Stem Cell Research & Therapy

REVIEW

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Fate and long-lasting therapeutic effects of mesenchymal stromal/stem-like cells: mechanistic insights

Akram Hoseinzadeh[1], Seyed-Alireza Esmaeili[2], Reza Sahebi[3], Anahita Madani Melak[4], Mahmoud Mahmoudi[2,6], Maliheh Hasannia[5] and Rasoul Baharlou[1*]

Abstract

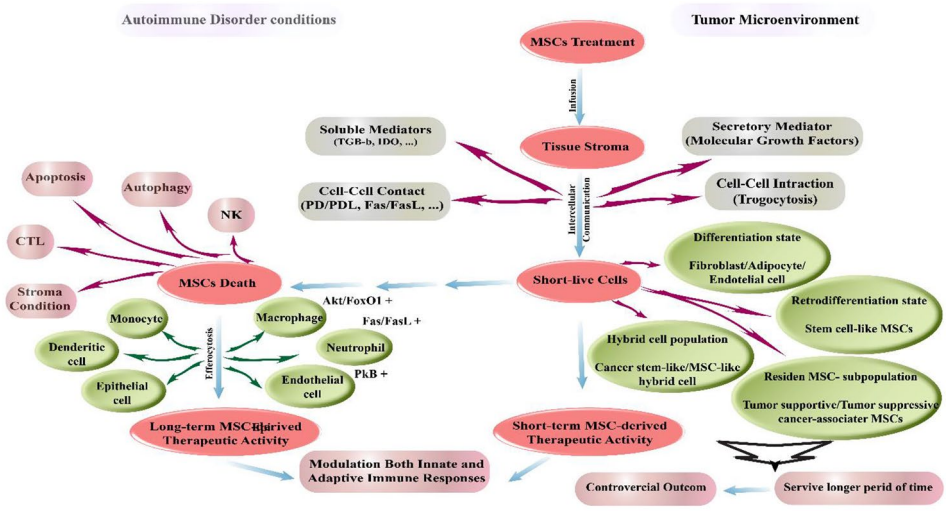
A large body of evidence suggests that mesenchymal stromal cells (MSCs) are able to respond rapidly to the cytokine milieu following systemic infusion. This encounter has the potential to dictate their therapeutic efficacy (also referred to as licensing). MSCs are able to rapidly react to cellular damage by migrating to the inflamed tissue and ultimately modifying the inflammatory microenvironment. However, the limited use of MSCs in clinical practice can be attributed to a lack of understanding of the fate of MSCs in patients after administration and long term MSC-derived therapeutic activity. While the known physiological effectors of viable MSCs make a relative contribution, an innate property of MSCs as a therapeutic agent is their caspase-dependent cell death. These mechanisms may be involving the functional reprogramming of myeloid phagocytes via efferocytosis, the process by which apoptotic bodies (ABs) are identified for engulfment by both specialized and non-specialized phagocytic cells. Recent studies have provided evidence that the uptake of ABs with a distinct genetic component can induce changes in gene expression through the process of epigenetic remodeling. This phenomenon, known as 'trained immunity', has a significant impact on immunometabolism processes. It is hypothesized that the diversity of recipient cells within the inflammatory stroma adjacent to MSCs may potentially serve as a biomarker for predicting the clinical outcome of MSC treatment, while also contributing to the variable outcomes observed with MSC-based therapies. Therefore, the long-term reconstructive process of MSCs may potentially be mediated by MSC apoptosis and subsequent phagocyte-mediated efferocytosis.

Keywords Mesenchymal stem cells, Apoptosis, Efferocytosis, Apoptotic bodies, Long-term effectiveness, Immunoregulation, Antimicrobial peptide, Autophagy

*Correspondence:
Rasoul Baharlou
Rasoulbaharlou195@gmail.com

Full list of author information is available at the end of the article

Graphical Abstract



Introduction

A multipotent and self-renewing population of spindle-shaped progenitor cells that can be isolated from various tissues and are capable of giving rise to multiple cell lineages under the appropriate differentiation media are known as marrow stromal cells or mesenchymal stromal cells (MSCs) [1, 2]. Their unique properties, including regenerative capacity, immunomodulatory activity and relatively non-immunogenic nature, have made MSC-based immunotherapy a promising novel therapeutic intervention in many areas, including hematological disorders, organ transplantation, tissue engineering, neurodegenerative diseases and conditions requiring immune system restoration [3–6]. MSCs have been extensively studied in various research endeavors within the field of managing pathological states. A major focus has been directed towards reducing inflammatory processes [7]. The verification of the safety of infusing culture-expanded allogeneic MSCs has been confirmed in the majority of clinical studies. However, the efficacy is reliant on various factors including the quantity of cells administered, the choice of MSCs donor, cell-passage number, the method of transplantation, and other associated variables [8]. Besides their potential to differentiate into multiple cell lineages (including osteoblasts, adipocytes and chondrocytes), MSCs have shown the ability to acquire adaptive properties to direct the functional fate of the cells, a phenomenon termed "trained immunity" [9].

It must be pointed out that trained immunity could be introduced as a nonspecific adaptive characteristic (functional reprogramming) in innate immune cells such as monocytes [10, 11], neutrophils [12], and macrophages [13]. Trained immunity is mediated by extensive metabolic and precise changes in chromatin structure facilitated by histone modification (epigenetic remodeling) [9, 14–17], resulting in enhancement/reduction in immune responses [18, 19]. Initially, trained immunity was believed to operate through mature myeloid cells. This posed a puzzle, as fully developed myeloid cells, including monocytes and dendritic cells (DCs), along with various receptors, can facilitate the initiation of trained immunity [9]. Trained immunity may manifest in bone marrow progenitor cells, known as central trained immunity [20, 21], and in blood monocytes and tissue macrophages (MQs), referred to as peripheral trained immunity. This phenomenon can persist in myeloid cells for extended periods, ranging from several months to years, and in some instances, even decades [21–31]. As indicated in monocytes functional fate, trained immunity may represent either enhanced or refractory innate immune status (tolerance), depending on various factors such as the type and concentration of targets they encounter [9, 25, 32, 33]. *Ifrim et al.* have revealed that the differential engagement of pattern recognition receptors (which are context-dependent) could decide the functional reprogramming of monocytes [9]. Accordingly, trained immunity has been suggested as an important new approach to improve current vaccines or to develop novel vaccines which lead to an augmented immune response [34]. However, various stimuli may lead to varied trained immunity of innate immune cells that have potential implications in improving phagocytosis of myeloid cells [35, 36] or homeostasis [37–39].

It is important to note that the outcome of MSCs transplantation is influenced by matrix-derived signals, particularly depending on the type and concentration

of inflammatory cytokines they are exposed to [40]. In addition, MSCs might also exhibit distinct differentiation characteristics in reaction to stimuli or cues within their stroma, resulting in epigenetic modifications and phenotypic alterations in MSCs [41]. This effect is evidenced by the reprogramming of their responses towards either immunogenic or tolerogenic phenotypes, two distinct and opposing functional pathways [40]. By analyzing the impact of the cytokine milieu on MSCs, current knowledge has suggested a link between the downstream activation of specific intracellular signaling cascades and their participation in acquired immunomodulatory functions. For example, MSCs primed with interleukin-1β (IL-1β) exhibit immunosuppressive activation through the nuclear factor kappa β (NF-κβ) pathway [42, 43]. Meanwhile, MSCs exposed to interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) show activation of the JAK/STAT1 signaling pathways [44], leading to the generation of various molecules responsible for orchestrating the immunomodulatory role of MSCs [45, 46]. Moreover, stimulation of toll-like receptor-3 (TLR-3) or TLR-4 has been found to potentially activate the immunosuppressive capacity of MSCs through the involvement of the Notch and NF-κβ pathways, respectively [47]. Therefore, the response to the surrounding stroma is the first step before the interaction between MSCs and their target cells.

Contact-dependent mechanisms involving intercellular interactions mediated by surface molecules, as well as the production of soluble components and subcellular particles, are thought to be the main mode of action of the short-lived immunomodulation mediated by MSCs [48]. Several biomarkers have been shown to be involved in MSC-mediated immunoregulation, some of which are produced constitutively (e.g., transforming growth factor-beta (TGF-β)), while others are released following MSC exposure to target cells or the matrix-derived cues (e.g., indoleamine 2,3-dioxygenase (IDO) and nitric oxide (NO)). MSCs display the presence of diverse cell surface proteins, including TLRs, ATPases, nucleosidases, receptors implicated in differentiation pathways, as well as molecules possessing immune regulatory capabilities, such as programmed death ligand 1 (PD-L1) and Fas ligand (FasL). By means of distinct surface proteins, MSCs are able to directly interact with immune cells including monocytes and modulate their functionality [49–51]. We have conducted a comprehensive analysis and documented the almost exhaustive short-term therapeutic properties of MSCs [52, 53]. Based on extensive pre-existing data, the paracrine capacity of MSCs is also influenced by various positive feedback and autoregulatory loops. MSCs may not have enough time to secrete a sufficient quantity of immunomodulatory factors to exert their effects over long distances, given their limited half-life [48]. Therefore, the hypothesis that the therapeutic activity of MSCs is mediated by their secretome is challenged by the relatively short lifespan of MSCs following intravenous infusion. However, there is evidence to support the concept that certain molecular mechanisms responsible for the therapeutically important immunomodulatory effects of MSCs are mediated or enhanced by direct or close communication with immunocytes [54, 55]. Recently, there is increasing evidence that MSCs possess an additional mechanism responsible for exerting a potent immunomodulatory effect on both innate and adaptive immune cells [7].

Emerging research suggests that exogenous MSCs undergo apoptosis after administration. This process is thought to represent a novel mechanism that contributes significantly to the therapeutic benefits of MSC therapy [48, 56–60]. The elimination of apoptotic cells by phagocytes, both professional and non-professional, referred to as "efferocytosis," is crucial in maintaining tissue homeostasis. The interconnection between efferocytosis and the resolution of inflammation in both healthy and pathological conditions, as well as in the regeneration of organs and immune responses, has been well-documented. Efferocytosis exhibits morphological and mechanistic differences compared to conventional phagocytosis processes [61–66]. In addition to preventing subsequent necrosis, efferocytosis can inhibit inflammatory reactions, promote self-tolerance, and initiate the stimulation of proresolving pathway [67–71].

Intrinsic antimicrobial efficacy of MSCs

The immunomodulatory features of MSCs have raised concerns regarding potential adverse outcomes linked to unintended suppression of antimicrobial immune reactions, thereby heightening the susceptibility to infections. In line with this, recent studies have proposed that MSCs exhibit antimicrobial capabilities against multidrug-resistant strains, although the mechanisms underlying this activity are not fully understood. Chow and colleagues have provided evidence that MSCs generate diverse mediators with the ability to eliminate bacteria, such as secretory antimicrobial peptides and proteins (AMPs) [72]. Indirectly, AMPs play a role in the innate immune response of the host against pathogens, specifically in the dynamic regulation of pro- and anti-inflammatory factors of the immune system or in the promotion of phagocyte function, leading to the mediation of immunological bactericidal effects by MSCs [73–79]. The evidence supports that MSCs have a direct antimicrobial capacity, as MSC-derived AMPs exhibit inherent abilities to eradicate microbial infections. Notable examples of these AMPs include cathelicidin peptide LL-37, hepcidin, β-defensin, cystatin C, elafin, and lipocalin. Interestingly, these AMPs are commonly found in neutrophils or epithelial

cells [79–82]. Antimicrobial peptides, also referred to as "host defense peptides," have exhibited various immunomodulatory roles in the context of resolving inflammation or infection, which are distinct for each type of AMPs [83, 84]. Particularly, cathelicidins and β-defensins exhibit chemotactic characteristics towards monocytes, neutrophils, MQs, and lymphocytes. Given the apparent involvement of vitamin D3 in increasing the production of LL-37, the incorporation of this vitamin during the cultivation of MSCs may enhance the expression of endogenous LL-37, offering a promising and innovative therapeutic strategy [85, 86]. Defensins are bioactive peptides displaying antimicrobial and antiviral properties. Consequently, regulating the endogenous synthesis of defensins, especially in scenarios characterized by reduced antimicrobial peptide generation, highlights their potential as therapeutic agents [73]. Hepcidin, functioning as an endogenous host defense peptide, demonstrates a broad spectrum of antimicrobial efficacy towards various fungal strains and clinically relevant bacteria. As a result, it has garnered considerable interest as a promising focal point for the advancement of novel therapeutic strategies [84].

Compared to other AMPs, lipocalins have garnered significant attention as a key area of research due to their unique biomarker characteristics, which act as an indirect defense mechanism against pathogens [87]. Lipocalins act as transporters, displaying a preference for attaching to small organic molecules that play a role in various essential biological processes like immune response, cell growth, proliferation, metabolism, prostaglandin synthesis, and iron transport. Elafin is recognized for its capacity to eradicate bacteria by disrupting their membrane structure, while the mechanism of action of cystatin C is not fully understood [82].

