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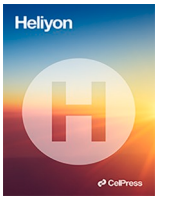

Review article

The role and research progress of macrophages after heart transplantation

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ABSTRACT

Since the 60s of the 20th century, heart transplantation has been the best treatment for patients with end-stage heart failure. Due to the increasing number of patients, how to expand the number of donor organs and enhance immune compatibility has become an urgent problem to be solved at this stage. Although current immunosuppression is effective, its side effects are also quite obvious, such as opportunistic infections and malignant tumors. In this review, we focus on the important role in macrophages after heart transplantation and their potential targets for achieving allogeneic graft tolerance, in order to improve effective graft survival and reduce infection and the occurrence of malignant tumors.

1. Introduction

Since the inception of the first heart transplantation in 1967, organ transplantation has emerged as the most viable treatment option for patients experiencing end-stage organ failure. Nevertheless, the escalating demand for organ transplants, intensified regulatory oversight, and expanding waiting lists have rendered the accessibility of donated organs a pressing concern. Consequently, it is imperative to broaden the scope of organ donation and enhance immune compatibility in the realm of transplantation. The process of organ transplantation has given rise to numerous research foci, primarily centered on mitigating post-transplantation complications. These complications include ischemia-reperfusion injury (IRI), primary transplant dysfunction, acute cell rejection (ACR), antibody-mediated rejection (AMR), and graft vascular lesions. The current immunosuppressive strategies employed worldwide are effective in suppressing the immune response of the recipient; however, they often lead to various complications, including kidney failure, infection, and malignancy. Consequently, the challenge lies in achieving allogeneic organ transplantation while safeguarding the recipient from the risks associated with infection and malignancy. Recent studies have revealed the significance of macrophages in the post-transplantation phase of heart transplantation. The classification of macrophage subtypes into tissue-resident macrophages and

Abbreviations: ischemia-reperfusion injury, IRI; acute cell rejection, ACR; antibody-mediated rejection, AMR; regulatory macrophages, Mregs; C–C chemokine receptor 2, CCR2; interferon-γ, IFN-γ; tumor necrosis factor-α, TNF-α; granulocyte-macrophage colony-stimulating factor, GM-CSF; lipopolysaccharide, LPS; inducible nitric oxide synthase, iNOS; reactive oxygen species, ROS; reactive nitrogen species, RNS; T helper cell 1, Th1; arginase-1, Arg1; tumor-associated macrophages, TAMs; peroxisome proliferator-activated receptor, PPARγ; transforming growth factor β, TGF-β; regulatory T cells, regs; cyclic GMP-AMP synthase, cGAS; damage-associated molecular patterns, dAMPs; colony-stimulating factor receptor-1, CSF-1R; RNA-induced silencing complex, RISC; Extracellular vesicles, EVs.

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infiltrating monocyte-derived macrophages has gained widespread recognition in transplantation research. Notably, tissue-resident macrophages primarily originate from donors, whereas monocyte-derived macrophages stem from recipients. This study describes in detail the changes of macrophages in the local immune microenvironment of allogeneic transplantation after heart transplantation, and focuses on the effect of macrophage-targeting immune tolerance on survival time after heart transplantation, and deeply discusses the future development direction and potential risks and challenges of these treatments.

2. Populations of macrophages after heart transplantation and their role in the immune inflammatory response

The heart harbors diverse subsets of macrophages, differing in their origin and functionality. Macrophages exhibit various phenotypes depending on their in vivo stimuli and microenvironment, broadly categorized as classically activated macrophages (M1-like), activated macrophages (M2-like), and regulatory macrophages (Mregs). Furthermore, cardiac macrophages can be subclassified based on the expression of C–C chemokine receptor 2 (CCR2) into CCR2+ and CCR2-subpopulations [1].

2.1. Functions and properties of M1 macrophages

M1-like macrophages play a pivotal role in defending against pathogens and initiating inflammatory responses. These cells are typically stimulated by interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), granulocyte-macrophage colony-stimulating factor (GM-CSF), or lipopolysaccharide (LPS). They express markers such as CD86, CD68, MHC class II molecules, and inducible nitric oxide synthase (iNOS). Additionally, they secrete high levels of pro-inflammatory cytokines like IL-1β, IL-6, IL-12, IL-23, and TNF-α, which promote inflammation and cytotoxicity [2]. Furthermore, M1-like macrophages generate abundant reactive oxygen species (ROS) and reactive nitrogen species (RNS) to counteract the invasion of pathogens. Concurrently, they secrete pro-inflammatory cytokines and stimulate the differentiation of T helper cell 1 (Th1), demonstrating a robust inflammatory response aimed at eradicating pathogens. However, excessive pro-inflammatory and cytotoxic effects of these macrophages can result in severe tissue damage [3].

2.2. Functions and properties of M2 macrophages

In contrast to M1-like macrophages, M2-like macrophages play a central role in tissue repair, immune modulation, and fibrosis. These cells are typically activated by T helper 2 (Th2) cytokines and exhibit reduced antigen-presenting capacity along with elevated levels of anti-inflammatory cytokines such as IL-10 and TGF-β [4]. This anti-inflammatory profile helps counterbalance excessive inflammatory responses. However, overactivation of M2-like macrophages can lead to tissue fibrosis, potentially resulting in organ dysfunction. With the advancement of knowledge regarding macrophages, M2-like macrophages have been further categorized into M2a, M2b, M2c, and M2d subtypes, based on distinct gene expression patterns.

