut microbial imbalance can lead to immune imbalance, which in turn participates in the pathogenesis of GVHD [184]. A decreased abundance of Lachnospiraceae and Ruminococcaceae and an increased abundance of Enterobacteriaceae are associated with a Treg/Th17 imbalance, possibly via H3 acetylation in CD4 + T cells [185]. Therefore, the gut microbiota

may cause aGVHD by affecting the Treg/Th17 balance. Furthermore, in a mouse model of cGVHD, bacterial extracts of the gut microbiota from cGVHD patients were found to induce murine splenic T cells to differentiate into Th1 cells and inhibit their differentiation into Treg cells, leading to a Th1/Treg imbalance. This finding was significant. It is associated with the onset of cGVHD. Therefore, these studies have shown that intestinal microbial imbalance can lead to immune imbalance, which in turn participates in the pathogenesis of GVHD [186]. Metabolites such as SCFAs, tryptophan, TMAO, tyrosine, and BAs play important roles in the occurrence and development of GVHD. Moreover, they represent an important opportunity for a novel therapeutic strategy for the treatment and prevention of GVHD. However, the specific mechanisms by which gut microbiota-derived metabolites regulate GVHD is not completely clear (Table 2) [186].

Therapeutic targeting of macrophages in GVHD

Macrophages are a heterogeneous population that engage in multifaceted interactions with other immune cells—such as T and B lymphocytes—and nonimmune cells, such as fibroblasts, within the GVHD microenvironment. These interactions have significant potential to influence the outcomes of GVHD [18]. Macrophage infiltration is a biomarker for GVHD incidence and is regulated by various cytokines. CXCL2 plays a key role in macrophage recruitment, whereas IL-17, in contrast, suppresses macrophage infiltration [105, 163]. Macrophage infiltration affects the organs involved in GVHD and alters the phase and severity of the disease. Low levels of macrophage recruitment reduce Th1 cells but increase Th2 and Treg cells in affected organs, ultimately alleviating acute GVHD [120, 133]. These effects promote the differentiation of macrophages into the M1 phenotype in GVHD mice, where they then secrete pro-inflammatory mediators to induce the differentiation of allogeneic T cells

Table 2 Microbial species and correlation with GVHD

Microbiota composition in GVHD	Change in abundance	Details	References
Lachnospiraceae (e.g., Blautia)	Reduced	Lachnospiraceae ferment dietary fibers, producing short-chain fatty acids that can enhance immune function and overall gut health	[212]
Ruminococcaceae	Reduced	The imbalance in Treg/Th17 ratios due to reduced Ruminococcaceae may contribute to the pathogenesis of aGVHD Significant reductions in short-chain fatty acids (SCFAs) like butyrate, which are produced by these bacteria were seen in severe aGVHD	[213]
Clostridia	Reduced	The disruption of mucosal microbiota including a decrease in Clostridia and an increase in Proteobacteria leads to shift in microbial composition that is linked to infectious and inflammatory complications in GVHD	[214]
Faecalibacterium prausnitzii	Reduced	Produce butyrate, which contributes to gut barrier integrity and immune modulation Stimulates IL-10 production in monocytes, promoting an anti-inflammatory response Its extracellular vesicles enhance macrophage repair mechanisms, reducing pro-inflammatory cytokine production	[215–217]
Actinobacteria	Reduced	Modulate immune responses affecting GVHD outcomes	[218]
Enterococcus (e.g., Enterococcus faecium)	Increased	May disrupt intestinal barrier integrity, leading to increased permeability and inflammation Alter the production of short-chain fatty acids, which are crucial for maintaining gut health and immune regulation	[214, 219]
Proteobacteria	Increased	Includes Gammaproteobacteria and Enterobacteriales linked to inflammation	[220, 221]
Verrucomicrobia	Increased	Notably Akkermansia; may influence gut barrier function and immune modulation	[222]
Firmicutes	Increased	Includes opportunistic pathogens like Lactobacillus, Staphylococcaceae, and Enterococcus SCFAs like butyrate, produced by Firmicutes, support gut barrier integrity and modulate immune responses by reducing inflammation and promoting regulatory T cell differentiation	[223]

