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Therapeutic Prospect of Adipose-Derived Stromal Cells for the Treatment of Abdominal Aortic Aneurysm

Mojtaba Parvizi and Martin C. Harmsen

Aneurysm refers to the dilation of the vessel wall for more than 50%. Abdominal aortic aneurysm (AAA) refers to the dilation and weakening of all three layers of the abdominal aorta, which mostly occur infrarenally. The population aged above 50 years is at risk of AAA development, while a familiar history doubles the risk. Progression of AAA can cause imminent rupture of the vascular wall and has a high mortality and morbidity risk. They are additional risk factors for AAA development such as gender, smoking, and dyslipidemia. In general, pathological features of AAA include inflammation, degradation of the extracellular matrix (ECM), and smooth muscle cell apoptosis. The main pathophysiology of AAA development is still unknown. Besides available treatment modalities for large AAA, which associate with a high mortality risk, effective, alternative, and safer treatments are required, preferably already at an early stage of AAA. For the last decades, tissue engineering and regenerative medicine showed promising potential therapeutic effects for various (cardio-vascular) diseases, including AAA. Adipose tissue-derived stromal cells (ADSC) are a candidate source of stem cells for regenerative medicine. ADSC are isolated from adipose tissue with low risk and are easily cultured and expanded while maintaining their multipotency. In addition, due to their differentiation capacity and trophic factor production, ADSC serve an important role in tissue engineering and regenerative medicine modalities. In this review, we will highlight the main pathobiology of AAA and introduce ADSC as a new promising therapeutic source for small AAA.

Introduction

CARDIOVASCULAR DISEASE is the leading cause of mortality in the world [1]. One of the major vascular diseases is aneurysmal arterial disease, in which the vessel wall progressively weakens and dilates. Excessive dilation frequently associates with acute rupture of the arterial wall with an 80% risk of death [2,3]. Aneurysms most commonly occur in the cerebral arteries and the aorta. The underlying cause of aortic aneurysms (AA) remains largely unknown, yet several risk factors associate with AA, including male gender, smoking, age, and family history [4,5]. The incidence of abdominal aortic aneurysm (AAA) varies between one and three percent among the elderly, with a prevalence for men over women [6]. This is likely to change because the fraction of women who smoke increases compared with man [4]. To date, screening for aortic dilation by ultrasonography is recommended for male smokers in the age group of 65–75 years. In a steadily increasing number of countries, screening programs are under development. Unfortunately, the onset and development of AAA are commonly asymptomatic. On routine examination, AAA is usually detected as a palpable, pulsatile, expansible, and nontender mass. In some cases, AAA is a coincidental

finding during an abdominal X-ray or ultrasound study performed for other reasons. At a later stage, AAA may become painful due to the dilation of the vessel lumen. Acute aneurysmal pain, often together with hypotension, is the early warning and indication of imminent rupture and, thus, requires immediate medical intervention [7]. Nevertheless, acute rupture of AAA mostly occurs without any prior warning, which is life threatening. Ultrasonography is the most commonly used technique to diagnose aneurysm. In general, patients with a large aneurysm, that is, at high risk of rupture, are treated with open surgery or endovascular repair. However, patients with small-sized AAA are monitored using a watch and wait strategy. Clearly, novel therapeutic modalities such as pharmacological intervention, gene therapy, and stem cell therapy are warranted [8].

In this review, we will discuss the pathogenesis of AAA, current treatment strategies, and the beneficial potential of ADSC for the treatment of small AAA.

Pathological Features of Aneurysms

AAA is characterized by chronic inflammation and extracellular matrix (ECM) degradation by proteolytic enzymes,

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mostly matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) [9,10]. In due time, this may result in severe dilation of the aortic wall and its rupture, which obviously has a high mortality rate. The abdominal aorta is an elastic artery that consists of three layers: *tunica intima*, *media*, and *adventitia* [8]. The *tunica intima* is the inner layer of the vessel, which consists of a monolayer of endothelial cells and subendothelial connective tissue over an intact internal elastic lamina (IEL) also known as *lamina elastic interna* (Fig. 1a). The *tunica intima* is the first layer of cells that is in contact with the circulation and, as such, also the first layer to be challenged with pathological triggers, such as hyperlipidemia, hyperglycemia, high blood pressure, inflammation, to mention a few. It is no surprise that the *tunica intima* is involved in the onset and progression of several vascular disorders, such as atherosclerosis and, importantly, aneurysm. The onset of these diseases involves the activation of the endothelial cells in the *tunica intima*. Activated endothelial cells secrete chemoattractants and express cell surface adhesion molecules that together promote at-

traction and adhesion of monocytes (Fig. 1b). These monocytes migrate into the *tunica media* of the aortic wall and differentiate into macrophages, which initiate aneurysm formation, through unknown mechanisms (Fig. 1b). The *tunica media* is the middle layer of an artery and is composed of about 28–30 concentric lamellar subunits buildup of smooth muscle cells (SMC) that are embedded in connective tissue-related ECM molecules such as collagen and fibronectin (Fig. 1a). Medial layers of SMC are separated by rings of elastin that span the circumference of the media. The elastic layer in the *tunica media* contributes to approximately two-thirds of the thickness of the entire *tunica media*. In comparison to the thoracic aorta, the abdominal aorta has less collagen and elastin fibers, which renders it more prone to develop aneurysms with an increased risk of rupture [11] (Fig. 1b). The outermost layer of the aorta, the *tunica adventitia*, is largely composed of collagen and other ECM proteins, which are deposited by fibroblasts (Fig. 1a). The *tunica adventitia* contains inflammatory cells too that serve a sentinel function. Large arteries such as the aorta have their