AMPs exhibit preferential efficacy toward a diverse spectrum of microorganisms, encompassing bacteria, yeasts, fungi, viruses, and even malignant cells. This efficacy of AMPs may be subject to additional regulation by the influence of infection-associated microbial structures and inflammatory agents [83]. The compact three-dimensional structures of biofilms create a conducive stroma for evading host immune responses, impacting macrophage polarization, bacterial phagocytosis inhibition, and neutrophil-mediated recruitment and killing limitation [72, 88]. Nevertheless, exposure to factors secreted by MSCs leads to an increase in monocyte recruitment, along with enhanced phagocytosis by MQs and neutrophils. Therefore, it is feasible that the use of MSCs could potentially enhance traditional antibiotic treatment.

AMPs interact with a variety of molecular targets, either on the cellular membrane or within the cytoplasm, to exert their antimicrobial activities and produce subsequent biological effects. In the research conducted by Sung and colleagues, microarray analysis was used to identify clusters of genes or molecular pathways potentially associated with the antimicrobial characteristics of MSCs. The results of their study indicated that the TLR-4 signaling pathway serves as a central paracrine factor that enhances the antimicrobial actions of MSCs and plays a crucial role in their anti-inflammatory and antibacterial effects [89].

The production of antimicrobial agents appears to be an inherent attribute of MSCs, as evidenced by bactericidal assays. Furthermore, the activation of MSCs appears to represent a critical stage in optimizing the interaction between MSCs and the host's innate immune defense to enhance the killing of bacteria. It is noteworthy that levels of AMPs have been observed to increase following priming with pro-inflammatory triggers such as IFNγ, IL-1β, or IL-12. Several investigations have indicated that MSCs can directly inhibit bacterial growth, even when using conditioned medium [78, 82–84, 87, 89]. These results suggest that MSC supernatants could serve as an adjunctive therapy alongside traditional antibiotics.

A recent study conducted by *Yagi et al.*, has linked the secretome of MSCs to a biocompatible adjunctive treatment for musculoskeletal infections. Based on their research findings, the antimicrobial activity of MSCs could be influenced to some extent by the MSC-vitamin D receptor and its effect on the expression level of LL-37 (cationic antimicrobial peptide) [90]. Moreover, *Yamamuro et al.*. carried out a study using a rat periprosthetic joint infection model, revealing that the antimicrobial properties of MSCs (when combined with antibiotic treatment) could be attributed to the upregulation of cathelicidin expression and downregulation of inflammatory cytokine expression, IL-6 and TNF-α, at the infection site [91]. Johnson and colleagues previously concluded that combining activated MSCs with antibiotic treatment in mouse and dog models of chronic biofilm infection could offer a novel non-antimicrobial approach for managing drug-resistant infections [88]. According to their data, MSC therapeutic potency is, at least in part, mediated by the increased secretion of cathelicidin, which is significantly enhanced in the presence of antibiotics. On a mechanistic level, it was noted that MSCs have the capacity to accumulate at the peripheries of wounds, leading to the polarization of macrophages towards the M2 phenotype within infected tissues. Hence, the antimicrobial properties exhibited by MSCs when combined with antibiotic therapy can lead to enhanced efficacy of antibiotics, consequently reducing the necessary dosage for bacterial eradication. On the other hand, *Harman et al.* have identified that MSCs could serve as active reservoirs of antimicrobial properties, which impede the proliferation of bacteria and disrupt the structural integrity of bacteria typically present in skin wounds [82].

Interestingly, Bonfield and colleagues have extensively documented the antimicrobial, anti-inflammatory, and antifibrotic capabilities of MSCs, along with their effectiveness against intracellular bacterial pathogens, both in laboratory settings and living organisms [78, 92–95].

Overall, MSCs from various sources show both antimicrobial and anti-inflammatory effects in preclinical models, though the underlying mechanism remains unclear [78, 96]. However, according to Rabani's research and others, enhancing AMPs and promoting an inflammation-resolving phenotype in immune cells may explain the paradoxical effects of MSCs during in vivo infections [78, 88, 97]. This insight could help guide clinical trials exploring MSCs as an adjunct to antibiotics in treating highly resistant infections.

Internalization capacity and clearance of apoptotic cells by MSCs

There is a shortage of literature on studies focusing on the phagocytic, pinocytic, and endocytic capacities of MSCs, suggesting a potential role in the functions attributed to MSCs [98]. MSCs, as a heterogeneous subset of stromal stem cells [99], have demonstrated the capacity to interact with a range of microorganisms and non-biological substances, potentially via distinct receptors, including specific TLRs, NOD-like receptors, scavenger receptors (SRs), mannose receptors (MRs), and retinoic acid-inducible gene [83, 99–105]. Notably, the function of MRs and SRs remains unclear in most tissues and cells, including MSCs. However, Xu and colleagues have described that the proliferation of MSCs can normally be regulated by SRs involving MAPK/ERK1/2 and PI3K/Akt pathways mediated by high-density lipoprotein [106]. According to the findings of *Khan et al.*, MSCs internalize different components through SRs than those endocytosed through MRs, which has a critical role in an intrinsic control of bacterial growth [98, 105]. Additionally, the results from Costela Ruiz et al. have shown that MSCs can engulf particles of various sizes and types [107], and this phagocytosis enhances their modulatory function.

Zhang and co-authors confirmed that MSCs possess the capacity to internalize ABs, enhancing MSC-mediated immunosuppressive activity. Mechanistically, stimulation of the NF-κB pathway by ABs mediates the activation of the cyclooxygenase 2/prostaglandin E2 (COX2/PGE2) pathway, enhancing the immunoregulatory function of MSCs [108]. Studies on MSCs obtained from human bone marrow have elucidated that these cells exhibit discriminate between the living and the dead, suggesting a preservation of MSC efferocytosis [109–111]. Importantly, MSCs are equipped with the necessary receptors for programmed cell removal [112], including Axl and Tyro3, whose expression is regulated in an efferocytic manner, independent of the apoptotic target [113]. The process of MSC-efferocytosis may play a role in maintaining the homeostasis of the bone marrow environment, particularly in relation to bone loss [113]. On the other hand, research has demonstrated a progressive elevation in MSC-MHC-II expression during a narrow window of IFN-γ levels. The diverse functions of MSCs, including their role as antigen-presenting cells (APCs) [114] and immune response regulators, can be attributed to their inherent adaptability, which is influenced by cytokine levels such as IFN-γ [99].

Moreover, the infused MSCs could be passively or actively induced to become ABs by a number of mechanisms. MSCs seem to have the ability to induce the phagocytic activity of other cells, such as polymorphonuclear cells or MQs [72]. Interestingly, promoting the engulfment of ABs has shown to be beneficial in immunologically mediated diseases [115, 116]. Finally, Zhang and colleagues observed that under transwell conditions, a slight increase in PGE2 levels was detected when the phagocytic activity of MSCs, acting as non-professional phagocytes, was inhibited. This finding is consistent with situations where the phagocytic activity of MSCs remained unaffected. Therefore, apart from phagocytosis, there might be certain factors released by ABs that contribute to augmenting the immunosuppressive functions of MSCs and potentially other phagocytes. This aspect warrants further exploration [108].

Hence, the process of phagocytosis involving ABs may signify a crucial juncture in the regulatory mechanisms employed by MSCs. Exploring approaches targeted at ABs offers the prospect of uncovering groundbreaking therapies to enhance the efficacy of MSC-mediated immunotherapy. Taken together, the role of MSCs as non-professional phagocytes -both "eaters" and "eaters of others"- may provide a novel basis for immunotherapy in managing localized and systemic infections, as well as immune-mediated conditions like systemic lupus erythematosus (SLE), which is characterized by impaired removal of dying cells. The complex characteristics of MSCs, including their role in immune regulation, phagocytic abilities, and expression of MHC II molecules, are crucial for maintaining tissue homeostasis, defending against infections, and modulating inflammatory reactions.

MSC apoptosis/efferocytosis/long-term effectiveness

Over the past few decades, MSCs have emerged as the most clinically studied experimental cell therapy, with significant advances achieved in their development as a promising solution for addressing a wide range of unrelated diseases [117]. However, concerns have been raised regarding the fate of these infused therapeutic cells,

which have received considerable attention under in vivo conditions, with the finding that MSCs are therapeutically effective despite the lack of engraftment [58, 118]. According to data, the survival of MSCs following transplantation could be affected by the response of the host [119, 120]. Moreover, it is worth noting that research has indicated that MSCs' survival rates also increase under conditions of oxidation, heat stress, hypoxia, and nutrient deprivation [121].

Research has provided evidence that distribution of MSCs after systemic delivery reveals a rapid entrapment of MSCs in the microvascular system of the lungs in rodent models, followed by their elimination from the circulatory system. According to findings presented by Pang and colleagues, MSCs located in the lung experience caspase 3-dependent apoptosis, followed by elimination by phagocytic cells [48]. In humans, however, intravenous infusion of MSCs would result in their widespread biodistribution and subsequent clearance from the circulation [8]. On the other hand, analysis of apoptotic markers in MSCs revealed that MSCs are resistant to several classical apoptosis inducers, and that apoptosis requires 6–18 h to be initiated. Subsequently, they accumulate in the liver and spleen, which are tissues where damaged or aged circulating blood cells are typically eliminated [48, 122–125]. These data have concluded that the majority of MSCs are no longer intact shortly post-transplantation and have a restricted lifespan [1, 126]. However, following transplantation, host effector cells would not immediately respond avidly to eliminate transplanted MSCs.

It is important to note that MSCs are resistant to allo-specific cytotoxic lymphocyte cells (CTLs), which is consistent with the low surface expression of HLA class I molecules [127]. However, the expression of low levels of HLA class I molecules, regardless of autologous or allogeneic source, renders MSCs a susceptible target for natural killer cell (NK)-mediated lysis [128]. Importantly, NK-mediated lysis of MSCs is only efficient when NK cells are present in an active state [7, 128]. In particular, during inflammatory responses, MSCs are exposed to pro-inflammatory cytokines (such as IFN-ɣ), which upregulate the surface expression of HLA class I on them, rendering MSCs resistant to NK-mediated lysis. Therefore, NK-mediated lysis of MSCs could be induced as a consequence of the downregulation of inflammatory cytokines and, consequently, the downregulation of HLA class I expression. On the other hand, MSC apoptosis can be triggered by the engagement of activating NK receptors by their respective ligands expressed on the surface of MSCs [128].

Galleu et al.. have found that MSCs are actively induced to undergo perforin-dependent apoptosis by recipient CTLs, whereby MSC-induced immunosuppression in patients with graft-versus-host disease is directly related to the high cytotoxic activity against MSCs [58]. Subsequently, recipient phagocytic cells, both specialized and non-specialized, engulf apoptotic MSCs and generate bioactive mediators that can facilitate the termination of inflammatory responses, promote self-tolerance, and activate pro-resolving pathways to restore homeostasis [129–132]. As a result, apoptosis of MSCs and the subsequent efferocytosis process may mediate the second phase of communication between MSCs and target cells (Fig. 1). Additional research has indicated that the process of perforin-dependent apoptosis of MSCs by CTLs or inducible autophagy [133, 134] is significantly involved in facilitating immunosuppression post-infusion [58, 118]. A distinct investigation has provided insights into the mechanism of apoptosis in MSCs, where the process takes place in a non-specific manner dependent on NO, inducing MSCs to exhibit programmed cell death [40]. Recognition of MSCs by CTLs is not antigen-specific as it does not require HLA engagement or result from alloreactive rejection, supporting the current practice of using allogeneic MSCs. Although an immunological synapse is not required, MSCs must be in physical contact with activated CTLs to undergo apoptosis. This supports a bystander role for the cytotoxic granules released by the activated CTLs. In the literature, lytic granule secretion has been described to precede the formation of the cytotoxic T lymphocyte/target cell synapse.

One plausible explanation is that, in the context of hot inflammation, activated T cells and NK cells interact by secreting IFN-γ, which induces the production of IDO by MSCs. This IDO production subsequently inhibits the functionality of activated T cells or NK cells. In contrast, in the stroma of cold inflammation - characterized by exhausted or inactivated T cells and NK cells - MSC-mediated immune regulation is reciprocally decreased [134–136]. These findings are consistent with data that has indicated a notable impairment in the proliferation of NK cells in the presence of MSCs, with no evidence of apoptotic or necrotic cell death [7]. Despite the well-established roles of TGF-β, IL-10, PGE2, and IDO in this context, there remains a significant gap in understanding the specific mechanisms by which MSCs inhibit NK cell activity [128].