into Th1 and Th17 subsets; this differentiation ultimately causes worsening of GVHD. Moreover, rationally altering the composition of the intestinal microbiome toward high-butyrate producers was also shown to mitigate GVHD. The administration of high butyrate-producing Clostridia organisms results in increased intestinal regulatory T cells (Tregs), which play an important role in regulating gut inflammatory responses through several mechanisms, including the release of anti-inflammatory cytokines, such as IL-10. Tregs are protective against GVHD, likely through suppression of alloreactive T cells that mediate GVHD.66 Furthermore, SCFAs can induce IL-22 responses in innate lymphoid cells (ILCs), which can exert anti-inflammatory and regenerative effects on IECs [187]. However, it cannot be concluded that the removal of macrophages leads to complete recovery, as further studies have shown that depleting macrophages from the GVHD microenvironment decreases TGF-β levels, which subsequently exacerbates GVHD [138]. Although the macrophage population in the GVHD microenvironment is derived from various sources—such as monocyte-derived macrophages, host residual macrophages, and donor macrophages—research suggests that they exhibit different polarizations in acute and chronic GVHD [1]. Traditionally, macrophages are categorized into two main groups: M1 and M2 macrophages. M1-polarized macrophages originate from M0 macrophages that are stimulated by IFN-γ, and they produce proinflammatory cytokines while also effectively presenting antigens [188]. M1 macrophages play a crucial role in the pathogenesis of GVHD by contributing to tissue damage in targeted organs. In the context of GVHD, M1 macrophages are characterized by their proinflammatory phenotype, driven by the secretion of cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6). These cytokines promote an inflammatory environment that can lead to tissue injury, particularly in organs such as the skin, liver, and gastrointestinal tract [125]. For example, studies have shown that the accumulation of M1 macrophages in the oral mucosa is correlated with the severity of acute GVHD, as these cells secrete matrix metalloproteinases that degrade the extracellular matrix, facilitating further inflammation and damage to epithelial tissues [94]. Additionally, the polarization of macrophages toward the M1 phenotype is exacerbated by factors such as the microbial metabolite trimethylamine N-oxide (TMAO), which enhances M1 macrophage activation and contributes to the overall inflammatory response observed in GVHD [112]. Thus, M1 macrophages not only amplify the immune response against host tissues but also directly mediate damage through inflammatory mediators and tissue remodeling processes, leading to significant morbidity associated with GVHD. M2 macrophages play a dual and complex role in GVHD and are associated primarily with tissue repair and immunoregulation. These macrophages are characterized by their anti-inflammatory properties and are involved in the resolution of inflammation following the initial immune response [176]. In the context of GVHD, M2 macrophages secrete a variety of cytokines, such as interleukin-10 (IL-10) and transforming growth factor-beta (TGF-β), which promote tissue healing and modulate the immune response by inhibiting the activation of proinflammatory T cells [129]. Additionally, M2 macrophages contribute to the maintenance of tissue homeostasis by facilitating the clearance of apoptotic cells and promoting extracellular matrix remodeling [189]. However, their role can be context dependent. While they can mitigate tissue damage and promote recovery, an excessive or dysregulated M2 response may also contribute to chronic GVHD by supporting a fibrotic environment in affected organs [190, 191]. This balance between their protective and potentially harmful effects underscores the importance of M2 macrophages in the pathophysiology of GVHD, highlighting their potential as therapeutic targets for modulating this disease.

Multiple in vitro and in vivo studies have revealed that M2 macrophages possess considerable potential to suppress acute GVHD and improve this condition. In this context, Yan Su et al. reported that extracellular vesicles (EVs) containing arsenic trioxide (ATO) shift M1-polarized macrophages toward the M2 phenotype through the mTOR-autophagy pathway. The results demonstrated the ameliorating effects of M2 macrophages on acute GVHD in a mouse model [97]. These functions are attributed to several mechanisms, such as anti-inflammatory cytokine production, tissue repair and remodeling, and the regulation of immune responses [148]. Notably, endeavors are being made to identify naturally occurring compounds and pharmacological agents that convert macrophage polarization in vitro and, subsequently, in vivo. Although these investigations are at the cell line and animal model levels, promising results have been achieved. Celastrol, luteolin (3′,4′,5,7-tetrahydroxy flavone), curcumin, and crocin are reported to influence macrophage polarization toward the M2 phenotype [192].