2.2.1. The M2a subtype is stimulated by cytokines IL-4 and IL-13, acting through the co-receptor IL-4Rα to activate the STAT6 pathway [5,6]. This pathway predominantly mediates tissue repair and antifungal responses, inducing the production of arginase-1 (Arg1). Subsequently, Arg1 degrades into polyamines and proline, promoting cell proliferation and collagen deposition, essential for tissue repair. On the other hand,

2.2.2. M2b macrophages emerge in response to immune complexes and agonists of IL-1R or TLR, primarily involved in immune response regulation [7].

2.2.3. The M2c subtype is induced by interleukin 10, expressing the Mer receptor tyrosine kinase noted for its significant anti-inflammatory and phagocytic capabilities.

2.2.4. M2d macrophages, also referred to as tumor-associated macrophages (TAMs), constitute a newly discovered subtype triggered by TLR agonists and IL-6. The identification of M2-like macrophages is facilitated by markers such as the mannose receptor (MR/CD206), Arg1, IL-10, MHC class II molecules, peroxisome proliferator-activated receptor (PPARγ), Fizz1, and members of the YM1/2 family. Furthermore [8], M2-like macrophages play a significant role in tumor progression and metastasis. Tumor-associated macrophages (TAMs), the most abundant immune cell type within tumors, primarily derive from circulating monocytes and contribute to an immune microenvironment conducive to tumor invasion. These macrophages typically express markers characteristic of M2 macrophages, including CD163 and CD206 [9]. The mechanism underlying this process involves cancer cells secreting various TAM-inducing factors, leading to the activation of AKT/mTOR and ERK/STAT3 signaling pathways [10,11]. TAMs secrete growth factors such as TNF-α, TGF-β, epidermal growth factor, and platelet-derived growth factor, all of which promote cancer tissue growth. The activation of EGF/STAT3 signaling and TNF-α/NF-κB signaling pathways via TAMs enhances tumor growth and progression [12,13]. Additionally, TAMs secrete angiogenesis-inducing factors, facilitating angiogenesis, tumor nourishment, and the establishment of metastatic pathways. Notably, the conversion of TAMs to an M1-like phenotype offers promising potential for cancer treatment [14,15].

2.3. Functions and properties of regulatory macrophages

Of particular note are regulatory macrophages (Mregs), as a special subtype of macrophages, which are formed primarily through the differentiation of bone marrow precursor cells and peripheral blood mononuclear cells in a specific in vivo microenvironment. These cells have a unique immunosuppressive function and play a key role in the immune response, particularly during tissue damage repair [16–18]. A distinctive feature of Mregs is their high expression of MHC-II and CD80 molecules [19], which allows them to not only have strong immunosuppressive abilities, but also to efficiently present antigens. In addition to producing large amounts of the

anti-inflammatory cytokines IL-10 and transforming growth factor β (TGF-β) [20], Mregs also inhibit the immune response in both direct and indirect ways. They can directly inhibit the proliferation of activated allogeneic and xenogeneic T lymphocytes, while also indirectly inhibiting the immune response by inducing regulatory T cells (Tregs) or secreting molecules such as IL-10, TGF-β, iNOS, and IDO [21, 22]. These properties allow Mregs to play an important role in the regulation of innate and adaptive immune systems. Therefore, in-depth research on the function and regulatory mechanisms of Mregs is expected to provide important clues for the development of new immunotherapeutic approaches, especially in the treatment of autoimmune and inflammatory diseases. By regulating the number and function of Mregs, we may be able to modulate the immune response more effectively for the purpose of treating diseases.

2.4. Functions and properties of CCR2 macrophages

In the heart, there exist distinct macrophage subpopulations, each characterized by a unique phenotype and functional role. Typically, the adult heart harbors CCR2+ MHC-II^{hi} macrophages, CCR2-MHC-II^{lo} macrophages, and CCR2-MHC-II^{hi} macrophages [23, 24], all of which play varying parts in maintaining cardiac homeostasis and mediating disease processes. In contrast to cardiac macrophages, monocytes express CCR2+ MHC-II^{lo} and lack the Mer receptor tyrosine kinase. The important role of CCR2-macrophages (CCR2-macrophages) and CCR2+ macrophages (CCR2+ macrophages) in cardiac transplant immunity. During a heart transplant, the immune response is a key factor that affects the survival of the graft and the health of the host. Among them, macrophages play an integral role in this process. In particular, CCR2-macrophages and CCR2+ macrophages, two types of macrophages with different characteristics, play an important role in the immune response after transplantation.

2.4.1. CCR2-macrophages are derived from embryonic hematopoietic progenitor cells, which implant in the heart during fetal development and have a long lifespan. Throughout life, they maintain their numbers independently of circulating monocyte inputs, through local proliferation. These cells were activated by factors such as CCL2 and CCL7 and expressed cell surface signaling molecules such as CCR2 and CD11b. They secrete factors such as interleukin-10 (IL-10) and transforming growth factor-β (TGF-β), which have the ability to promote coronary angiogenesis and cardiomyocyte proliferation, while also having potential anti-inflammatory effects. In