In addition, evidence from clinical trials has shown that the three complement pathways (classical, alternative, and lectin) play a critical role in recognizing and directly damaging both autologous and allogeneic MSCs following their administration [137–139]. Of particular interest is the fact that MSCs can constitutively inhibit complement activation in their native tissue environment by locally secreting factor H [140]. This action helps to maintain MSC function and prolong their half-life [137]. Most interestingly, the chemotactic and immunomodulatory

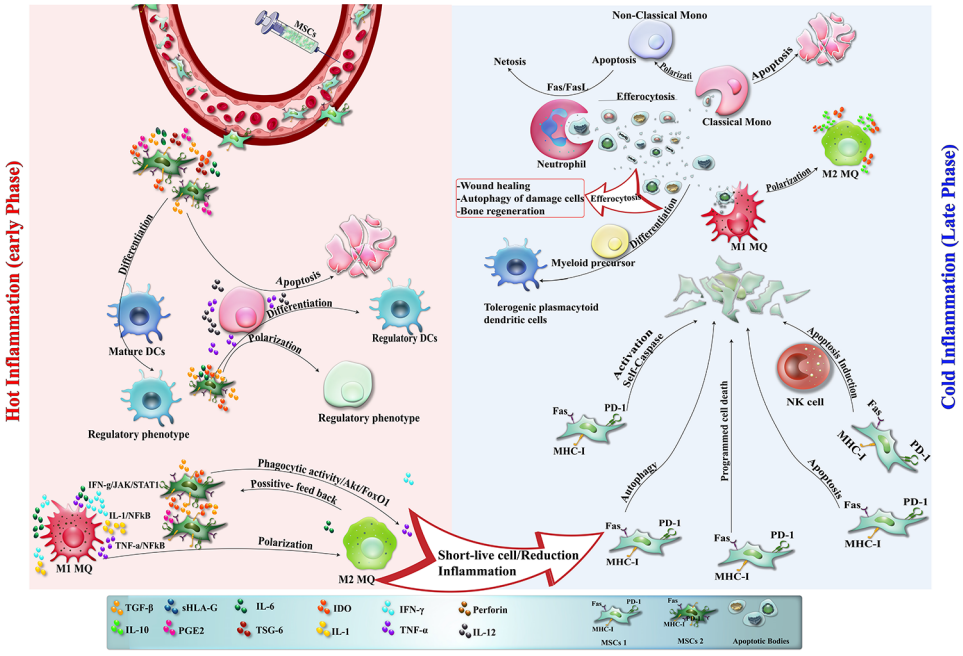


Fig. 1 Linking MSCs apoptosis to immunosuppression mechanisms. The combination of cues to which MSCs are subjected within the tissue stroma, and the resulting intracellular signaling cascade determine the particular immunomodulatory repertoire responsible for orchestrating the MSC-derived therapeutic activity [158]. The immunotherapeutic effect of MSCs is thought to occur in two interrelated phases. However, the fate of MSCs is only partially understood. Experimental data suggest that a hot inflammation (term for a stroma with high levels of inflammatory mediators/inducers such as IFN-γ (phase 1)) [159–161] may favor the protection of MSCs from cell-cytotoxicity by distinct mechanisms. While in cold inflammation (term for a stroma with intermediate levels of inflammatory mediators/inducers (phase 2)), the MSCs would behave in a different way. This may have relevant implications for the clinical application of MSCs. Importantly, the presence of different subpopulations of living MSCs, deceased MSCs, late apoptotic MSCs, early apoptotic MSCs, and autophagic cell death should be considered simultaneously [117, 162]. In the early phase, infused MSCs migrate to the focus of inflammation and injury to promote tissue repair and regeneration through paracrine effects, in the next phase apoptotic MSCs effectively regulate various aspects of the recipient cells. The short lifespan of MSCs gradually affected the transplanted cells [163, 164], with MSCs undergoing apoptosis through various mechanisms (described in detail throughout the document). Subsequently, MSC-derived apoptotic bodies are cleared by cells of the myeloid lineage (mainly neutrophils, MQs, and circulating monocytes) [117, 123]. Based on the "dying stem cell hypothesis," efferocytosis of apoptotic MSCs induces phenotypic and functional changes in phagocytes, which then modulates both innate and adaptive immune responses [123, 164]

properties of MSCs can also be triggered by complement activation products, which are produced in the blood after systemic infusion of these cells. Conversely, it has also been observed that MSCs weakly stimulate the complement system of innate immunity. The observation that even autologous MSCs have the ability to induce complement suggests that naturally occurring apoptosis of MSCs is beneficial and may partially explain the significant improvement seen with MSC-based interventions [137]. However, when MSCs undergo apoptosis in hemolytic complement-deficient mice, complement activation is unlikely to be the primary mechanism involved [141]. Activated complement overwhelmed the protective function of intrinsic complement regulators on the cell surface of MSCs, resulting in cytotoxicity, subsequently recruits and activates leukocytes and enhances phagocytosis [137].

Pang et al.. intriguingly unveiled a mechanism of action in which the host's response to dying MSCs plays a crucial role in their therapeutic benefits, even in the absence of cytotoxic host cells [48]. It is worth noting that MSCs are generally considered to be independent of major histocompatibility complex or species barriers, exhibiting a phenotypic profile that shields them from alloreactive T-cell reactions. The findings from Pang's research team indicate that the elimination of BAK and BAX inhibits the death of MSCs and diminishes their immunosuppressive activity [48]. These results are particularly interesting as they propose a connection between MSCs' apoptosis and peripheral immune responses, subsequently impacting distant tissue damage.

Given that caspase activation in MSCs may induce alternative pathways that promote the production of immunomodulatory molecules independently of efferocytosis signal generation, it is plausible that both early and late phases of MSC-driven immunomodulatory functions could occur simultaneously. This notion is supported by studies demonstrating that MSCs stimulate IL-1 signaling through caspase activation, leading to increased release of specific biomolecules [142].

It is clear that the involvement of recipient cellular networks plays a crucial role in facilitating the therapeutic

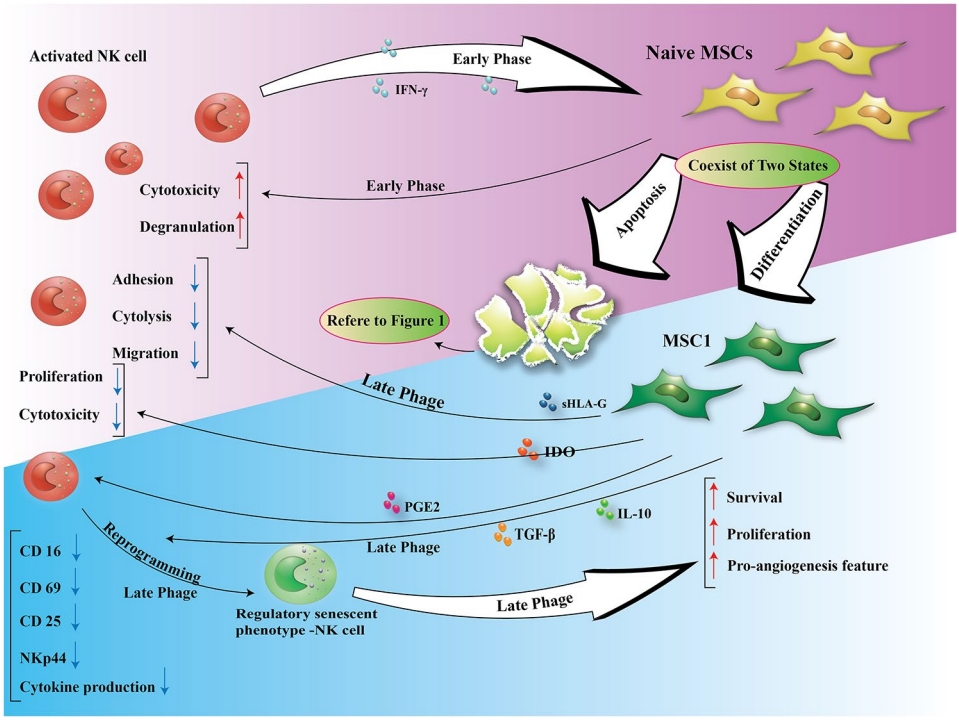


Fig. 2 Intercellular communication and potential outcomes between NK cells and MSCs. During the stage phase, MSCs upregulate the cytotoxic capabilities of NK cells. NK-MSC interactions lead to the secretion of IFN-γ by NK cells. Activated MSCs with IFN-γ (MSC1) increase their levels of MHC class I. Boosting MHC class I presentation may provide MSCs with protection against the destruction caused by NK cells [230, 231]. This is accomplished by shifting the balance towards the inhibition of NK cell activity, which results in the transmission of inhibitory signals and the hindrance of the cytotoxic capabilities of NK cells [232]. Nevertheless, there is evidence indicating that MSCs could be regarded as targets sensitive to lysis by activated NK cells [128, 218, 233]. On the contrary, during the resolution phase of inflammation, MSCs in a bioactive mediators-dependent manner induce an inhibitory effect on NK cell proliferation, cytokine secretion, expression of surface-activating receptors, and coreceptors, as well as induce senescence of inflammatory NK cells with a less responsive phenotype [55, 208]. IDO, PGE2, TGF-β, IL-10, and sHLA-G5 are key regulators of the mentioned effects. Furthermore, scientific evidence has demonstrated that MSCs exhibit the capacity to directly hinder the cytotoxic capability of NK cells through the downregulation of perforin and STAT3 [218, 234]. Overall, MSCs have the capability to enhance the cytotoxic activity of NK cells during the initial stages. Subsequently, they have the potential to promote a regulatory phenotype or trigger senescence in activated NK cells [208]. Importantly, it has also been postulated that MSCs exhibit inherent heterogeneity, which may influence the MSC-NK cell interaction [235]. Additionally, MSCs demonstrate varied crosstalk patterns with different NK cell populations [236]

benefits of MSCs and their derivatives. The study by Ko and colleagues has shown that MSCs induce immune tolerance by activating the recipient's endogenous immune regulatory system, thereby providing long-term therapeutic benefits [143]. In line with this, some studies have shown that MSCs induce the expansion of myeloid cells with immunosuppressive activity and modulate the immunophenotype of monocytes/macrophages as important effectors of immune regulation and tolerance [123, 144]. These findings suggest that MSCs can induce long-lasting suppressive adaptive immune responses by inducing non-specific innate tolerance, even when MSCs are no longer present in the system.

The use of pharmacological compounds that specifically target modulators of apoptosis could enhance our understanding of the molecular components involved in MSC apoptosis. Recent work has shown that inactivated MSCs, which have lost their ability to secrete factors, still retain their regulatory capacity, suggesting that cell membrane-dependent interactions with immune cells may contribute to these regulatory effects [118, 145]. Importantly, because dead MSCs lack active cell metabolism, they cannot differentiate into other cell types in vivo [117]. In conclusion, despite the wealth of preclinical and clinical data, elucidating the underlying mechanisms of MSCs as a potential cell-based immunotherapy for a wide range of diseases remains a challenging endeavor. The ability to influence innate immune functions through epigenetic changes provides a promising way for MSCs to gain adaptive characteristics after being activated in disease states. This mechanism may explain how MSCs promote long-lasting immune tolerance, showcasing their potential for therapeutic use [79, 146, 147].

Modifying hepatic/splenic macrophages functional properties

The exact composition of the organ microenvironment differs according to the specific tissue types,

characterized by the attributes of the immunocytes in their reaction to therapeutic strategies like MSC engraftment. The transplanted MSCs are mainly localized in the pulmonary tissue; however, a small number of MSCs traveled to the liver through different migration routes [151]. Moreover, the infused MSCs can modulate the function of splenic cells and enhance reparative mechanisms in distant organs. Macrophages play a key role in enhancing the recruitment and homing of MSCs. Conversely, the infusion of MSCs has been shown to increase the number of macrophages in the fibrotic liver [165]. Macrophages, being the predominant cell type engaged in efferocytosis, are pivotal in various physiological processes. A compilation of research works delineating the intercommunication between macrophages within the hepatic and splenic systems, and therapeutic MSCs has succinctly been presented in Table 1.

