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Review Article

Progress and prospect of mesenchymal stem cell-based therapy in atherosclerosis

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Abstract: Atherosclerosis is a chronic inflammatory disease of the arterial intima, occurring usually in the aged populations who are suffering from hypertension, dyslipidemia and diabetes for a long time. Research on atherosclerosis has shown that macrophage foam cell formation, inflammation, dyslipidemia and immune cells infiltration are all involved in regulating the onset and progression of atherosclerosis. Mesenchymal stem cells (MSCs) originated from different kinds of tissue are a group of cells possessing well-established self-renewal and multipotent differentiation properties as well as immunomodulatory and anti-inflammatory roles. Recent studies have displayed their dyslipidemia regulation functions. Transplantation of MSCs to atherosclerotic patients might be a new multifactorial therapeutic strategy to improve atherosclerosis. This review updates the advancement on MSCs and atherosclerosis.

Keywords: Mesenchymal stem cells, foam cells, macrophages, differentiation, t cells, atherosclerosis

Introduction

Despite the remarkable achievements have been made in the past decades, cardiovascular diseases still remain to be one of the leading causes of death worldwide, leading to immense health and economic burdens globally [1]. Atherosclerosis is the major cause of cardiovascular diseases.

Although the pathogenesis of atherosclerosis is not completely clear, current evidence indicates that macrophage at least involves in the pathogenesis. Under some conditions, the monocytes are recruited into the vascular intima, where they take up of modified low-density lipoprotein (LDL), particularly low-density lipoprotein cholesterol (LDL-C), to form macrophage foam cells. These lipid overloading foam cells mark the initiation of atherosclerosis and their accumulation and necrosis or apoptosis further promote the development of atheromatous plaques and eventually lead to serious cardiovascular diseases [2].

Lipid lowering, especially by statins, has been the most effective way to reduce risk of atherosclerotic cardiovascular diseases currently [3]. Despite the great progress achieved in the pharmacologic treatments with statins, several large controlled clinical trials, from the long-term intervention with pravastatin in ischemic disease study to the improve-it trial, from the cholesterol and recurrent events trial to the Scandinavian simvastatin survival study, all support that cardiovascular risk reduction including treatments with statins remains far from satisfactory [4-6]. Moreover, approximately two thirds of patients under the treatment of statins continue to suffer from the expected cardiovascular disease events and many patients cannot tolerate statin or follow a long term stains treatment to reach optimal LDL-C levels [3]. Thus, additional therapies for cardiovascular diseases, particularly for effective lipid lowering to inhibit foam cells formation so as to improve atherosclerosis are needed.

Inflammation and immunity are also known to be intimately involved in all stages of atherosclerosis, which links multiple risk factors covering aging, hypertension, dyslipidemia and diabetes for atherosclerosis. Moreover, inflamma-