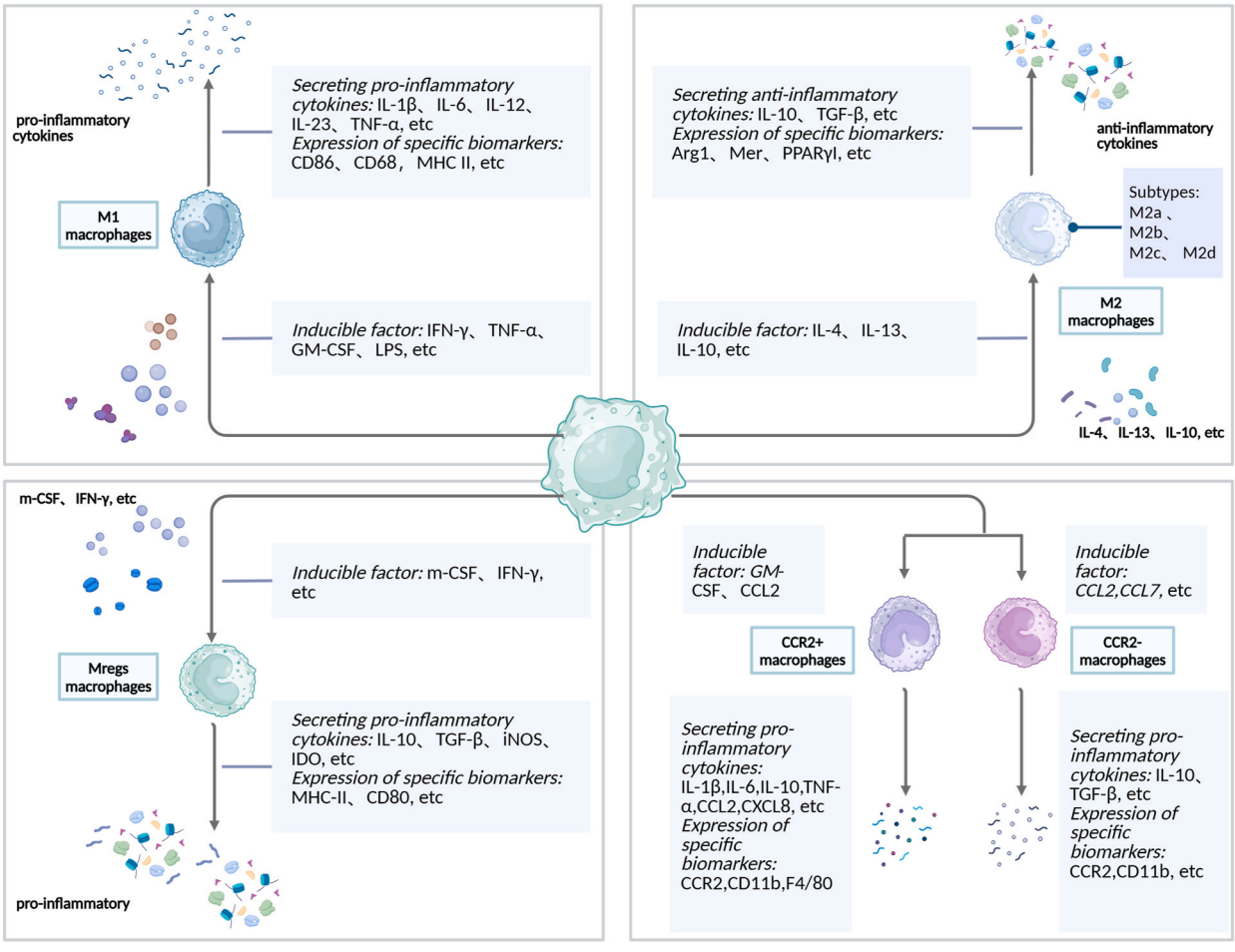

Fig. 1. Summarizes the activation stimulators, cell surface markers, and secreted cytokines, chemokines of various types of macrophages.

addition, CCR2-macrophages inhibit the recruitment of neutrophils and monocytes, thereby helping to maintain homeostasis in the transplanted heart [23–27].

2.4.2. In contrast, CCR2+ macrophages are derived from circulating monocytes. They are activated by factors such as CCL2 and GM-CSF, and express high levels of CCR2, CD11b, F4/80 and other molecules. Unlike CCR2-macrophages, CCR2+ macrophages are rich in pro-inflammatory genes, and their activation is an important mechanism driving the inflammatory response. These cells secrete factors such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), IL-10, CCL2, CXCL8, and other factors, which play an important role in pro-inflammatory and immunomodulatory processes [28]. In the context of heart transplantation, the pro-inflammatory effects of CCR2+ macrophages may negatively affect the graft, leading to transplant failure. However, the anti-inflammatory and angiogenesis-promoting effects of CCR2-macrophages may have a protective effect on the graft and improve the success rate of transplantation. Therefore, regulating the number and function of these two types of macrophages may be an effective strategy to optimize the immune response to heart transplantation and improve graft survival.

2.4.3. Overall, CCR2-macrophages and CCR2+ macrophages play a key role in the immune response after heart transplantation. The former helps protect the graft through its anti-inflammatory and angiogenesis-promoting abilities, while the latter may negatively affect the graft through its pro-inflammatory effects. Understanding the properties and functions of these two types of macrophages will help us develop new therapeutic strategies to optimize the immune response to heart transplantation and improve graft survival (Fig. 1) (Table 1).

3. Induce graft tolerance with macrophages as targets

Ischemia reduction is paramount in heart transplantation, as it strongly correlates with perioperative clinical outcomes in post-transplant patients. A major contributor to primary graft dysfunction, early graft loss, and mortality is ischemia-reperfusion injury. Recent investigations have demonstrated that ischemia enhances the recruitment and activation of macrophages.