A recent investigation conducted by *Liang et al.* has revealed that systemic delivery of MSCs effectively regulates the ratio of M1 and M2 MQs within the liver, spleen, and bone marrow. This modulation is accomplished by reducing the quantity of M1 and increasing the population of M2 MQs [148]. Furthermore, a decrease in the levels of IL-1β and TNF-α has been documented, concomitant with an increase in the levels of IL-10. The synergistic impact of these changes leads to an enhancement in platelet counts and alleviates underlying immune system irregularities [148]. *Fiore et al.* demonstrated that macrophages derived from the fibrotic livers of mice treated with MSCs exogenously expressing IGF-I showed reduced expression profiles of pro-inflammatory and pro-fibrogenic genes and overexpressed HGF, indicating a restorative phenotype [165]. In addition, MSCs have exhibited a significant influence in alleviating hepatic injury and inflammation through their extracellular vesicles (EVs), containing IL-10, which accumulate in the hepatic parenchyma [155]. Upon reaching the liver, EVs are engulfed by Kupffer cells, leading to the upregulation of PTPN22. Consequently, this sequence of events prompts Kupffer cells to transition into an anti-inflammatory phenotype, thus mitigating hepatic inflammation and injury. Moreover, MSC-EVs have been observed in circulation towards the liver subsequent to the transplantation of MSCs. The mechanism underlying the protective effect of MSC-EVs against liver fibrosis involves the transmission of miR-148a, which regulates the functions of intrahepatic macrophages via the KLF6/STAT3 signaling cascade. This interplay presents a promising avenue for targeting liver fibrosis therapeutically [157]. Besides, research has revealed that intravenously delivered MSCs tend to primarily accumulate in the spleen, leading to an increase in M2 MQs within the kidneys via the complex spleen-kidney immune interplay [150]. EVs originating from MSCs have been shown to induce distinct patterns of gene expression linked to hyperpolarization and stimulation of PGE2 in M2 MQs situated within the spleen. It is noteworthy that the clinical effectiveness of EVs has been observed to be less favorable in comparison to the direct administration of MSCs.

Furthermore, MSC-primed macrophages experience a shift from M1 to M2 immunophenotype, with distinguishable differences observable at the molecular epigenetic level. This transition triggers the activation of diverse immunological pathways that result in the production of numerous immunomodulatory molecules like TGF-β, IL-10, IDO, and PGE2. Consequently, macrophage depletion has been shown to abolish the beneficial effects of MSC therapy [165]. *Liu et al.* have reported on the discovery of the Akt/FoxO1 pathway's role in facilitating the transition of macrophage phenotype and augmenting their phagocytic capability through MSC-mediated mechanisms. This process seems to play a part in reducing exaggerated inflammatory reactions. FoxO1, as one of the key downstream targets of the Akt pathway, serves as an essential nuclear transcription factor involved in various cellular processes such as cell differentiation and cellular metabolism. The subcellular localization of FoxO1 in the nucleus is observed in its non-phosphorylated state, whereas phosphorylation mediated by Akt induces its nuclear export, thereby dynamically regulating macrophage polarization through the process of phosphorylation [166]. These findings are consistent with the conclusions drawn from different experimental conditions, which indicate that the cells of both the innate and adoptive immune systems play a crucial role in facilitating and disseminating the immunomodulatory properties of MSCs.

Overall, transplanted MSCs have shown the ability to influence the polarization, phagocytic activity, and metabolic functions of macrophages through a variety of mechanisms. Among the mechanisms that have been elucidated are enhancing the activation of M2 MQs and the expression of MMP13 and IL-10, alongside inhibiting M1 MQs and the expression of IL-12, IFN-γ, TNF-α, and IL-6. Furthermore, MSCs have been found to possess the ability to enhance the production of PGE2, thereby mitigating organ damage in both in vivo and in vitro investigations [152, 153, 156]. More importantly, modified MSCs, such as those that have undergone cultivation in hypoxic environments (5% O2) or have been activated with pro-inflammatory cytokines, exhibited heightened efficacy in their therapeutic outcomes compared to naive MSCs [151, 167, 168].

Apoptotic MSCs and macrophages cross-talk following efferocytosis

While the infusion of MSCs initially has a significant impact on immunocytes, inducing changes in metabolic

Table 1 A selection of studies indicating crosstalk between macrophages and therapeutic MSCs

Product	Disease/Experimental model	Target Cell (s)	Effect (s), Biomolecule (s)	Mechanism (s)	Finding (s)	Ref
MSCs (Viable cells)	Mouse immune thrombocytopenia model	MQs	Polarizing to an anti-inflammatory phenotype	Regulating the M1/M2 MQs ratio, decreasing IL-1β and TNF-α, increasing IL-10	Elevating platelet count, ameliorating innate immune disorders	*Liang et al.*, 2023 [148]
	Mouse intramuscular implantation model	MQs	Gradual infiltration Chemokines (CCL2, 3)	ERK signaling pathway	Bone regeneration	*Wang et al.*, 2018 [149]
		MSCs	Accelerating migration, increasing CCR1, CCR2			
	Rat glomerular nephritis model	MQs	Promoting activation of M2 MQs, inhibition of M1 MQs MSC-EVs	Inducing M2 MQs via PGE2	Ameliorating of renal damage	*Shimamura*, et al., 2022 [150]
	Mouse liver cirrhosis model	MQs	Polarizing to an anti-inflammatory phenotype MSC-PGE2, MSC-miR210	ND	Reducing serum alanine aminotransferase, oxidative stress, and fibrosis	*Kojima et al.*, 2019 [151]
	Mice liver fibrosis model	MQs	Promoting M2 MQs activation with M1 MQs inhibition	Promoting MMP13 expression, suppressing the expression of α-SMA, Sirius red, and collagen-1	Ameliorating liver fibrosis	*Luo et al.*, 2019 [152]
	Mouse ischemia/reperfusion model	MQs	Reprograming MQs polarization toward M2	Enhancing NLRP3/caspase-1 activity, Reducing mammalian Ste20-like kinase ½, Augmenting YAP and β-catenin expression with increased PGE2 production	Reducing hepatocellular damage	*Li et al.*, 2019 [153]
	Mouse ischemia/reperfusion model	ND	ND	Increasing CD47 expression	Ameliorating liver ischemia/reperfusion injury	*Sheng et al.*, 2021 [154]
MSC- conditioned media	JS1 cell Line (hepatic stellate cell strain)	ND	ND	ND	Elevating IL-10, and reducing IL12, IFN-γ, TNF-α, and IL-6 gene expression, reducing TGF-β1 and collagen-1 secretion, increasing caspase-3	*Luo et al.*, 2019 [152]
MSC-exoxoms	Mice Traumatic hemorrhagic shock model	MQs	Shifting to an anti-inflammatory phenotype MSC-IL-10	Inducing the expression of PTPN22	Attenuating hepatic injury and inflammation	*Zhang et al.*, 2021 [155]
	Rat nonalcoholic steatohepatitis model	MSCs	Reducing in KC number and HSCs activation	Attenuating the mRNA expression of IL-1β, TNF-α, TGF-β	Ameliorating inflammation and fibrogenesis	*Ohara et al.*, 2018 [156]
	Mice liver fibrosis model	MQs	Promoting M2 MQs MSC-miRNA-148a	KLF6/STAT3 signaling pathway	Alleviating liver fibrosis	*Tian et al.*, 2022 [157]

Kupffer cells (KCs), Prostaglandin E2 (PGE2), Hepatic stellate cells (HSCs), Matrix metalloproteinase 13 (MMP-13), Kruppel-like factor 6 (KLF6), Protein tyrosine phosphatase non-receptor 22 (PTPN22), Not determined (ND), Tumor necrosis factor (TNF), Macrophage (MQ), Mesenchymal stem/stromal cells (MSCs), Interleukin (IL), Prostaglandin E2 (PGE2), C-C chemokine receptor type 2 (CCR2), C-C chemokine ligand (CCL), Yes-associated protein (YAP), NLR family pyrin domain containing 3 (NLRP3), Transforming growth factor beta (TGF-β), Interferon gamma (IFN-γ), hepatic stellate cell (HSC)

and signaling pathways, the subsequent apoptosis of MSCs leads to the formation of ABs containing various cellular components. Apoptosis, traditionally considered as an 'immunologically silent' mechanism of cellular death, is characterized by the lack of an inflammatory reaction [48]. In recent times, there has been a growing interest in investigating apoptosis as a process that stimulates intercellular communication, thereby facilitating the remodeling of adjacent tissues [169]. Apoptotic cells exhibit a number of distinct changes in their ultrastructural morphology, including the formation of plasma membrane blebs, nuclear fragmentation, and disintegration of the cell into apoptotic bodies. Subsequently, ABs containing fragmented organelles and other substances are engulfed and degraded by phagocytes [117]. It is noteworthy that ABs from the same apoptotic cell can

contain different components, potentially influencing various biological outcomes. Therefore, ABs as cell trainers may have little long-term effect on immune cell function or consistent long-term tolerance. This suggests that specific components at particular concentrations could potentially trigger short-/long-term tolerogenic immunity. The occurrence of cross-tolerance phenomena may be induced through active involvement. Nevertheless, further investigation is required to validate this assertion.

Several studies have emphasized the significant potential of MSC-ABs engulfed by phagocytes in treating various pathological conditions. These effects are closely tied to the biological properties of MSC-ABs and their interaction with target cells (see Table 2). As such, the communication of MSC-ABs with a variety of immune cells, such as natural killer cells, dendritic cells, T cells, among others, has been validated to augment the immunotherapeutic effectiveness of MSC [48]. Research on tolerized cells focuses on the signaling and molecular mechanisms behind their induction. According to the 'dying stem cell hypothesis' [170–172], engulfing MSC-ABs may reduce inflammatory cytokine secretion and inflammatory cell infiltration, promoting tissue regeneration and wound healing [173, 174]. According to reports, MSC-derived ABs have the potential to be internalized by pre-osteoclasts, consequently facilitating bone remodeling by transferring miR-223-3p to osteoclasts [175].

Dynamic changes in chromatin structure across immunological pathways might contribute to the process of tolerance induced by ABs. Monocytes are stimulated by MSCs to acquire an anti-inflammatory phenotype, characterized by the production of IL-10, high expression levels of B220 and CD11b, and display of T-cell suppressive functions that are independent of FoxP3 + regulatory T cells [143]. TNF-α-induced gene/protein-6 (TSG-6) has been recognized as a key pathway responsible for mediating the induction of the phenotypic shift towards immune regulatory monocytes. In this context, MSCs with TSG-6 knockdown have been shown to be unable to induce an immunoregulatory state in monocytes. It has been established that TSG-6 can induce the differentiation of bone marrow-derived myeloid precursors into plasmacytoid dendritic cells with immunosuppressive activity, characterized by the markers $CD11c^+$ $B220^+$ $CD8\alpha^+$ [176].

Moreover, MSCs release chemoattractant protein-1 (MCP-1) that attracts monocytes/macrophages to the injury site [177]. Evidence has revealed that MSCs promote monocyte viability via direct physical interactions [178]. Importantly, monocytes possess the capacity to generate pro-inflammatory cytokines, including TNF-α and IL-1β, upon exposure to MSCs, consequently triggering the immunosuppressive activity of MSCs. This process can skew the polarization of macrophages towards a specialized state, characterized by increased phagocytic capability and elevated secretion of IL-10 and TGF-β [178–181].

MSCs also possess the ability to modify the immune response through specific interactions with classical monocytes, either by triggering selective apoptosis or by prompting a transition to a non-classical phenotype characterized by elevated levels of PD-L1 expression [49, 123]. It is imperative to underscore that these particular modifications are closely linked to the monocytes' capacity to efferocytose the apoptotic MSCs, rather than being influenced solely by the soluble factors released by MSCs [123]. Monocytes containing apoptotic MSCs undergo systemic migration through the circulatory system, with a notable affinity for the hepatic and splenic regions. Additionally, remnants of MSCs are captured within the hepatic microenvironment and subsequently phagocytosed by Kupffer cells [123]. Consequently, monocytes are capable of inducing long-term adaptive immune responses when they differentiate into macrophages.

Furthermore, the immunosuppressive secretome of MSCs inhibits monocyte differentiation into dendritic cells, reduces DC maturation, and promotes a regulatory and strong phagocytic phenotype in mature DCs (as outlined in [52]). On the other hand, DCs possess the capacity to efficiently engulf apoptotic bodies, thereby impeding the progression of phenotypic maturation within these cells. Additionally, antigens originating from engulfed apoptotic cells can be cross-presented by DCs, suggesting that apoptotic cells might selectively present immunologically important antigenic determinants [182, 183]. Nevertheless, the impact of MSC-derived apoptotic bodies on DCs remains to be investigated.