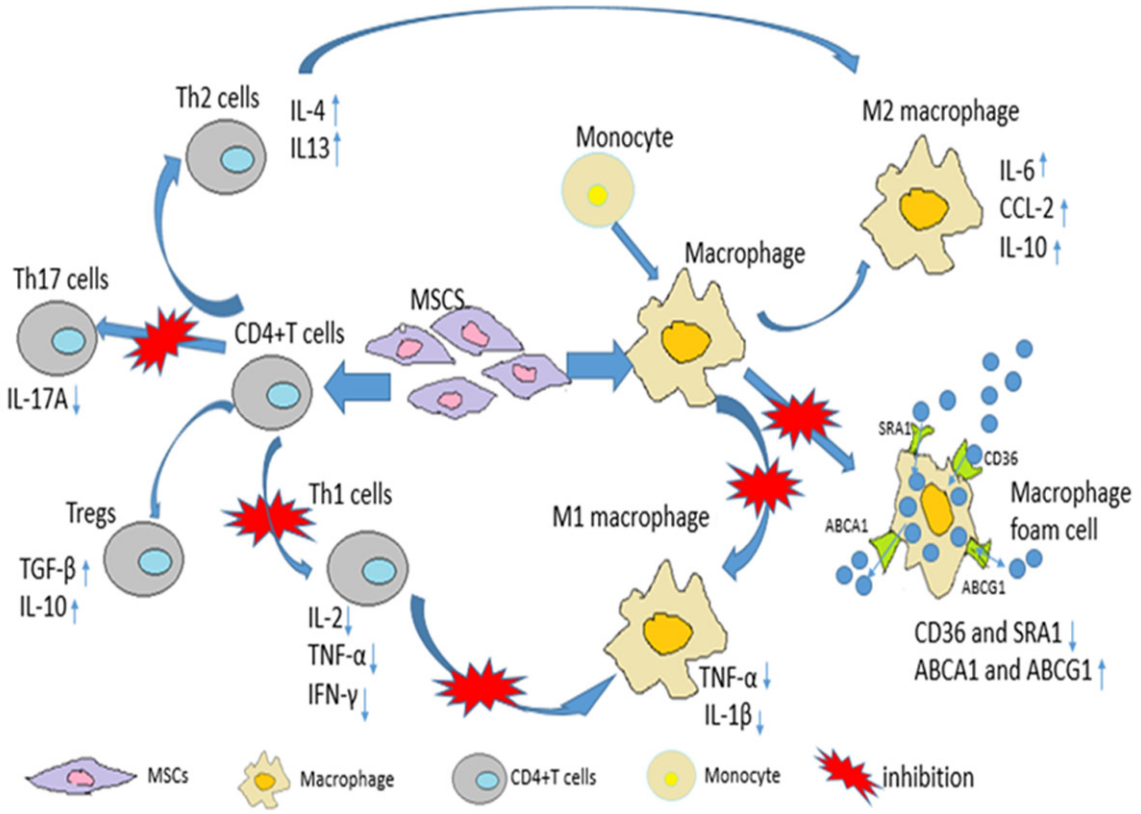

Figure 1. The immunomodulatory and anti-inflammatory effects of MSCs on immune cells involved in atherosclerosis. MSCs suppress Th1, Th17 whereas promote Th2 and Treg cells. MSCs also regulate the balance between M1 and M2 macrophage. MSCs inhibit M1 but facilitate M2 differentiation. MSCs also curb the formation of foam cells from macrophage in atherosclerosis.

tory and immunological signaling can alter the behavior of the intrinsic cells of the artery wall like endothelium and smooth muscle cells, leading to lipid peroxidation, endothelium dysfunction and further recruitment of inflammatory and immune cells to the vascular intima [2, 7-10]. The roles of anti-inflammation and anti-immunity therapies in mitigation of atherosclerosis have been support by increasing studies [11-13].

Given the underlying pathological and physiological development of atherosclerosis, current and future treatment strategies should more focus on lowering plasma cholesterol and attenuating inflammation and balancing immunity. Mesenchymal stem cells (MSCs), also known as multipotent mesenchymal stromal cells, are a cluster of well-established cells characterized with non-hematopoietic, self-renewal and multipotent differentiation properties. Bone marrow is considered to be original source of MSCs. More studies have showed that MSCs can be isolated from the different tissues including umbilical cord, placenta, adipose tissue and human gingiva [14-16]. Recently, the anti-inflammatory and immunomodulatory effects of MSCs on autoimmune and inflammatory diseases have been increasingly appreciated [17-22]. Additionally, MSCs also took part in the lipid metabolism, reducing serum cholesterol strikingly [23]. These properties of MSCs may open a new avenue for the treatment of atherosclerosis. This review will update the study progress and discuss the possibility to apply MSCs for improving atherosclerosis. The review also proposes some questions that are needed to be overcome.

MSCs inhibit foam cell formation

Multiple lines of evidence, from genetic, experimental, epidemiological to clinical studies,

have converged on plasma cholesterol, particularly low density lipoprotein cholesterol (LDL-C), as the primary driver of the initiation and progression of the atherosclerotic plaque. After being recruited to the intima by activated or damaged endothelial cells, the monocytes differentiate into macrophages. These macrophages are then able to take up modified low-density lipoproteins (LDL) particles such as oxidized LDL (ox-LDL) and thereby transform into foam cells. Foam cells, mostly arising from monocytes, are recognized as the early pathological changes of atherosclerosis [24]. During the foam cell formation, two steps are critical in maintaining lipid homeostasis in macrophages: 1) cholesterol uptake mediated by scavenger receptors such as CD36 and scavenger receptor A (SR-A), and 2) cholesterol efflux mediated by ABCA1/ABCG1 [25]. When the balance was disturbed, the foam cells are formated.