During heart transplantation, ischemia-reperfusion injury elevates ROS levels, leading to the accumulation of cytosolic double-stranded DNA (dsDNA). This accumulation triggers the cyclic GMP-AMP synthase (cGAS)/interferon gene stimulator (STING) signaling pathway, which in turn elicits various immune inflammatory responses [29–31]. These responses release inflammatory mediators such as IL-β and TNF-α, promoting further cytosolic dsDNA release and activating the cGAS-STING pathway, thereby exacerbating tissue damage [32,33]. Recent studies have revealed that in allogeneic heart transplant mouse models, the absence of cGAS leads to reduced inflammatory cytokine production, significantly prolonging transplanted heart survival [34]. Hence, inhibiting the dsDNA sensor cGAS may represent a promising new approach to mitigating the inflammatory response triggered by ischemia-reperfusion damage during the initial stages of organ transplantation.

Targeting the reduction of CCR2+ macrophages has been effective in mitigating infarct size and improving cardiac remodeling following myocardial infarction [35,36]. Prior research has indicated that transplanted donor CCR2+ macrophages exhibit a robust MYD88/NF-κB signaling cascade [37]. Furthermore, macrophages lacking MYD88 in the donor heart exhibit improved outcomes, suggesting that blocking MYD88 signaling in these macrophages may alleviate allograft rejection.

Chronic allograft rejection is characterized by an inflammatory response centered around coronary artery vessels. This process involves the migration and proliferation of various inflammatory cells into the intima, ultimately leading to intimal thickening and coronary artery stenosis or occlusion [38]. Currently, there are limited therapeutic options available to prevent chronic transplant rejection, despite the existence of drugs primarily targeting acute rejection [39]. Macrophages play a pivotal role in chronic rejection, with their infiltration degree strongly correlating with the incidence of chronic rejection. In heart transplantation models, M2-like

Table 1
Summarizes the activation stimulators, cell surface markers, and secreted cytokines, chemokines, and functions of various types of macrophages.

phenotye		stimuli	Cell expression markers	Cytokines, chemokines, and other secreted mediators	functions
M1		IFN-γ,TNF-α,GM-CSF,LPS	CD14,CD80,CD86,CD68,MHC-II,iNOS	IL-1β,IL-6,IL-12,IL-23,TNF-α CXCL9, CXCL10, CXCL11, CXCL16, CCL5	Promotes inflammation and cytotoxicity, Antitum-or response, induces Th1 cell differentiation
M2	M2a	IL-4,IL-13	Human: MMR/CD206, IL1Ra; IL-1R II Mouse: Arg-1, FIZZ1, Ym1/2	IL-10, TGF-β, CCL17, CCL18, CCL24	Tissue repair and antifungal response
	M2b	IL-1R, TLR agonists, immune complex	IL-10 high, IL-12 low, CD86	TNF-α, IL-1β, IL-6, IL-10	Immunomodulation
	M2c	IL-10, TGF-β	Human: MMR/CD206, TLR-1, TLR-8 Mouse: Arg-1	IL-10, TGF-β, CCL16, CCL18	Anti-inflammatory and phagocytic effects
	M2d	TLR agonists, IL-6	CD163, CD206,IL-10, MHC II	TGF-β, Epidermal growth factor, platelet-derived growth factor	Angiogenesis, tumor progression
Mreg		LPS, immune complex, TLR agonists	CD80, MHC-II	IL-10,TGF-β	Immunomodulation, Antigen presentation, Defensive features
CCR2+		CCL2,GM-CSF	CCR2,CD11b,F4/80	TNF-α,IL-1β,IL-6,IL-10,CCL2, CXCL8	Inflammatory response, Immunomodulation
CCR2-		CCL2,CCL7,CCL8, CCL12,	CCR2,CD11b	IL-10,TGF-β	Anti-inflammatory,Promotes heart repair

macrophages emerge as the dominant innate immune cell type mediating chronic rejection [40].

During chronic rejection, M2-like macrophages exhibit distinct functions [40,41], including the production of growth factors like TGF-β and PDGF. These growth factors trigger vascular changes by inducing smooth muscle cell proliferation, myofibroblast activation, and extracellular matrix deposition [42,43]. Furthermore, studies have shown that α-SMA + myofibroblasts contribute to interstitial fibrosis in chronic kidney transplant injury, with approximately 50 % of myofibroblasts derived from M2-like macrophages [44]. These findings underscore the significance of M2-like macrophages in chronic allograft rejection. Recent research has focused on the role of specific signaling pathways within macrophages [45]. For instance, conditional deletion of TRAF6 and mTOR in mouse models promotes macrophage polarization towards the M2 phenotype and is associated with intensified immune rejection. Additionally, deleting the macrophage mTOR gene in the heart enhances the differentiation of Foxp3+ Tregs cells, thereby suppressing immune rejection. These findings provide insights into potential therapeutic targets for chronic allograft rejection. These findings offer promising new targets for treating chronic transplant rejection. Furthermore, recent research has demonstrated that macrophages infiltrating chronic rejection heart grafts primarily exhibit an M2 phenotype and express the purine receptor P2X7 [46]. Blocking this receptor suppresses M2 polarization, potentially halting the progression of chronic allogeneic rejection. This suggests that targeting the P2X7-M2 pathway may offer a therapeutic strategy to mitigate M2-like macrophages induced graft inflammation and chronic rejection [41].