In brief, macrophages are recognized as the predominant population of mononuclear phagocytes residing in various tissues, while DCs are considered the primary APC responsible for T cell priming [184, 185]. The generation of apoptotic bodies is a consequence of the process of apoptosis [180, 181, 186, 187]. Upon introduction into the systemic circulation, apoptotic cells are rapidly taken in by MQs and DCs located in the spleen, liver, and lungs [188–190]. The important role of macrophages, a specific type of white blood cells responsible for engulfing and eliminating apoptotic bodies, is significant in the initiation, advancement, and resolution of inflammation through undergoing phenotypic conversion. It is noteworthy that there is a suggestion that apoptotic cells tend to be phagocytosed by MQs [191]. Various surface markers present on apoptotic bodies (ABs) have been shown to stimulate the recruitment of MQs to sites of cell death. The internalization of ABs may result in the transformation of MQs into a phenotype that resembles M2 MQs, known for their involvement in anti-inflammatory processes. An example of this phenomenon is

Table 2 Summary of studies on the role of MSC-ABs in multiple pathophysiological settings

Application	Source	Disease/Experimental model	Mechanism	Role	Target cell	Clues Remarks	Ref
Proregenerative /Organ repair	M-BM	Aged mice	Activating autophagy, contributing to autolysosome formation	Rab7	BM-MSCs	Bone homeostasis maintenance	*Lei et al.*, 2022 [276]
	M-BM	Rat bone defect model	Promoting the viability of endogenous BMSCs	ROS/JNK Signaling	BM-MSCs	Promoting new bone formation	*Li et al.*, 2022 [279]
	H-UC, H-BM	Mice apoptosis-deficient Fasmut and Bim−/− model	Assembling multiple nuclear DNA repair enzymes, such as the full-length PARP1, into ABs	PARP1	Recipient cells	Rescue of DNA damage and elimination of senescent cells.	*Hung et al.*, 2024 [278]
	M-BM	Mice model	Metabolization in the integumentary skin and hair follicles	Wnt/β-catenin	Skin and hair follicle MSCs	Promoting wound healing and hair growth	*Ma et al.*, 2023 [171]
	R-BM	Rat myocardial infarction model	Regulate autophagy	Activation lysosome function	EC	Improve angiogenesis and cardiac function	*Liu et al.*, 2020 [274]
	M-BM	Mouse skin wound healing model	Polarization M1 MQ toward M2	Migration and proliferation of fibroblasts	MQs, Fb	Accelerating wound healing process	*Liu et al.*, 2020 [174]
	M-BM	Mouse acute liver failure model	Form a chimeric organelle complex with recipient Golgi, Golgi recovery and ploidy transition	SNARE-mediated membrane interaction	HC	Liver regeneration and protect against acute hepatic failure	*Sui et al.*, 2021 [303]
	H-UC	Rat acute lung injury model	Reducing lung neutrophil infiltration, total protein leakage, IFN-γ, boost in IL-4	Lowering myeloperoxidase activity	Neutrophils	Reducing the degree of injury in acute lung injury	*Liu et al.*, 2016 [282]
	M-AD	Rat sepsis syndrome model	Reduction TNF-α and creatinine levels, expressions of ICAM-1, MMP-9, TNF-α, NF-κB, Bax, caspase-3, PARP, increasing Bcl-2	Suppressing inflammation, apoptosis, oxidative stress and enhancing anti-oxidation and anti-apoptosis	Inflammatory cells $CD68^+$	Protecting lung and kidney from damage	*Sung et al.*., 2013 [304]
	H-DP	Mice and dog tooth implantation model	Elevating angiogenesis	Mitochondrial Tu translation elongation factor	EC	Pulp revascularization and / dental pulp regeneration	*Li et al.*, 2022 [275]
	H-AD	Rat model/in vitro	Enhancing proliferation and migration of EC and Fb. promoting adipogenic differentiation and inhibit the fibrogenic differentiation of Fb	Modulating fibroblasts and endothelial cells	Fb and EC	High-quality skin wound healing/reduce the area of scars	*Dong et al.*, 2023 [277]
	M-BM	Cardiotoxin injury tibialis anterior model	Increasing the fusion index of myoblasts	Pannexin channel	Myoblasts	Promoting muscles regeneration	*Ye et al.*, 2023 [283]

Table 2 (continued)

Application	Source	Disease/Experimental model	Mechanism	Role	Target cell	Clues Remarks	Ref
Immunomodulation	H-BM	Rabbit hypertrophic scar model	Secretion of TSG-6	Activation of caspase-3	CD45- and CD3-positive cells	Regulated inflammation and preventing Scar formation	*Liu et al.*, 2014 [281]
	H-BM	Murine graft-versus-host disease model	Engulfment ABs by phagocytes	Production IDO	Phagocytes	Effecting immunosuppression	*Galleu et al.*, 2017 [58]
	M-BM	In vitro	Inhibition MQs to skew into M1, Suppressing the secretion of TNF-α	AMPK/SIRT1/NF-κB pathway	MQs	Inhibition osteoclast formation	*Ye et al.*, 2022 [131]
Disease Treatment	M-BM	Mice periodontitis model	Interferes with the function of osteoclasts	miR-223-3p targets Itgb1	Osteoclasts	Inhibited osteoclast differentiation, alveolar bone resorption	*Liu et al.*, 2023 [175]
	H-DP	Mice allergic airway inflammation model	Rousting immunosuppressive reaction	Reduction eosinophil infiltration	EO	Suppressing allergic airway inflammation	*Laing et al.*, 2018 [305]
	H-UC	Murine acute endometrial damage and Rat intrauterine adhesions models	Induction macrophage immunomodulation, cell proliferation, and angiogenesis	Reduce fibrosis and promote endometrial regeneration	MQs	Endometrial regeneration and intrauterine adhesions treatment	*Xin et al.*, 2022 [291]
	M-BM	Murine lupus/ Murine arthritis	Inhibited CD25 expression and IL-2 production, Preventing Th17 differentiation and memory	PS	$TCD4^{+}$	Amelioration of inflammation, lupus activity, and joint erosion in murine arthritis	*Wang et al.*, 2023 [280]
	H-BM	Mouse type 2 diabetes model	Induce macrophage reprogramming	Calreticulin	MQs	Counteract T2D	*Zheng et al.*, 2021 [264]
	M-BM, H-AD, H-BM	Mice haemophilia A model	Activate platelet functions	Fas/FasL linkage	Platelets	Ameliorate haemophilia A	*Zhang et al.*, 2022 [263]
	M-BM	Mice osteopenia model	Activate the Wnt/β-catenin pathway	Ubiquitin ligase RNF146/miR-328-3p/Wnt	BM-MSCs	Rescue MSC impairment and ameliorate osteopenia	*Liu et al.*, 2018 [172] [170–173]
	H-UC	Mice sepsis model	Accumulate in the bone marrow of septic mice, switch neutrophils NETosis to apoptosis	Fas ligand, electrostatic charge	Neutrophil	Amelioration multiple organ dysfunction and improve survival	*Ou et al.*, 2022 [298–301]
Antitumor activity	M-BM	Mice multiple myeloma model	Restoring Fas-mediated apoptosis	Fas ligand	Multiple myeloma cell	Inhibition multiple myeloma cell growth	*Wang el al.*, 2021 [306]

Human bone marrow (H-BM), Human umbilical cord (H-UC), Human adipose tissue (H-AD), Lysosomal associated membrane protein 1 (LAMP1), c-Jun N-terminal kinase (JNK), Thymic stromal lymphopoietin (TSLP), Murine bone marrow (M-BM), Human dental pulp (H-DP), Endothelial cells (EC), Fibroblasts (Fb), Macrophage (MQ), Rat bone marrow (R-BM), Hepatocytes (HC), Eosinophil (EO), Phosphatidylserine (PS), Indole amine 2,3-dioxygenase (IDO), Ras-related protein 7 (Rab7), Reactive oxygen species (ROS)

the efferocytosis of MSC-derived ABs by alveolar MQs, a mechanism that has been found to mediate immunomodulation and decrease the severity of autoimmune disorders. The aforementioned observations indicate that apoptosis serves as a highly immunomodulatory phenomenon in the context of MSC transplantation, operating through the intermediary role of MQs. The secretion of soluble mediators by MQs subsequent to the phagocytosis of apoptotic cells plays a crucial role in influencing the activities of dendritic cells, manifesting a potent inhibitory effect on the maturation process of dendritic cells [185–187, 192, 193]. DCs within organs play a crucial role in maintaining peripheral T cell tolerance [183, 186, 189, 194–197], process that restrains both innate and adaptive immune responses to antigens presented on dying cells and upholds immune tolerance [178]. Various factors such as the intrinsic characteristics of cells undergoing apoptosis, the efficacy of their clearance, the specific environment in which apoptosis occurs, and the presence of opsonins bound to the apoptotic cells collectively shape the influence of apoptotic cells on the recipient APCs. This, in turn, impacts the ability of APCs to either enhance or suppress immune reactions [198, 199].

Natural killer cell-MSCs crosstalk facilitated by monocyte/macrophages

The interaction between MQs and NK cells suggests that transplanted MSCs are likely to modulate NK cells through both direct and indirect mechanisms, which involve regulating MQs [200–202]. Rather than inherently functioning as immunosuppressant cells, MSCs can display immunostimulatory characteristics that depend on the specific microenvironment. MSCs have been shown to exhibit distinct immunomodulatory abilities that affect the functionality of mature immune cells, including the NK cell lineage. A notable example of this phenomenon is the requirement for MSCs to depend on specific components in their microenvironment, such as IL-10, to facilitate the expression of the soluble HLA-G5 protein [203]. Monocytes, as a substantial source of IL-10, are essential contributors to efferocytosis, a process potentially regulated by the collective activation of receptors that serve as primary mediators. *Selmani et al.* have illustrated that the interaction with HLA-G-specific receptors, such as KIR2DL4 found on NK cells, inhibits the cytotoxic, adhesion, and migratory functions of NK cells [203]. It is well-established that MSCs are hypoimmunogenic cells in an undifferentiated state, leading to a potentially specific close contact with immunocytes that demonstrate a specific affinity for MSCs.

In this particular context, it is widely believed that TLR4 plays a crucial role in initiating the immune response to eliminate pathogenic microorganisms. However, a study by *Lu et al.* found that TLR4^{+} MSCs located in the tumor stroma exhibited greater efficacy in inhibiting the function and proliferation of NK cells compared to TLR4^{-} MSCs [204]. Two distinct subpopulations of NK cells have been identified, with one exhibiting heightened cytotoxicity and the other characterized by high amounts of cytokine secretion. NK cells possess the capacity to eliminate virally infected, stressed, and cancerous cells through their cytotoxic function. Moreover, there has been documented evidence of the emergence of NK cells with regulatory roles during pregnancy [205]. Moreover, the liver has been identified as the predominant immunological solid organ enriched in distinct lineages of NK cells. The therapeutic potential of MSCs interacting with NK cells may play a role in influencing the immune response in liver damage, autoimmune liver conditions, and liver tumorigenesis. On the other hand, NK cells are considered to be one of the major effector cells mediating reactions such as graft-versus-leukemia [206, 207]. Hence, MSCs could potentially act as accessory cells in modulating the activity of NK cells in a manner that depends on the subtype. NK cells have been studied for their crucial role in the effective lysis of both self and non-self MSCs, leading to the subsequent elimination of MSCs post-transplantation. This process is further characterized by heightened cytokine secretion and activation of NK cells [128]. Notably, the outcomes of separate research endeavors converge to support and validate each other. Based on the findings of the *Petri et al.* [208] and the *Martinez et al.* [209], a complex and time-dependent model of coordinated interplay involving polarization and licensing between MSCs and NK cells has been postulated. During the early phases, MSCs are capable of enhancing the functional capacity of NK cells, leading to increased cytotoxicity and degranulation. Correspondingly, *Cui et al.* have reported that MSCs contribute to the upregulation of IFN-γ release from activated NK cell lineages through a positive feedback loop established between activated NK cells and MSCs. This interaction occurs in a manner that is dependent on both cellular contact and lack thereof, resulting in a heightened inflammatory response of NK cells [210]. Early events may then lead to the upregulation of immunosuppressive and pro-angiogenic properties in MSCs, influenced by a more pronounced reduction in ligands that activate NK cells (such as ULBP1, B7H6, and ULBP2) following exposure to IFN-γ. At the same time, the expression of the immunosuppressive molecule galectin 1 may be heightened. Consistent with reports, MSCs possess various ligands for both activating and inhibiting NK cell receptors. *Abomaray et al.* have discussed the potential interactions between these ligands and NK cell receptors [211]. At subsequent time points, NK cell functionality is restricted by TGF-β and IL-6, leading to the cessation of inflammatory reactions through the induction of a

regulatory senescent-like NK cell phenotype characterized by the absence of CD16. Moreover, the presence of TGF-β on activated NK cells has been shown to hinder cytokine production and reduce the expression of certain activation markers such as NKp44, CD69, and CD25 [212–220]. Additionally, numerous investigations have outlined the impact of MSCs on suppressing NK cell activity [55, 221]. The results of *Ishida et al.* showed that co-transplantation of expanded MSCs (with IFN-γ, TNF-α, IL-1β) suppressed NK cell function and migration in the liver of experimental mouse models of islet transplantation. Their results indicated that PGE2 (derived from MSCs) mediated the suppression of NK cells and subsequently improved islet graft survival [222]. On the other hand, Pradier and colleagues have investigated the effect of allogeneic MSCs on activated NK cell function in vitro and report a reduction in the proliferation and cytotoxic behavior of NK cells mediated by IDO in a long-term contact to promote exhaustion of NK cell function [223]. Surprisingly, the feedback of regulatory NK cells to MSCs promotes their survival, proliferation, and pro-angiogenic properties. During this feedback, senescent-like NK cells favor tissue-regenerative functions of MSCs over MSC killing. In support of this hypothesis, the *Dastagir et al.'s* findings on NK cell-mediated repair and regeneration also revealed that the regenerative role of mesenchymal progenitor cells is dependent on NK cytotoxicity [224]. Notably, the survival of MSCs from NK cytotoxicity was enhanced with senescent-like NK cell effectors. Hence, the interaction between NK cells and MSCs amplifies the regulatory response of NK cells and promotes the proliferation of MSCs. These findings suggest that NK cells play a crucial role in promoting regeneration by positively regulating MSC differentiation, proliferation, and viability. Consequently, MSC-NK crosstalk may link the initial inflammatory immune response to the subsequent resolution of inflammation and tissue repair. In addition to the secretion of various factors like elevated levels of IL-15, IDO, and TGF-β [55, 223–226]. that result in NK cell inhibition, the indirect influence of MSCs on NK cells might be facilitated through MQs [226, 227].