Interestingly, a recent study shows that bone marrow derived mesenchymal stem cells (BM-MSCs) can inhibit the formation of macrophage foam cells *in vitro* and in ApoE-KO mice, and the mechanism underlying this therapeutic effect might partly be related to the downregulation of scavenger receptors CD36 and SRA in response to infusion of BM-MSCs (**Figure 1**). Also, anti-inflammatory cytokines IL-10, which is thought to be able to modulate the lipid metabolism and protect from atherogenesis, was significantly upregulated in the BM-MSC treatment [25, 26]. Moreover, BM-MSCs-treated mice displayed a significant reduction in circulating monocytes and serum cholesterol level [23].

MSCs induce the polarization of M2 macrophage

Inflammation plays an important role in all stages of atherosclerosis, which involves different kinds of immune cells. Inside the atherosclerotic plaque, macrophages also account for the vast majority of immune cells [27, 28]. Normally, arterial endothelial cells resist the attachment of leukocytes cells streaming past them. When the endothelium is subjected to harmful stimulus such as dyslipidemia and hypertension, monocytes are recruited to the intima where they differentiate into M1 macrophages, produce and release pro-inflammatory cytokines. Further, some of them engulf lipoprotein to become macrophage foam cells.

Interestingly, some studies showed that mesenchymal stem cells can reprogram macrophages into anti-inflammatory phenotypes, M2 macrophages [29, 30]. When co-cultured with macrophages, human gingiva-derived mesenchymal stem cells (GMSCs) can convert macrophages into M2 phenotype, inducing the secreting of IL-6, CCL-2, IL-10, and decreasing the production of TNF-α. After systematically injection, GMSCs can home to the wound site to accelerate the wound healing by secreting anti-inflammatory cytokines and enhance macrophage phagocytic capacity [29]. Additionally, cardiac adipose tissue-derived mesenchymal stromal cells (AT-MSCs) also can polarize macrophages toward an M2 anti-inflammatory phenotype, and this function was mediated partly by IL-6 (**Figure 1**). Interestingly, these AT-MSCs are shown to weaken macrophage phagocytic capacity [30]. Whether macrophage phagocytic capacity is weaker or stronger when co-cultured with MSCs and the signaling pathways by which MSCs play to reprogram macrophage may need further research.

Skin-derived MSCs (S-MSCs) are also able to migrate to the atherosclerotic plaque to modulate the function of macrophages after tail-vein injection and reduce the formation of atherosclerotic plaque in Apo $E^{-/-}$ mice. This modulatory function of S-MSCs in macrophage is thought to partly depend on the impairment of the NF-κβ signaling pathway in S-MSCs and the increased release of COX-2 or PGE2 from S-MSCs. In turn, these changes stimulated the release of anti-inflammatory cytokine IL-10 and decreased the release of inflammatory cytokines TNF-α and IL-1β, leading to the reduction of atherosclerotic lesions in Apo $E^{-/-}$ mice eventually [31].

However, it is noteworthy that aortic smooth muscle cells can be transformed into a dysfunctional macrophage-like phenotype by cholesterol loading [32]. When one tries to evaluate the macrophage inside atherosclerosis, it should be taken into consideration to avoid underestimating the function of macrophage involved in the atherosclerotic plaque [32].

Effects of MSCs on T cells

It was widely thought that immune cells played little role in atherogenesis until Hansson et al

reported the presence of lymphocytes within atherosclerotic lesions before 1986 [2]. Although in much lower number than macrophages, accumulating evidences now show that adaptive immune cells, mainly T and B lymphocytes, also exist and involve in atherosclerotic plaque and atherosclerosis. To date, the CD4+ effector T cells that play a role in atherosclerosis include Th1, Th2, Th17 cells and Tregs [2, 33-35].

Among these cells, Th1 cells are predominately involved in aggravating atherosclerosis, no matter whether in a plaque of human or mouse [36]. A variety of inflammatory cytokines are produced by Th1 cells, such as IFN-γ, TNF-α and IL-2. Among these inflammatory cytokines, IFN-γ is closely related to the instability of atherosclerotic plaque and reduces the collagen production of smooth muscle cells. IFN-γ also increases the expression of adherence factors and the lipid absorption of macrophage and further increases the rupture of unstable plaques. Cleaning up of CD4+ T cells showed a 70% reduction in plaque size [37], further highlighting the importance of T cells in the pathogenesis of atherosclerosis.