The preclinical and clinical research has been conducted to evaluate the efficacy and application of the macrophage-based therapy. GFP + M2 macrophages could improve GVHD in BALB/c mice; the proposed approach to this outcome was inhibiting the expansion of alloreactive T cells through cell contact. Additionally, the infused macrophages express the high level of Areg-1 enzyme that depletes L-arginine, which is introduced as an iNOS substrate [25]. Macrophage infiltration to the organs is the first step in acute-GVHD macrophage-based pathology. A conducted study evaluates the inhibition of macrophage infiltration by the Spahngosine-1 receptor agonist, CYM-5442. Although, the intervention failed to inhibit T cell infiltration then could not prevent acute GVHD, reduce the number of macrophages in GVHD without directly affecting their proliferation and

cause inhibited GVHD [193]. The same consequences were reported by Jing Du et al. that applied Pirfenidone. The FDA-approved drug reduces macrophage infiltration to GVHD organs and TGF-β. Additionally, the drug dampens antibody production through the decline of splenic Germinal Center B cell and Follicular T cell frequencies [108]. Training macrophages to follow a proposed target is a new avenue in medical approaches. As Jeljeli et al. have shown the LPSlow-trained macrophages significantly reduce T cell activation and proliferation through IL-10, and decrease release of IL-2, IFN-γ, TNF-α, and IL-6. The trained macrophage intervention improves GVHD mice survival and alleviates the clinical signs of GVHD in mice by suppressing T cells and reducing autoantibody production, and tissue fibrosis. It is important to note that this approach did not influence anti-tumoral features in mice [138]. The brain is one of the organs that are influenced by chronic GVHD. The depletion of macrophages in the periphery and macroglia in the brain by Pexidartinib (CSF-1R) inhibitor PLX3397 showed a significant improvement in clinical, histological, and cognitive impairments in mice [194].

The last mentioned preclinical study approach has made its way into clinical studies. The two independent studies have applied CSF-1R blocker monoclonal antibody (Axatilimab). In the phase I/II clinical trial of Axatilimab that was conducted by Kitko et al., 40 patients (17 in phase I; 23 in phase II) received at least one dose. Phase I established 3 mg/kg administered every 4 weeks as the optimal biologic dose, with dose-limiting toxicities observed at 3 mg/kg given biweekly. Treatment-related adverse events (TRAEs) occurred in 30 patients, with grade ≥ 3 TRAEs reported in eight patients, predominantly attributable to on-target CSF-1R inhibition. No cytomegalovirus reactivations were noted. The phase II cohort achieved the primary efficacy endpoint, with a 50% overall response rate (ORR) at cycle 7 day 1. An ORR of 82% was observed within the first six cycles, supporting regulatory approval. Responses were observed across all affected organs irrespective of prior therapies, and 58% of patients reported significant symptom improvement on the Lee Symptom Scale. Decreased skin CSF-1R-expressing macrophages further indicated the on-target activity of axatilimab (NCT03604692) [195]. In another study evaluating axatilimab, 241 patients were enrolled across three dose groups: 0.3 mg ($n = 80$), 1 mg ($n = 81$), and 3 mg ($n = 80$). The primary efficacy endpoint was achieved in all groups, with overall response rates (ORR) of 74% (95% CI 63–83), 67% (95% CI 55–77), and 50% (95% CI 39–61) for the 0.3-mg, 1-mg, and 3-mg groups, respectively. Significant symptom reduction, indicated by a ≥ 5-point improvement on the modified Lee Symptom Scale, was observed in 60%, 69%, and 41% of patients in the respective dose groups. The most common adverse events were dose-dependent, transient laboratory abnormalities consistent with CSF-1R blockade. Treatment discontinuation due to adverse events occurred in 6%, 22%, and 18% of patients in the 0.3-mg, 1-mg, and 3-mg groups, respectively (NCT04710576) [196]. Axatilimab demonstrates promising efficacy and manageable safety across various dosing regimens for the treatment of chronic graft-versus-host disease (cGVHD), with significant symptom improvement and on-target activity observed.