In a unique scenario, MSCs have the potential to induce macrophage polarization towards an M2-like phenotype. These polarized MQs, in turn, exert inhibitory effects on NK cells and other components of the immune system. Within NK cells, their primary effects are on activation status and cytokine production [178]. Emerging findings have demonstrated that MSCs play a crucial role in supporting the viability of monocytes as they differentiate into MQs. Moreover, MSCs have been shown to influence the polarization of these MQs towards a peculiar M2-like functional phenotype, characterized by enhanced phagocytic capabilities and elevated secretion of IL-10 and TGF-β. Specifically, in interactions with activated NK cells, M2-like MQs were found to suppress the expression of activating molecules like NKp44, CD69, and CD25, as well as the production of IFN-γ [178]. On the other hand, the M2-like MQs express the ligands of PD-1, which bind to the inhibitory receptor identified on the membrane of immune effector cells such as NK cells. The engagement of PD-1 and PD-Ls serves to limit both the magnitude and the duration of the immune response. Interestingly, the M2-like MQ has an unstable functional phenotype and has the ability to respond to changes in the environment (such as pro-inflammatory triggers) and undergo polarization towards a pro-inflammatory/immunostimulatory functional phenotype. Consequently, upon interaction with these cells, NK cells may retain the expression of activating receptors and the capacity to secrete cytokines [79, 178]. Indeed, within a complex and reciprocal interplay, the generation of various factors like MCP-1 is amplified by the stimulation of MSCs through IFN-γ originating from NK cells, a process linked to the existence of IL-12 and IL-18 cytokines. The chemotactic agents discharged by MSCs, notably CCL2/MCP-1, have the ability to recruit cells expressing the C-C chemokine receptor type 2 (CCR2), such as MQs and NK cells, thus uniquely influencing the activities of various cell populations within the immune system and their subsets. Several lines of evidence support the notion of the ability of MSCs to attract granulocytes and MQs [79, 228].

Qu et al.. (2015) proposed that MSCs have the ability to suppress NK cell activation and their chemotactic function by inhibiting the expression of sphingosine-1-phosphate receptor type 5 (S1PR5), a crucial receptor involved in the trafficking of NK cells in vivo [203, 207]. Consistent with a prior investigation, the cytotoxicity, adherence, and migratory capabilities of NK cells may be impaired through interactions with HLA-G-specific receptors such as KIR2DL4 and ILT-2 present on NK cells [203].

Overall, NK cells play a critical role as innate cellular effectors in the elimination of virus-infected cells and in the regulation of tumor cell proliferation. Nevertheless, NK cells have been implicated in the pathogenesis of graft-versus-host disease, which can be fatal in some cases. In such situations, the administration of MSCs may be considered as a novel therapeutic option for patient intervention. It is important to highlight that the considerable inhibition of cytotoxic activity triggered by MSCs should not be a cause for concern, as its correlation with the reduction in immune surveillance remains unclear [211]. Hence, owing to the reciprocal interplay between MSCs and NK cells, the utilization of MSCs might not interfere with the favorable outcomes of allogeneic hematopoietic stem cell transplantation and could also alleviate acute liver injury (as demonstrated in mouse models of polyI: C-induced liver injury) [224]. MSCs possess the capacity to attract NK cells through the secretion of

chemokines and trap them via adhesion molecules such as ICAM-1 and VCAM-1. They also promote the process of revascularisation and subsequently enhance suppression through direct cell-cell interactions [229]. These results collectively imply that crucial attributes associated with phenotypic alterations and the onset of senescence/apoptosis in NK cells may potentially be induced by activated MSCs. Interestingly, MSCs exert a variety of immunomodulatory effects on NK cells, thus influencing immune-related conditions in which NK cells play an important role.

Comparison of the three classical types of extracellular vesicles from MSCs

In recent times, there has been a growing interest among various research teams in the exploration of MSC-derived ABs as a novel type of therapeutic biological vehicles containing functional biomolecules aimed at organ regeneration and safeguarding. Various cell types, including but not limited to stem cells, immunocytes, precursor cells, osteoblasts, and endothelial cells, have the capacity to produce EVs [237, 238]. The content of EVs is contingent upon their origin, dimensions, and biogenesis pathway, characterized by surface markers. Similar to cytokines and growth factors, EVs have emerged as a novel mode of intracellular communication, which could facilitate the transport of their bioactive cargo to nearby or distant target cells. Consequently, EVs influence cellular behavior through paracrine and endocrine signaling mechanisms. Evidently, the diverse cargo enclosed within EVs is not random, suggesting specific processes for packaging components within these vesicles [239]. For instance, specific EVs have the capability to convey functionally active receptors between different cells [240].

Three subpopulations of classical EVs have been identified: apoptotic bodies, microvesicles (MVs), and exosomes. They vary in diameter from around 50 nm to over 5 μm, with the larger vesicles often referred to as ABs. Each subpopulation has distinct biophysical properties and physiological functions [239, 241–244]. They are similar lipid bilayer membrane regulatory nanoparticles and exert their own messages by delivering their cargo (vast repertoire of molecules) to targeted immune effectors and other non-immune recipient cells. Exosomes, formed by budding into endosomes and released through exocytosis, and MVs, shed by outward blebbing of the plasma membrane, are released from metabolically active cells, whereas ABs are generated during apoptosis [245]. Natural exosomes, which are small-sized membrane-bound structures, exhibit a preference for targeting immunocytes over phagocytes through blocking phagocytosis. Conversely, engineered exosomes could be associated with the phagocytic system [246]. MVs are characterized by their larger size compared to exosomes and also possess the ability to evade phagocytosis. Moreover, MVs demonstrate a closer resemblance to their parental cell in terms of protein composition when compared to exosomes. The impact of exosomes and MVs can vary significantly, ranging from immunostimulation to immunosuppression, depending on the origin cell. Furthermore, the internalization process of apoptotic cells, also referred to as efferocytosis, plays a crucial role in triggering intrinsic mechanisms like tissue growth, restructuring, regeneration, and specifically, the resolution of inflammation for the maintenance of homeostasis (Table 2).

In contrast, ABs are larger than MVs and exosomes, influencing both professional and non-professional phagocytes. As a result, MQs and DCs constitute their primary target cells. MVs and exosomes, on the other hand, perform a function similar to their originating cells, while ABs are recognized predominantly for their anti-inflammatory properties aimed at preserving homeostasis. Research by *Florek et al.*. and by *Williams et al.* has demonstrated that apoptotic cells can promote immune tolerance by modulating the maturation of APCs, resulting in reduced dendritic cell activation [183, 247]. In relation to the size of EVs, exosomes are identified as the smallest subtype of EVs, with a size of approximately 50 nm, implying enhanced oral bioavailability and stability. Conversely, the majority of ABs have a size of about 1 μm, potentially suggesting a propensity to target MQs via the mechanism of phagocytosis, which involves the engulfment of particles larger than 0.5 μm. Although the antigen-presenting feature of MSCs and their significance in immunomodulation and immune tolerance have been elucidated, exosomes' evasion of the mononuclear phagocyte system leads to reduced antigen presentation.

Similar to exosomes, MVs derived from MSCs exhibit immunomodulatory properties and have been shown to regulate inflammatory responses [248, 249]. Both MVs and exosomes exhibit a multi-targeting characteristic, along with an extended circulation period. These vesicles possess high potential for delivery, increased cellular uptake (excluding the mononuclear phagocyte system), and a notable targeting ability, particularly post modification or engineering. Consequently, exosomes and microvesicles find application in a variety of therapeutic approaches [250–252]. Despite this, the complex and ineffective process of isolating and purifying exosomes poses difficulties for their scale-up in both production and clinical application [253, 254].

The production of apoptotic cell-derived extracellular vesicles, known as ABs, is notable for being regulated in a time-dependent manner, leading to variations in their molecular composition, cargo, and overall profile [255–257]. A recent study conducted by *Zhang et al.* has revealed that different types of apoptotic vesicles play

specific biological roles in regulating stem cell functions and responses, as well as influencing tissue rejuvenation. This influence is primarily linked to the diverse protein and mRNA content found within the various subtypes [258].

The dimensions of the small blebs, known as apoptotic microvesicles or exosome-like entities, exhibit a gradual augmentation during the process of apoptosis, culminating in the creation of substantial extracellular vesicles denoted as apoptotic bodies. Specifically, the allocation of cellular constituents within apoptotic bodies encompasses a diverse array of potentially bioactive components [237]. An example of this is the presence of RNA in apoptotic bodies without concurrent presence of DNA, indicating a potential selective mechanism for the entry of certain biomolecules into EVs [259]. Furthermore, it has been noted that the number of EVs produced per cell in MSCs is higher in comparison to other cell types [260–262]. EVs predominantly contain elements like the extracellular matrix, endoplasmic reticulum, immune response factors, and cell adhesion proteins, whereas ABs are enriched in functional proteins related to cellular functions, metabolism, transportation, and the management of different diseases [263–265].

ABs typically exhibit low immunogenicity, a phenomenon influenced not only by signals originating from themselves but also by the specific cell type responsible for their capture (e.g., phagocytes lodged in the spleen and liver) [179, 266]. Indeed, the process of phagocytosis involving cellular remnants serves to attract inflammatory cells and consequently serves as a mechanism to mitigate inflammation [169, 267].

Apoptosis is intricately linked with cell differentiation, exemplified by the transformation of monocytes into MQs [268]. Thus, apoptosis and differentiation may act synergistically in initiating the immunoregulatory response, a concept applicable to various pathological conditions like autoimmunity, cancer, and infection [269]. Notably, the engulfment of apoptotic cells through phosphatidylserine-mediated mechanisms stimulates MQs to secrete TGF-β1, thereby facilitating inflammation resolution [56, 270].

Autoantigens are likewise incorporated into small ABs at the onset of apoptosis. As a result, ABs may regulate antigen presentation in various disease contexts, including autoimmunity. The engulfment of ABs containing intracellular autoantigens by MQs suggests that ABs might play a role in autoimmune regulation through the presentation of autoantigens [56]. ABs transfer autoantigens to neighboring or distant cells in different tissues via the circulation system. Consequently, through the incorporation of autoantigens into MSCs, it is plausible that their ABs could play a role for biotherapeutics in restoring tolerance in autoimmune disorders. Modified ABs may exhibit superior efficacy in transporting genes, proteins, and chemical compounds to MQs when compared to smaller liposomes, attributed to their distinctive surface molecules such as phosphatidylserine (PS) and calreticulin, as well as their larger dimensions [271]. Remarkably, the integration into liposomal bilayers has been utilized to further augment the alterability of natural vesicles and generate composite vesicles [272].

Extracellular vesicles, known as exosomes originating from MSCs and their distinctive attributes concerning the regulation of the immune system and facilitation of tissue repair, have been comprehensively examined in recent literature [253, 254, 273]. Consequently, in the context of this review, we have tried to present the recent knowledge on mechanisms of action involved in the therapeutic effects of MSC-ABs as an innovative therapeutic intervention. Overall, EVs originating from MSCs are recognized as crucial paracrine modulators that mimic the functionalities of their parent cells. The manipulation of MSCs and their secreted factors could potentially improve the efficacy of EVs including ABs in therapeutic applications, achieved by modulating their properties in a laboratory setting or by modifying the parent cell lineage.

Proregenerative behavior of apoptotic vesicles-MSC

In this context, increasing studies underline the role of stem cells as powerful instruments in organ regeneration. An interesting finding is that apoptotic bodies can stimulate the expansion of resident stem/progenitor cells, thereby facilitating tissue regeneration and replacing dysfunctional cells (Table 2) [261].