In contrast, few Th2 cells are found in atherosclerotic plaques, their roles in atherosclerosis remains unclear [38-40]. However, polarizing leukocytes to a Th2-like profile can inhibit of experimental atherosclerosis [41, 42]. Clinical evidence also supports a protective role of Th2 cells in cardiovascular disease such as myocardial infarction [34].

Th17 cells also exist in atherosclerotic plaques, studies to date are inclined to support that Th17 cells play an atherogenic role in atherosclerosis [33, 39, 43-45]. However, the role of IL-17A, one cytokine mainly produced by Th17 cells, remains somewhat controversial in atherosclerosis. Some studies suggested that IL-17A is one of the pathogenic factors in atherosclerosis, and inhibiting of IL-17A can reduce atherosclerotic lesion development in ApoE-deficient mice *via* weakening its widespread of pro-inflammatory and pro-apoptotic effects in atherosclerosis [46]. In contrast, Gistera and her colleagues observed that IL-17A can induce a stable plaque phenotype by stimulating the collagen production of human vascular smooth muscle cells, and blocking IL-17A receptor could increase the cardiovascular events in patients [47].

T regulatory cells (Tregs) are another subset of T cells. They play an important role in maintaining the immune tolerance and immune homeostasis [48-51]. The protective function of Tregs in atherosclerosis has been confirmed in multiple studies [43, 52-55]. In unstable atherosclerotic lesions, the number of Tregs is much lower compared to stable ones [54]. Accordingly, an increase in Tregs can alleviate atherosclerosis in animal models [43, 53].

Accumulating studies showed that MSCs can wake up the immune activity of different kinds of immune cells including T cells, B cells, NK cells and so on. This immunosuppressive effect provides MSCs an advantage in cell-based therapy. *In vitro*, studies indicated that GMSCs are able to suppress the activation and proliferation of Th1 and Th17, and enhance the differentiation of regulatory T cells [56]. Also, BM-MSCs are found to promote the expansion of Tregs as well as improve the function of Tregs in atherosclerotic mice [23]. Moreover, BM-MSCs also inhibit the inflammatory cytokines secreted by effector T cells [57]. All together, these studies indicate that MSCs are able to regulate the balance between inflammatory effector T cells and anti-inflammatory Tregs to maintain a stable plaque or reduced atherosclerosis, implicating that MSCs maybe a potent candidate for atherosclerosis therapies (**Figure 1**).

Impairment of MSCs by age and age-related diseases

Atherosclerosis is a multifactorial-induced chronic disease and usually accompanied by age and age-related diseases. However, age and age-associated conditions also impair the properties and functions of MSCs [58-62]. Although transplantation of human MSCs from all patients can improve the heart function in rats with myocardial infarction (MI), the ability to ameliorate MI is significantly reduced in MSCs from aged subjects than that from younger ones [58]. Also, the angiogenic potential of adipose-derived mesenchymal stromal cells from aged patients declines, even though they can maintain stable mesenchymal stromal cell properties [60]. Moreover, human MSC-mediated T-cell suppression also markedly reduced

in aged, T2DM and atherosclerotic subjects [61]. Further, MSCs isolated from experimental type II diabetes displayed the impaired regenerative capacity in post-ischemic neovascularization [62]. The hyperinsulinemia-induced oxidant stress brought by experimental type II diabetes in MSCs may help to explain the impairment of MSCs. Nonetheless, hypoxic stimulation conversely promotes the immunomodulatory properties of GMSCs by inhibiting the proliferation of PBMCs as well as increasing the apoptosis of PBMCs, which was thought to associate with the Fas ligand (FasL) expression of GMSCs [63].

Given to the anti-inflammatory and immune regulatory function, as well as their effect on restoring endothelial function [64], one can conclude that MSCs may be a promising candidate for the treatment of atherosclerosis. Since the vast majority of patients that may benefit from MSCs therapies in atherosclerosis are elderly individuals, clarifying the underlying molecular mechanism by which MSCs function and by which aged-MSCs are weaken, as well as proper MSCs donors selection are important to maximize the therapeutic effect of MSCs in atherosclerosis.

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Disclosure of conflict of interest

None.

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