It has been established that Mregs promote the production of IL-10 through DC-SIGN and TLR4 interactions, ultimately fostering graft immune tolerance by suppressing $CD8^+$ T cell proliferation while concurrently stimulating the expansion of $CD4^+$ Treg cells [47]. Additionally, macrophages involved in antibody-dependent tumor cell phagocytosis can transition into Mregs, exerting immunosuppressive effects through the overexpression of PD-L1 and IDO [48]. Mregs further contribute to immune tolerance by reducing the

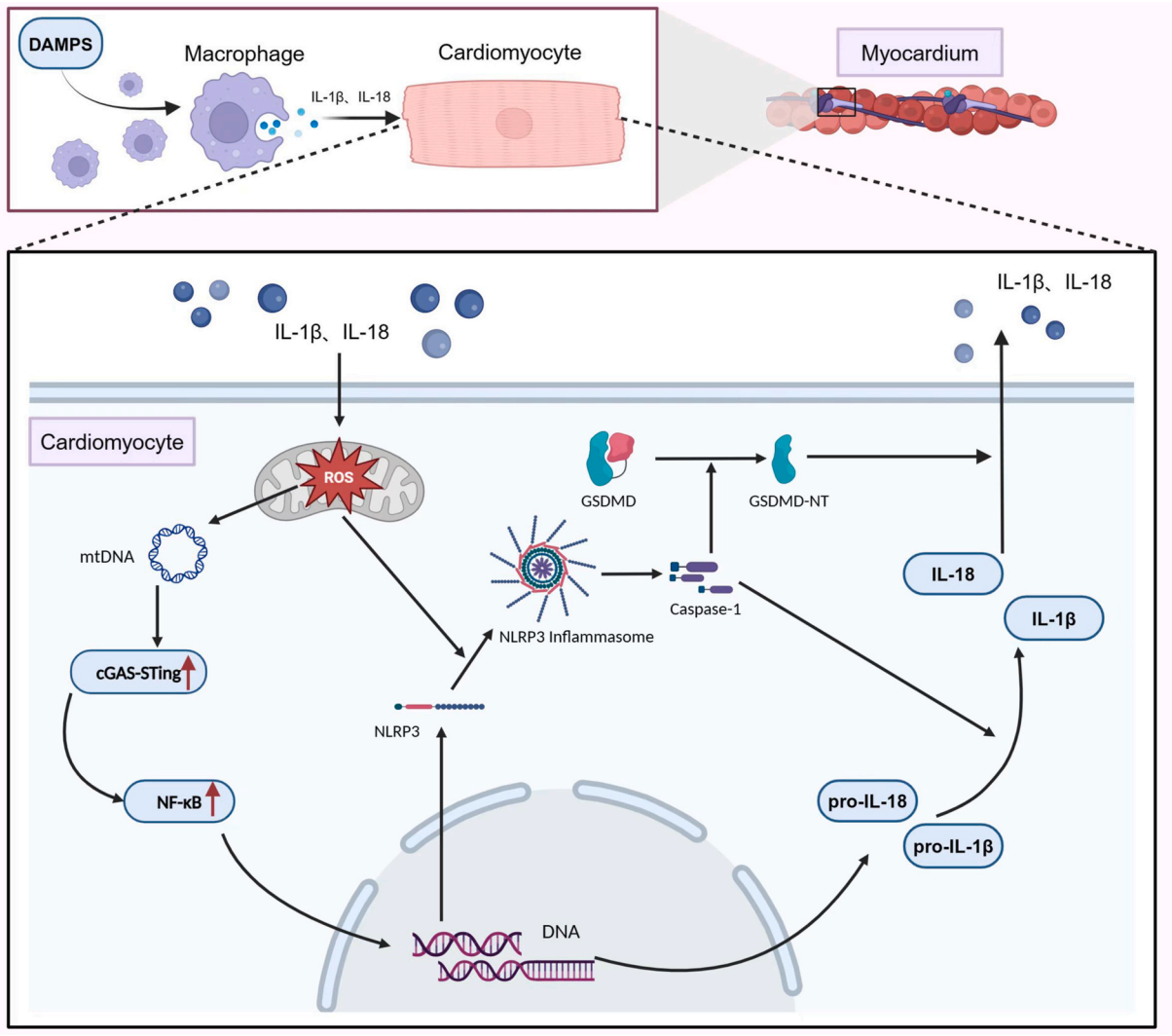

Fig. 2. Pattern of signaling pathways in macrophage-induced cardiomyocyte pyroptosis.

number of activated allogeneic T cells through phagocytosis and inhibiting their ability to secrete IL-2 and IFN-γ.Research has demonstrated that human myeloid-derived suppressor cells (Mregs) hinder the proliferation of allogeneic T cells activated by phytohemagglutinin (PHA) through an indoleamine 3,3-dioxygenase (IDO)-dependent mechanism, leading to a significant decrease in the number of activated T cells [49]. Furthermore, Mregs indirectly impede the maturation of dendritic cells (DCs) via the Treg pathway [50]. These mechanisms collectively enhance Mregs' capacity to foster immune tolerance among organ transplant recipients, providing further insights into potential therapeutic avenues.

4. Mechanism of action of macrophages in transplantation-induced immune responses

In essence, chronic allogeneic rejection is a complex interplay of immune cells and signaling cascades. A deeper comprehension of these mechanisms could lead to the development of more targeted treatment approaches for preventing or managing chronic transplant rejection. Regarding the role of macrophages in transplantation-induced immune responses, the immune reaction to heart injury is a multifaceted process that remains partially enigmatic. This response is influenced by various factors, such as the type of rejection, receptors involved, and environmental cues. Upon heart damage, a cascade of sterile inflammatory responses ensues, relying on diverse signaling molecules. Central to this process are damage-associated molecular patterns (dAMPs) released by dying cells, the cytosolic DNA sensing pathway mediated by cGAS-STING, and the NLRP3 inflammasome within cardiomyocytes. Together, these components contribute to creating a proinflammatory milieu. Furthermore, numerous immune cell types interact with fibroblasts, endothelial cells, and cardiomyocytes within the heart, forming a crucial component of the post-injury immune response. This intricate network of interactions underscores the complexity of the immune response following heart injury.