Based on this data, the findings suggest that MSC-ABs, when taken up by recipient endothelial cells, play a role in stimulating angiogenesis and helping to improve cardiac performance by modulating autophagy [274]. *Li et al.*. demonstrated that apoptotic vesicles from MSCs could be ingested by endothelial cells (ECs), leading to increased expression of genes related to angiogenesis. This led to the revascularization of dental pulp and regeneration of dental-pulp-like tissue. At a molecular level, the apoptotic vesicles, containing mitochondrial Tu translation elongation factor, can transport and regulate the angiogenic stimulation of ECs through the transcription factor EB-autophagy pathway [275]. Additionally, literature has shown that apoptotic vesicles from youthful MSCs can rejuvenate senescent MSCs by reinstating autolysosome formation, offering a promising approach to age-related bone deterioration [276]. According to *Dong et al.*, apoptotic vesicles derived from MSCs can accelerate skin wound healing, improve the quality of granulation tissue, and reduce scar size by influencing fibroblasts and endothelial cells. Mechanistically, these vesicles could be ingested by fibroblasts and ECs, significantly boosting their proliferation and movement.

Furthermore, they could also promote adipogenic differentiation and inhibit fibrogenic differentiation of fibroblasts [277]. Previous studies have shown that externally sourced apoptotic vesicles from MSCs stimulate metabolic processes in the integumentary system, including the skin and hair follicles. For instance, these vesicles stimulate the Wnt/β-catenin pathway which is crucial for promoting wound healing and hair growth [171].

Impaired programmed cell death has been established to be closely associated with age-related disorders. Nevertheless, apoptotic vesicles originating from young MSCs have successfully revitalized the nuclear abnormalities observed in aged MSCs from bone marrow. Furthermore, they have also restored the diminished ability of these cells to self-renew and differentiate into bone or fat cells by triggering autophagy. In terms of the underlying mechanisms, the young apoptotic MSCs have the capacity to produce and accumulate a considerable quantity of Ras-related protein 7 within the apoptotic vesicles derived from MSCs, which can be taken up and utilized by the recipient aged MSCs to re-establish the formation of autolysosomes. Consequently, this facilitates the initiation of autophagy flux and plays a role in the revitalization of MSCs, contributing to the rejuvenation of MSCs [276]. Huang and colleagues have clarified the vital role of apoptotic vesicles derived from MSCs in protecting tissues from DNA damage [278]. Apoptosis can facilitate the accumulation of various nuclear DNA repair proteins, such as full-length PARP1, within these apoptotic vesicles. Subsequently, the vesicles are transferred directly to target cells, leading to the restoration of DNA damage and the elimination of senescent cells. In an independent investigation, Liu and colleagues have revealed that ABs originating from MSCs have the ability to be internalized by recipient ECs, leading to the stimulation of lysosomal activities and increase in TFEB (transcription factor EB) expression. TFEB functions as a crucial controller in lysosomal formation and autophagy. The consequent elevation of autophagy in ECs has been linked to the facilitation of angiogenesis and the improvement of cardiac function restoration. The observed properties of MSC-derived ABs shed light on the potential therapeutic benefits of these vesicles in conditions characterized by abnormal angiogenesis and impaired tissue regeneration [274]. *Chen et al.* have verified that the local implantation of MSC-derived apoptotic vesicles effectively enhanced bone regeneration within the calvarial defect. The underlying mechanism involves the promotion of new bone formation by apoptotic vesicles-MSCs through the elevation of intracellular reactive oxygen species (ROS) levels to trigger JNK signaling, potentially stimulating the proliferation, migration, and osteogenic differentiation of recipient MSCs [279].

Immunomodulatory activity of apoptotic vesicles-MSC

ABs play a major role in immune regulation through their clearance by immune effectors. ABs' engulfment may support MSC homeostasis by modulating cell survival, proliferation, differentiation, and subsequent secretome. Similarly, ABs can also regulate antigen presentation via these mechanisms in various disease settings, including autoimmunity. *Wang et al.* recently revealed that MSC-derived ABs directly interact with $TCD4^+$ cells and prevent Th17 differentiation, while $Foxp3^+$ cells are maintained [280]. A mechanistic explanation lies in the exposed phosphatidylserine PS on apoptotic vesicles, which facilitates the interaction with T cells, disrupting the proximal signaling transduction of T cell receptors. This procedure inhibits the expression of CD25 and the production of IL-2, IFN-γ, IL17A, and IL-10, ultimately contributing to the mitigation of lupus and arthritis.

Indeed, the available literature indicates that MSC-ABs have demonstrated efficacy in suppressing the formation of hypertrophic scars by modulating the inflammatory response. The ability of MSC-ABs to boost the expression of TSG-6 has been reported as one of the underlying mechanisms, which is partially mediated through caspase-3 activation, underscoring the role of apoptosis in facilitating the anti-inflammatory properties of MSCs [281].

Liu et al.. found that ABs derived from MSCs promote cutaneous wound healing partially by converting MQs to an anti-inflammatory phenotype, which enhances the migration and proliferation of fibroblasts, thereby facilitating the wound healing process [174]. Importantly, apoptotic MSCs have been recognized as a promising advancement in clinical applications, particularly in the management of acute respiratory distress syndrome. This innovative strategy has demonstrated to target the reduction of the inflammatory exudate and vascular permeability in the pulmonary system. Experimental findings have provided insight into the underlying mechanism by which apoptotic MSCs operate, revealing their capacity to diminish pro-inflammatory cytokines, suppress IFN-γ gene expression, and enhance the production of anti-inflammatory cytokines such as IL-4 [282]. As a result, this chain of events leads to a decrease in the pathological damage observed in cases of acute lung injury. Sung and colleagues have documented that the application of apoptotic MSCs in the management of experimental sepsis syndrome may signify a pioneering therapeutic method because of their potential to shield pivotal organs such as the liver and kidney from harm. The central process involves the ability of apoptotic MSCs to downregulate the expression of various inflammatory factors (ICAM-1, MMP-9, TNF-α, NF-κB), as well as oxidative and apoptotic markers (Bax, caspase-3, PARP). Additionally, they decrease the population of inflammatory cells

($CD3^+$) in the pulmonary system and reduce the levels of the DNA damage biomarker (γ-H2AX) in the renal system [282]. In contrast, an increase in antiapoptotic markers (e.g., Bcl-2) and mitochondrial integrity markers (e.g., cytochrome C) has been observed. This is accompanied by the detection of antioxidant markers, both at the protein level (GR, GPx, NQO-1, HO-1) and the cellular level (GR, GPx). Furthermore, a study by *Ye et al.*. demonstrated that apoptotic vesicles derived from MSCs can enhance muscle regeneration by transmitting intercellular signals through the release of metabolites via the activated Pannexin 1 channel [283]. *Ye et al.*. have similarly noted that apoptotic vesicles derived from MSCs have the ability to be phagocytosed by MQs, resulting in a reduction of COX2 expression within proinflammatory MQs. This mechanism effectively inhibits the release of TNF-α and IL-6, while concurrently promoting the secretion of IL-10. From a mechanistic standpoint, apoptotic vesicles derived from MSCs inhibit the transformation of MQs into proinflammatory phenotypes by acting on the AMPK/SIRT1/NF-κB pathway. Moreover, these vesicles also suppress the generation of osteoclasts, bone resorption, and MMP-9 expression, thereby potentially providing valuable perspectives for addressing periodontitis [131]. *Zheng et al.* discovered that MSC-derived apoptotic vesicles, when efferocytosed by MQs, influence the homeostasis of liver MQs in response to type 2 diabetes (T2D). The mechanism underlying this phenomenon involves the ability of MSC-derived apoptotic vesicles to induce transcriptional reprogramming of MQs in an efferocytosis-dependent manner. This cascade of events ultimately leads to the suppression of macrophage accumulation and the transition of MQs toward an anti-inflammatory phenotype within the context of T2D in the liver [264]. The *Zhang ei al.*, has also offered an extensive proteomic overview of apoptotic vesicles derived from MSCs, highlighting their potential in the management of hemophilia A. Through a mechanistic lens, apoptotic vesicles derived from MSCs carry imprints including Fas, enabling interaction with platelets' FasL to trigger platelet activities, thereby ameliorating the hemostatic anomaly [263]. Altogether, as mentioned throughout this article, the process of clearing apoptotic bodies necessitates phagocytes to express specific receptors that can identify ligands associated with apoptotic bodies, subsequently prompting a proresolving phenotype in MQs by reducing the expression of proinflammatory cytokines and elevating the levels of proresolving mediators [67, 69, 284, 285]. Upon ingestion of apoptotic bodies, MQs inhibit the synthesis of proinflammatory cytokines and promote the generation of molecules that suppress inflammation and facilitate resolution and tissue repair. These crucial processes are compromised in conditions characterized by impaired efferocytosis [68, 69, 286–288]. While DCs engaging in efferocytosis of infected ABs can trigger the display of pathogen antigens and activation of effector T cells for host defense, this sequence is typically absent when non-infected ABs are engulfed by resolving MQs [66, 289]. Consequently, interventions directed at enhancing efferocytosis are anticipated to mitigate inflammation and promote resolution.

Using apoptotic MSC-derived vesicles to inhibit disease progression

Exogenous MSC-ABs have been shown to possess the capacity to reverse damage in endogenous MSCs, a process that is crucial for maintaining bone homeostasis and managing osteoporosis. Mechanistically, endogenous MSCs have the capability to internalize exogenous MSC-ABs via integrin avb3 and inhibit Axin. Moreover, the activation of the Wnt/β-catenin pathway is facilitated by ubiquitin ligases RNF146 and miR-328-3p originating from ABs [172]. On the other hand, apoptotic vesicles derived from MSCs have demonstrated the ability to integrate into hepatic tissue and undergo selective internalization by hepatocytes, indicating their potential use for hepatic regeneration and protection against acute liver failure. The mechanistic process involves the formation of a composite organelle structure between apoptotic vesicles and the recipient Golgi apparatus through SNARE-mediated membrane interaction, subsequently promoting the organization of microtubules and cytokinesis in hepatocytes [290].

In the same direction, the results obtained by *Li et al.*. have demonstrated that MSC-ABs inhibit osteoclast differentiation and alveolar bone resorption. From a mechanistic standpoint, miR-223-3p shows significant enrichment within those apoptotic bodies. Itgb1 is targeted by miR-223-3p, which interferes with the function of osteoclasts. Additionally, DC-STAMP is a key regulator that mediates membrane infusion. ABs and pre-osteoclasts express high levels of DC-STAMP on their membranes, mediating the engulfment of ABs by pre-osteoclasts. Collectively, MSC-derived ABs are directed towards internalization by pre-osteoclasts through the DC-STAMP pathway. This process results in the mitigation of alveolar bone loss by delivering miR-223-3p to osteoclasts, subsequently inhibiting their differentiation and bone resorption. These results could suggest that MSC-derived ABs are promising therapeutic agents for the treatment of periodontitis [175]. *Xin et al.*. have discovered that in situ injection of MSC-ABs could efficiently reduce fibrosis and promote endometrial regeneration, resulting in fertility restoration. Mechanistically, ABs could induce macrophage immunomodulation, cell proliferation, and angiogenesis [291]. Additionally, research has provided evidence that apoptotic MSCs can counteract the occurrence of a distinct variant of

programmed cell death known as excessive extracellular trap formation (NETosis) in neutrophils [292, 293]. NETosis is important in the pathogenesis of several diseases, including SLE and mortality in sepsis [294–298]. Surprisingly, MSC-ABs can initiate the Fas/FasL pathway, thereby altering the signaling patterns that guide neutrophils to undergo programmed cell death, shifting them toward NETosis instead. Besides, MSCs can increase the longevity of neutrophils and enhance their phagocytic activity, a process that is dependent on cell-cell contacts [299, 300]. This indicates that MSCs are a promising candidate for the treatment of neutropenia. Moreover, *Ou et al.* have shown that MSC-derived apoptotic vesicles can ameliorate multiple organ dysfunction and improve survival in sepsis. Mechanistically, MSC-derived apoptotic vesicles mainly accumulate in the bone marrow via electrostatic charge interactions with positively charged neutrophil extracellular traps, switch neutrophils NETosis to apoptosis via the FasL-activated Fas pathway, suggesting that cell death is associated with disease development and therapy [300].

Apoptotic vesicles-MSC in antigen presentation

The immunomodulatory properties exhibited by EVs are of great importance as they significantly contribute to the facilitation of antigen presentation, a pivotal component of the adaptive immune response. Research has shown that EVs play a role in promoting antigen presentation via direct and cross-presentation mechanisms [301]. Indeed, apoptotic EVs possess the capability to modulate the process of antigen presentation. The internalization of MSC-ABs by DCs, in a manner comparable to the original cell, has been demonstrated to have the potential to modify their antigen-presenting functions. Notably, EVs originating from apoptotic lymphoblasts have been proven to reduce immune responses by suppressing MHC class II molecules on DCs [302].