It is evident that conventional pharmacotherapy strategies to modulate macrophages in the context of GVHD encounter significant problems, including severe side effects and limited therapeutic efficacy. To address these challenges and improve clinical outcomes, the development of targeted drug delivery systems that specifically engage macrophages has emerged as a promising strategy. These advanced systems are designed to deliver therapeutic agents directly to macrophages, thereby reducing off-target effects and increasing the local concentration of the drug at the site of tissue-involved GVHD [197]. Among the most promising approaches are pH-responsive polymers, which exploit the acidic environment of inflamed tissues to release their cargo specifically where it is required [198]. Redox-responsive polymers, on the other hand, leverage unique redox conditions within macrophages to trigger drug release [199, 200]. Similarly, temperature-responsive polymers can be engineered to release drugs in response to the elevated temperatures characteristic of inflamed tissues [201, 202]. Smart nanoparticles, including liposomes and exosomes, offer another avenue for macrophage targeting, providing a versatile platform for the encapsulation and controlled release of various therapeutic agents. These nanoparticles can be engineered to recognize specific markers on the surface of macrophages, ensuring that the therapeutic payload is delivered precisely to the cells involved in GVHD pathology [203].

The application of nanoscale drug carriers, such as liposomes and polymeric nanoparticles, has led to improved pharmacokinetics and controlled release profiles. These carriers can be engineered to respond to specific microenvironmental cues, such as pH or redox potential, which are often altered in GVHD. This responsiveness allows for the precise timing of drug release, ensuring that therapeutic agents are delivered when and where they are most needed [204]. Drawing from the experience of cancer immunotherapy, exploring the integration of gene therapy with macrophage-targeted delivery systems may offer novel avenues for macrophage-based GVHD treatment [205]. Despite the promising nature of this strategy, further research is essential to optimize macrophage-targeted drug delivery systems for GVHD treatment.

Despite the promising potential of macrophage-based therapy in the context of GVHD, various challenges and limitations hinder its effectiveness. The primary challenge lies in the complex role macrophages play in GVHD

pathophysiology, as they can exhibit both pro-inflammatory (M1) and anti-inflammatory (M2) phenotypes [206]. This dual nature makes it difficult to precisely target and modulate macrophage activity without potentially exacerbating the condition.

One interesting approach to overcome these limitations is the use of arsenic trioxide (ATO) to modulate macrophage polarization. Research has shown that ATO can shift the balance toward an M2 phenotype, reducing the number of pro-inflammatory F4/80 + iNOS + cells and increasing anti-inflammatory F4/80 + CD206 + cells in GVHD mice. This modulation improved survival rates and reduced GVHD severity, suggesting that targeted manipulation of macrophage phenotypes could be a viable therapeutic strategy [96]. Research has demonstrated that macrophages can promote tumorigenesis through the secretion of cytokines, especially in individuals with a history of malignancy. These challenges have a significant effect on the safety of therapeutic interventions, particularly in specific groups [207]. Macrophage-based therapies have the potential to both promote and inhibit tumorigenesis, depending on the macrophage phenotype involved. TAMs, particularly those exhibiting the M2-like phenotype, can facilitate tumor progression by supporting processes such as inflammation, angiogenesis, and tumor cell invasion [208]. Conversely, M1-like macrophages are associated with antitumor activities. To mitigate the protumorigenic effects of TAMs, strategies are being developed to reprogram M2-like macrophages into the M1-like phenotype, thereby enhancing their antitumor functions [209]. Additionally, targeting the lipid metabolism of TAMs has emerged as a novel approach in cancer immunotherapy, aiming to alter their function and reduce tumorigenesis [210]. These approaches are under investigation to improve the efficacy of macrophage-based therapies in cancer treatment. Conversely, innovative methodologies involving the use of nanoscale drug delivery systems face an additional obstacle: the activation of the immune system by these agents, which can exacerbate GVHD [211]. Despite the encouraging results observed in preclinical investigations, there is a significant deficiency in rigorous clinical trial data to substantiate the effectiveness and safety of macrophage-based interventions in the context of GVHD. It is imperative that more extensive studies be conducted to assess their potential advantages and disadvantages across various patient populations.

Conclusion

This review comprehensively explored the multifaceted role of macrophages in GVHD, a severe complication following hematopoietic stem cell transplantation. By understanding their complex interplay with the GVHD microenvironment, researchers can develop novel therapeutic strategies that target macrophage polarization and utilize targeted drug delivery systems. Overcoming the challenges associated with macrophage-based therapies requires further research and development. By addressing safety concerns, refining targeting methods, and conducting rigorous clinical trials, we can pave the way for the clinical translation of these promising approaches. Ultimately, the successful integration of macrophage-targeted therapies into the management of GVHD has the potential to significantly improve outcomes for patients undergoing hematopoietic stem cell transplantation, reducing morbidity and mortality associated with this life-threatening complication.

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Declarations

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