4.1. Early immune response in HMGB1 and transplant rejection

DAMPs, or damage-associated molecular patterns, are early signals emitted by damaged cells that stimulate an immune response. Among them, HMGB1 stands out as a key player closely linked to transplant rejection [51]. Following organ transplantation, HMGB1 levels rise sharply, stimulating the pro-inflammatory pathway and inducing macrophages to release IL-23 in response to DNA from necrotic cells [52,53]. This cascade ultimately triggers the release of IL-17A from γδT cells, leading to cardiomyocyte apoptosis and neutrophil recruitment.

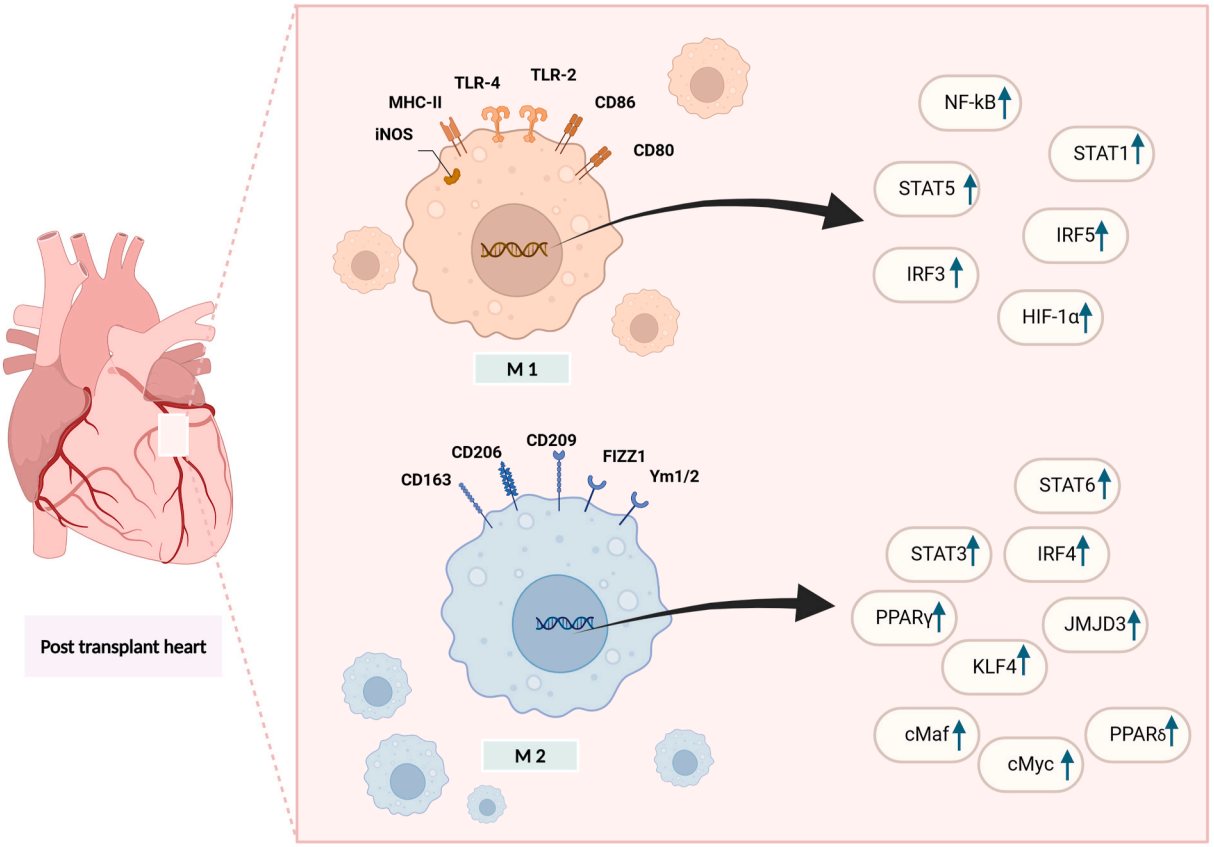

Fig. 3. Summarizes the different phenotypes and roles of M1 and M2 macrophages after heart transplantation.

4.2. The role of NLRP3 inflammasomes and pyroptosis in graft rejection

When inflammatory signals are sensed by cell membrane receptors, the NF-κB signaling pathway is activated, inducing the transcription of NLRP3 and IL-1β [54]. NLRP3 inflammasomes are crucial in this process, recruiting ASCs and pro-caspase-1 to initiate inflammasome assembly and activation. Activated caspase-1 then cleaves pre-IL-1β and pre-IL-18, promoting their maturation and release [55]. Concurrently, GSDMD is cleaved by caspase-1, and its N-terminal domain inserts into the cell membrane to form pores, facilitating the release of mature inflammatory factors and inducing pyroptosis [56](Fig. 2).