Autophagy and MSC-based inflammation and repair modulation

In the context of cytokines and growth factors released by MSCs, EVs may be highly concentrated in the supernatant. EVs have been widely utilized as delivery vehicles for many years due to their targeting capabilities, extended circulation time, and ability to overcome natural barriers [253, 254]. As mentioned, classical EVs, consisting of exosomes, microvesicles, and apoptotic bodies, demonstrate variations in size and the process of their generation. The correlation between the genesis of EVs and the cellular processes linked to autophagy exhibits a multifaceted and varied nature. The ability of EVs to serve as regulators of autophagy and the transfer of autophagic constituents to recipient cells suggest a potential reliance on the structural integrity of lipid rafts [307, 308]. Moreover, the regulation of autophagy could potentially impact not only the number of EVs but also their composition, thereby significantly shaping the ultimate pro-tumorigenic or anti-cancer outcomes of autophagy regulators [309]. Autophagy, also referred to as autophagocytosis, is a fundamental process occurring in most tissues to maintain cellular homeostasis. It can be influenced by stress factors and various immunoregulatory signaling molecules [310]. Moreover, autophagy serves as an inherent and evolutionarily conserved mechanism of cellular degradation that is essential for eliminating unnecessary or impaired components. Besides, it is integral in orchestrating antigen presentation, maintaining lymphocyte homeostasis, and modulating cytokine generation [242]. On the other hand, autophagy modulators influence the content of important signaling molecules in ABs [309]. Impaired autophagy, also operating as a form of type II programmed cell death, may potentially increase the susceptibility of individuals to tumor formation. Research has shown that autophagy provides recyclable materials to support cellular sustenance, which complicates the distinction between "cell death with autophagy" and "cell death by autophagy" [310, 311]. Autophagy, recognized as a process of intracellular self-regulation, is believed to exert a substantial influence on the self-renewal, pluripotency, and differentiation of various categories of stem cells [312]. Notably, the interaction between intrinsic autophagy and the secretion of oxidants has been identified as a mechanism by which MSCs limit the growth of internalized bacteria. Consequently, the use of "conditioned" autologous or heterologous MSCs, or "engineered" MSCs with enhanced autophagic activity, holds significant promise for advancing MSC-based immunotherapy [105].

Moreover, autophagy modulators influence the content of important signaling molecules in ABs [309]. The induction of autophagy is of great significance, as it plays a crucial role in enhancing MSC-mediated immunomodulation against neurological disorders by facilitating the clearance of ABs [313]. Notably, Dang and colleagues provided compelling evidence of autophagy's role in MSC functionality, highlighting the mechanistic induction of BECN1/Beclin 1 expression during their use in experimental autoimmune encephalomyelitis [134]. On the other hand, the suppression of autophagy may be facilitated by the downregulation of Beclin 1, leading to an increase in prostaglandin E2 production, ultimately augmenting the therapeutic effectiveness of MSCs. In the same direction, *Xia et al.*. have outlined that MSC-EVs play a role in facilitating angiogenesis following an ischemic stroke through the possible suppression of autophagy, a process that partly relies on the activation of STAT3 [314]. Recent studies have identified additional categories of EVs, including autophagic EVs, defined by

a mostly theoretical nature and a lack of total exclusivity among them [239, 241]. Autophagy significantly impacts the genesis and excretion processes of EVs. The autophagosome is capable of fusing with various endosomes, resulting in its release as autophagic EVs [239]. A study by Ahrabi and colleagues showed that EVs derived from autophagic MSCs can reduce inflammation and the accumulation of extracellular matrix in damaged renal tissue. Therefore, there is a potential for these EVs to be used as a therapeutic approach for treating renal fibrosis in the future [308].

Conclusion and future perspective

Over the last forty years, there has been a substantial amount of research carried out on MSCs resulting in the identification of their therapeutic potential. It is widely agreed upon that the immunotherapeutic capacity of infused MSCs is not solely reliant on their intrinsic characteristics, but rather necessitates the active involvement of other immune/non-immune cells, such as regulatory T cells and endothelial cells, which also subsequently facilitate tissue repair and regeneration. Additionally, the clearance mechanisms carried out by MSCs may enhance their therapeutic effects. In the meantime, the ultimate fate of administered MSCs continues to present as a substantial unresolved issue, despite their widespread application in clinical settings. As indicated in existing literature, the initial stage of immunotherapeutic action derived from MSCs encompasses intricate and multifaceted intercellular dialogues, whereas in the subsequent phase, MSCs have the capability to actively engage in programmed cell death and discharge ABs.

The formation of ABs as extracellular vesicles derived from apoptotic cells is a significant and efficient process. However, their use as carriers remains limited due to their variable sizes, complex contents, potential pro-apoptotic effects, and rapid clearance by phagocytes [291, 315, 316]. In addition to their enhanced production efficiency, ABs offer the advantage of being more manageable and scalable in production processes, thanks to a better understanding of cell apoptosis compared to the mechanisms involved in the formation of exosomes or microvesicles [264, 317–320]. Furthermore, because exosomes and ABs follow distinct biogenetic pathways, ABs exhibit a higher presence of cytomembrane-embedded protein complexes, which may enhance targeted delivery processes. On the other hand, the superior efficiency in loading components into ABs is linked to the natural encapsulation of molecules within ABs [316, 319–322]. Of importance, the existence of markers such as "find me" and "eat me" on the exterior of ABs aids in the precise recognition and engulfment by phagocytes [291, 315, 316]. A limited number of studies have concentrated on improving the targeting potential of apoptotic vesicles by investigating surface modification techniques or engineering strategies to enhance specificity for targeted cell or tissue delivery, potentially transforming drug delivery systems [316, 319, 320, 322–324]. For example, *Dou et al.*. have formulated chimeric ABs that are equipped with a natural membrane and a modular delivery system designed for the purpose of modulating inflammatory responses [322].

Therefore, it has been commonly noted that exogenous MSCs often undergo apoptosis following infusion. This phenomenon is regarded as a novel mechanism that significantly contributes to the therapeutic benefits observed in MSCs transplantation [120]. As supported by available evidence, this series of events is a crucial prerequisite for maintaining the immunoregulatory properties of MSCs and may be linked to the prediction of clinical responses. Apoptosis predominantly elicits tolerance, a process thought to underlie immunoparalysis. The development of immunoparalysis seems to be influenced by the nature and concentration of specific ligands, while tolerance may be an intrinsic feature. Research has shown that components of the innate immune system can trigger either trained immunity or tolerance-based immunity, independent of adaptive immune cells. Hence, the modulation of the immune response by apoptotic MSCs occurs through a series of sequential actions. The sequence of steps involves the internalization of apoptotic MSCs by phagocytic cells, reprogramming of their functionality, and modulation of specific intracellular signaling pathways. These mechanisms are facilitated by the innate functional plasticity of immunocytes and result in the enhancement of the host's immune regulatory networks, which is associated with the long-term therapeutic efficacy of infused MSCs [53]. By identifying the receptors and signaling pathways that govern the functional fate of immunocytes, these discoveries have the potential to enable the differentiation and continuous regeneration of a reservoir of memory-like immunocytes. Such developments could have significant implications for host responsiveness.

Existing evidence substantiates the notion that the death of MSCs is likely to be triggered by a variety of complex mechanisms as outlined in this manuscript, including cellular cytotoxicity and activation of the complement system, both of which have a limited impact and could occur simultaneously. Additionally, the condition of the tissue stroma may provide insights into the induction of caspase-dependent apoptosis in MSCs [142]. It has been clarified that a strong cytotoxic reaction to MSCs, along with elevated levels of pro-inflammatory cytokines, may serve as reliable indicators for identifying individuals suitable for MSC infusion. Furthermore, in cases involving immunological complications and impaired inflammatory resolution, a discrepancy can

occur in the abundance and effectiveness of cytokines, as varying cytokine concentrations may lead to different outcomes [325]. This disparity may explain the inconsistent findings regarding the influence of MSC infusions in patients suffering from the same conditions. As a result, the prediction of clinical response and the therapeutic effectiveness of transplanted MSCs depend on multiple factors, some of which may be influenced by the specific disease condition and the route of administration. In this regard, the beneficial aspects of infused apoptotic MSCs in the disease setting may be related to the specific inactivation method (programmed cell death) and the distinct homing niche characterized by a diverse array of hematopoietic cell types with efferocytic capacity [48, 58, 149, 282, 304, 326]. Going forward, the emphasis in forthcoming clinical trials should transition from merely selecting the most suitable population of MSCs to identifying the specific patients or diseases that are most predisposed to positively responding to MSC therapy, enhancing the MSC product, and formulating a treatment centered on the functional aspects of MSCs.

To summarize, MSCs possess the capacity to be prompted to release their secretome, which comprises extracellular vesicles, in response to signals present in their local microenvironment. These biologically active molecules play a pivotal role in expediting tissue healing by restraining stress reactions and programmed cell death. Subsequently, MSC-derived apoptotic bodies play an important role in the maintenance of tissue and organ homeostasis and in immune modulation in a wide variety of diseases. It is noteworthy that the characteristics of MSC-ABs can be modified through *ex vivo/in vitro* priming of MSCs, which carries extensive implications for the efficient translation of cell-based therapies and has been embraced as an attractive approach to augment the therapeutic target profile of MSCs [291, 322, 325, 327]. This might encompass the integration of cytokines, pretreatment with probiotics, introduction of genetic modifications, drug delivery systems, and other strategies. A key objective in expanding the use and impact of apoptotic body-MSCs, along with advancing treatments involving nanoparticles and drugs, is to explore the potential of apoptotic bodies as carriers for pharmaceutical delivery in various preclinical contexts beyond the current research scope. Consequently, regulating the apoptotic status of MSCs and ABs could significantly enhance the efficiency of MSCs in biomanufacturing and biotherapeutics for clinical applications.

Abbreviations

ABs	Apoptotic bodies
APC	Antigen-presenting cells
AMPs	Antimicrobial peptides and proteins
CCR2	C-C chemokine receptor type 2
CCL	C-C chemokine ligand
COX2	Cyclooxygenase 2
CTL	Cytotoxic T lymphocyte
DC	Dendritic cell
ECs	Endothelial cells
EVs	Extracellular vesicles
FasL	Fas ligand
HLA	Human leukocyte antigen
HSCs	Hepatic stellate cells
ICAM-1	Intercellular adhesion molecule 1
IDO	Indoleamine 2,3-dioxygenase
IFN-γ	Interferon gamma
IL	Interleukin
KCs	Kupffer cells
KLF6	Kruppel-like factor 6
MCP-1	Chemoattractant protein-1
MM	multiple myeloma
MMP 13	Matrix metalloproteinase 13
MQs	Macrophages
MRs	Mannose receptors
MSCs	Mesenchymal Stem/Stromal Cells
NETosis	Excessive extracellular traps
NFκB	Nuclear Factor Kappa B
NK	Natural killer cell
NO	Nitric oxide
PD-L	Programmed death ligand 1
PGE2	Prostaglandin E2
PS	Phosphatidylserine
PTPN22	Protein tyrosine phosphatase non-receptor 22
Rab7	Ras-related protein 7
ROS	Reactive oxygen species
S1PR5	Sphingosine-1-phosphate receptor type 5
SLE	Systemic lupus erythematosus
SRs	Scavenger receptors
T2D	Type 2 diabetes
TFEB	Transcription factor EB
TGF-β	Transforming growth factor beta
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TSG-6	TNF-α induced gene/protein-6
VCAM-1	Vascular cell adhesion molecule 1

Author contributions

Akram Hoseinzadeh, Rasoul Baharlou, and Mahmoud Mahmoudi designed and drafted the manuscript. Akram Hoseinzadeh, Seyed-Alireza Esmaeili, Reza Sahebi, Anahita Madani Melak, Mahmoud Mahmoudi, Maliheh Hasannia, and Rasoul Baharlou participated in manuscript writing. Akram Hoseinzadeh has drawn the Figures. All authors have fully read and approved the final manuscript.

Funding

Not applicable.

Data availability

No data was used for the research described in the article. This is a review article. Data sharing not applicable – no new data generated. Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

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.

Artificial intelligence (AI)
The authors declare that they have not use AI-generated work in this manuscript.

Author agreement
This manuscript has been read and approved by all the authors, and all authors listed on the manuscript have agreed to its submission.

Author details
[1]Department of Immunology, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, Iran
[2]Department of Immunology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[3]Department of Modern Sciences and Technologies, Mashhad University of Medical Sciences, Mashhad, Iran
[4]Department of Biology, Kavian Institute of Higher education, Mashhad, Iran
[5]Cancer Research Center, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, Iran
[6]Department of Immunology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Received: 27 April 2024 / Accepted: 21 January 2025
Published online: 04 February 2025

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