4.3. The central role of macrophages in graft rejection and its regulatory mechanisms

In grafts, macrophage aggregation is a hallmark of rejection [57,58]. Studies reveal that macrophages comprise 74.2–86.6 % of immune cells in chronic rejection [59]. Research has established that the Notch2 signaling pathway is pivotal in regulating the transition of Ly6Chigh monocytes into Ly6Clow CX3CR1 high monocytes under homeostatic conditions [60]. Additionally, the activation of the colony-stimulating factor receptor-1 (CSF-1R) signaling axis is essential for the differentiation of Ly6Chigh monocytes into Ly6Clow CX3CR1 high monocytes. These signaling pathways play a central role in the proliferation and chemotaxis of M2-like macrophages upon stimulation [61,62].

In conclusion, the immune response following cardiac injury is an intricate and finely tuned process that encompasses the interplay of diverse cells and signaling cascades. As research progresses, we aim to gain a deeper understanding of this complex mechanism and identify more effective therapeutic strategies(Fig. 3).

5. Prospects

5.1. Gene editing

Gene editing is a technology that can precisely modify specific genes in an organism's genome, and its core lies in the use of synthetic DNA fragments to achieve precise manipulation of the genome. This includes insertions, deletions, modifications, and substitutions of DNA fragments with the aim of adding, deleting, or replacing specific DNA sequences. Among the many technologies, CRISPR-Cas9, TALEN, and Zinc Finger Nucleases stand out. These technologies use specially formulated enzymes to pinpoint and edit the gene of interest, with the CRISPR-Cas9 system being particularly popular. It uses RNA-guided Cas9 protein to cleave DNA, enabling precise editing at the gene leve [63,64].

Gene editing technology has broad application prospects in the field of organ transplantation. Its potential is mainly reflected in improving the quality and quantity of donor organs, reducing immune rejection, and optimizing the function of post-transplant organs. When exosomes are combined with CRISPR/Cas9 technology, exosomes can be used as vectors to deliver components of the CRISPR/Cas9 system to target cells, enabling efficient gene editing while reducing potential damage to normal cells [65–67].

In particular, gene editing technology has shown unique advantages in inducing immune tolerance by macrophages after heart transplantation. By editing specific genes in macrophages, we can modulate their immune functions, such as regulating processes such as antigen presentation, signal transduction, or cytokine production, thereby promoting the induction of immune tolerance. In addition, enhancing the expression of molecules related to immune tolerance, such as PD-L1, IL-10, etc., or inhibiting the expression of pro-inflammatory factors, can also help reduce immune response and inflammation [68–70]. At the same time, the use of gene editing technology to construct gene knockout or knock-in models can further study the mechanism of macrophages in immune tolerance and provide a theoretical basis for the development of new immune tolerance strategies [71].

However, despite the significant advantages of gene editing technology in the field of organ transplantation, its practical application still faces many challenges. First, tools such as CRISPR/Cas9, while powerful, can also be subject to non-specific editing, i.e., off-target effects, leading to undesirable gene mutations. Second, gene editing may trigger an immune response because the edited cells may be perceived as abnormal by the immune system. Furthermore, since gene editing acts directly on genetic material, its potential risks and side effects cannot be ignored, such as the risk of gene transmission to offspring through germ cells and the risk of serious diseases such as cancer. Therefore, adequate safety assessment and long-term observation are required before application [67,72]. In addition, ethical issues are also important challenges for gene editing technology. This technology has the potential to alter human genetic information, leading to widespread concern and controversy about individual physiological characteristics, disease susceptibility, and other issues [73]. Finally, the difficulty and cost of gene editing technology also limit its popularity and application. The technology requires a high degree of expertise and skill, and the operation process is complex and relatively costly. In summary, gene editing technology has great application potential in the field of organ transplantation, but it also faces challenges such as technical limitations, ethical issues, safety considerations, and operational challenges. While promoting its development, we should maintain a cautious and prudent attitude to ensure the safe and rational application of technology.

5.2. siRNA

siRNA, the full name of small interfering RNA, that is, small interfering RNA, is a small RNA molecule with a length of about 19～25 nucleotides. It forms a functional complex by binding to an RNA-induced silencing complex (RISC) that specifically recognizes and binds to the target mRNA, leading to its degradation and thus silencing the expression of the target gene [74,75]. siRNAs are key transcriptional regulators of gene expression in most organisms, and have the advantages of strong specificity, multiple actionable

targets, and innate immune tolerance. It plays an important role in macrophage polarization, infection, tumor growth, inflammatory activation, proliferation, and phagocytosis [76–80].

Coronary artery disease is a major health problem, and existing treatments rely on surgical procedures such as coronary artery bypass grafting or coronary artery reconstruction. Studies have shown that down-regulation of vascular endothelial growth factor (VEGF) and up-regulation of tyrosine phosphatase-1 (SHP-1) in the Src homology region 2 domain may exacerbate the pathological progression of ischemic heart disease. Recently, Kim et al. presented an approach for the treatment of ischemic heart disease using an anti-apoptotic SHP-1 siRNA and a plasmid DNA of plasmid DNA from a hypoxia-inducible VEGF expression vector for angiogenesis. This polymer carrier interacts with multiple siSHP-1 molecules and packages pHI-VEGF pDNA to form stable bifunctional nanoparticles designed to attenuate cardiomyocyte apoptosis and enhance cardiac microvascularization to combat ischemic heart disease [81].

In the field of heart transplantation, the application of siRNAs is mainly focused on immune protection. By interfering with specific signaling pathways, such as the OX40/OX40L pathway, siRNAs are able to modulate the immune response and protect the transplanted heart from immune rejection. Due to immune differences between donors and recipients, immune rejection often occurs in heart transplantation, resulting in dysfunction and shortened survival time for the transplanted heart. As a gene silencing tool, siRNAs can specifically inhibit the expression of genes associated with immune rejection, thereby mitigating immune rejection and improving the survival rate of transplanted hearts. Studies have shown that siRNAs targeting the OX40/OX40L pathway can significantly prolong the survival time of mouse transplanted hearts. This is because the OX40/OX40L pathway plays a key role in macrophage activation and immune response, and by interfering with this pathway, it can inhibit macrophage overactivation and alleviate the immune damage of transplanted hearts [82].

A key advantage of siRNA therapeutics is their ability to potentially target the expression of virtually any single gene/protein of interest, which enables the use of traditional small molecule approaches to address targets that may not be medically treatable. In addition, although only a small amount of siRNAs are able to escape endosomes and enter the cytosol, once bound to RISCs, they are protected from nuclease degradation, prolonging their therapeutic effects [83]. This gives siRNAs a distinct advantage in the treatment of chronic diseases that require regular medication and has the potential to greatly improve medication adherence for better disease control. siRNA-based therapeutics are highly selective and less expensive to synthesize and manufacture than certain protein-based drugs such as antibodies, which gives siRNA-based therapeutics a clear advantage. siRNAs are initially synthesized in the external environment and bind to the RISC complex as a functional molecule that directs the degradation of the target RNA. However, siRNA delivery is a technical challenge because bare siRNAs are susceptible to nucleosome degradation. An effective way to do this is to encapsulate the siRNA in liposomes or nanoparticles, which protect the siRNA from degradation and allow it to enter the bloodstream smoothly until it reaches the target cell [84]. Another method of delivery is the use of modified retroviruses as vectors. These viruses are modified to carry targeted drugs while eliminating the genes in which the virus develops to ensure safety. However, choosing the right viral transporter to match a specific cell type while avoiding triggering a severe immune response is major challenge for this approach [85,86].

Despite the enormous potential of siRNAs, there are still some technical hurdles. First, as a novel drug, siRNA pharmacokinetic and pharmacodynamic studies are still in their infancy, and there may be unknown side effects. Second, the non-specificity of siRNAs can lead to off-target effects, interacting with other non-target genes, triggering unwanted gene silencing, and thus limiting their clinical application. In addition, the stability of siRNAs cannot be ignored and is susceptible to enzyme degradation and immune clearance in vivo. Finally, siRNAs may trigger an immune response that produces antibodies that reduce the efficacy of treatments [87,88]. Therefore, in order to realize the full potential of siRNAs, we need to address issues such as their delivery, nonspecificity, stability, and immunogenicity. This requires us to continuously explore new delivery methods, optimize the design of siRNAs, and delve into their biological mechanisms to ensure the safety and efficacy of siRNAs.

5.3. miRNA

MicroRNAs (miRNAs) are endogenous non-coding RNAs that are approximately 21–25 nucleotides in length. They are able to interact with target cell messenger RNA (mRNA) to induce degradation or translational arrest, thereby regulating gene expression and affecting cell growth, proliferation, differentiation, and apoptosis [89,90].

Exosomal miRNAs play an important role in atherosclerosis and cell-to-cell communication. The study found that when ApoE (−/−) mice were injected with exosomes from mesenchymal stem cells, these exosomes were able to migrate near atherosclerotic plaques and affect macrophages. By reducing macrophage infiltration of plaques and inducing M2-type polarization, MSC exosomal miRNAs were able to reduce atherosclerotic plaque area in mice [91,92]. In addition, it was found that the miR-let7 family was enriched in MSC exosomes, and its expression level was increased in the aortic root of ApoE (−/−) mice, and had an important effect on the function of macrophages [93].

In the immune response after heart transplantation, miRNAs affect macrophage function by binding to target mRNAs within macrophages. This binding typically occurs in the 3′ untranslated region (3′ UTR) of the mRNA, resulting in mRNA degradation that downregulates gene expression associated with immune responses and inflammatory responses [94,95]. However, the specific details of this mechanism and the types of miRNAs involved are still not fully understood, and further research and experimental verification are needed.

In conclusion, miRNAs play an important role in the regulation of cell function and the occurrence of diseases, but their specific mechanisms of action and the types of molecules involved still need to be further explored. Future research will help us to understand the biological functions of miRNAs more comprehensively, and provide new ideas and methods for the treatment of related diseases.

6. Conclusions

Macrophages play a crucial role in various aspects of transplantation, ranging from ischemia-reperfusion injury to allogeneic transplant rejection and tolerance. The current studies suggest that targeting donor macrophages prior to transplantation could potentially enhance transplant outcomes. To achieve this, a thorough comprehension of the roles played by both donor and recipient macrophages throughout the allograft's lifecycle is crucial. However, significant gaps exist in our understanding of this complex topic. Key challenges that need to be addressed include elucidating the functions of various macrophage subtypes in allogeneic rejection and transplant tolerance, understanding the precise mechanisms underlying macrophage activation and proliferation, exploring potential interactions between donor-derived macrophages and circulating macrophages, and examining how new lymphatic vessels formed between the donor and recipient impact transplant outcomes. By addressing these issues, we could potentially pave the way for safer and more effective organ transplantation practices, offering new hope to patients in need of transplants.

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Data availability statement

No data was used for the research described in the article.

CRediT authorship contribution statement

Yao Chen: Writing – original draft, Visualization, Methodology, Investigation. **JianPeng Wang:** Writing – original draft, Software. **Cheng An:** Writing – review & editing, Funding acquisition. **ShanQing Bao:** Writing – review & editing. **ChengXin Zhang:** Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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