FIG. 1. Development of abdominal aortic aneurysm. (a) Normal aortic tissue. (b) Abdominal aortic aneurysm (AAA) development and progression. Adhesion and infiltration of monocytes, differentiation of monocytes to macrophages, formation of intraluminal thrombi (ILT), phenotypic shift and apoptosis of aortic smooth muscle cells (SMC), degradation of elastin and collagen, accumulation of T lymphocytes and neovascularization are the best-known features of AAA. Color images available online at www.liebertpub.com/scd

own vasculature called *vasa vasorum*. For a long time, the *tunica adventitia* has been considered of minor importance in arterial disorders and disease development [12,13]. Yet, a growing body of evidence shows that adventitia-derived inflammatory cells and cytokines contribute to the development of both atherosclerosis and aneurysm [14]. Finally, most large arteries, including the aorta, are embedded in adipose tissue that serves a regulatory role [14–16]. Besides adipocytes, this tissue consists of endothelial cells, inflammatory cells, and stem/progenitor cells. Perivascular adipose tissue regulates the function of large arteries and, thus, its dysfunction coincides with the development of vascular disease. Perivascular fat is also involved in the formation of aneurysms [12,13,17–20].

Different extracellular proteases such as serine elastase, matrix metalloproteinase (MMP), and serum plasminogen are present at increased levels in human AAA tissues. The MMP comprises collagenases and elastases, MMP that can degrade both the *lamina elastic interna* as well as the interstitial ECM (Fig. 1b). Thus, these MMP are essential players in aneurysmal disease. Moreover, pharmacological inhibition of MMP, for example, with doxycycline, has been deemed as a possible treatment for AAA. Upregulation of a number of MMP family members (namely MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, and MMP-12) in human and animal AAA tissues has been shown [9,10,21–24]. Meanwhile, downregulation of tissue inhibitors of metalloproteinase (TIMP-1, 2) in AAA has been documented [25]. In the aorta, the main producers of MMP and TIMP are fibroblasts, SMC and, during the development of aneurysm, macrophages are the main source.

The MMP primarily degrade collagens, yet elastin is another important component of the aorta that is affected in aneurysm. Indeed, a plethora of proteases with elastase activity are overexpressed in AAA, including MMP-12, serine elastase, plasminogen activator, and cathepsins-S, L, and K [26–28]. Literature suggests that cathepsins are involved in the pathogenesis of aneurysm by degrading the elastin fibers in the aortic wall [29–35]. As mentioned, activated macrophages are present in AAA and contribute to a chronic inflammatory phenotype; this coincides with the ECM and elastin fiber degradation [25]. Moreover, several other components of both the innate and adaptive immune system have been found in human AAA tissue samples, including mast cells, neutrophils, and T lymphocytes [17,23,36] (Fig. 1b). The *vasa vasorum* is the main route for immune cells to gain access to the adventitia and media in the large arteries [18]. Upon migration and accumulation of immune cells in the aortic wall, they secrete proteases, proinflammatory factors among others, which cause the formation of reactive oxygen species (ROS) [13,23]. ROS is a strong inducer of apoptosis. Mast cells play a critical role in the progression of AAA, on the one hand, by recruiting other inflammatory cells such as neutrophils, macrophages, and T lymphocytes (CD4⁺ cells 3- to 20-fold greater than CD8⁺ cells) and, on the other hand, by secretion of MMP and proinflammatory cytokines. Furthermore, increased numbers of mast cells have been found in the adventitial and outer layer of the media in AAA [37]. The present mast cells also induce apoptosis of vascular SMC (VSMC) in AAA.

Cytokines such as IL-1 β , IL-10, IL-6, IL-13, TNF- α , and IFN- γ in the diseased aortic wall promote AAA develop-

ment through modulation of the secretion of MMP and serine proteases. For instance, it has been reported that in AAA, IFN- γ induces cathepsin S secretion from VSMC [26,28,30,32,33,35,38].

So far, all the studies that are based on the investigation of surgical samples from late stages of AAA or from animal studies, do not explain the initial trigger and initiation of AAA [39–42]. An important typical “chicken or egg” question that remains to be answered is whether immune cells such as lymphocytes and macrophages are a cause or a consequence of AAA, or perhaps both. During the onset of AAA, immune cells are likely attracted by activated endothelial cells and set the stage for the progression of AAA, which might be governed by yet other immune cells. Unfortunately, current clinical studies only allow investigating late- and end-stage AAA tissue samples, whereas animal models are limited to acute and artificial induction of aneurysms.

Besides, other factors such as atherosclerosis, genetic and epigenetic backgrounds are also involved in the pathogenesis of vascular diseases, including aneurysms. Despite reports that state an association between diabetes mellitus and the vascular disease in general, diabetes mellitus might have a protective role in the formation of AAA [43,44].

Besides endothelial cells, SMC are the main workhorse of an artery and regulate vascular contraction and tension. Alterations in the number and function of SMC, therefore, rapidly interfere with vascular function or the structural integrity of the arterial wall. In the media of the abdominal aorta, SMC are the main cellular component too. In this study, the SMC not only serve a contractile role but, in addition, produce, deposit, and continuously remodel the vascular ECM. They generate ECM components that provide structural strength, such as fibrillar collagens and proteoglycans, whereas SMC also generate the elastin fibers that make up the inner elastic layers that provide elasticity to the aorta. Thus, a loss or distortion of these structural proteins will contribute to the aneurysmal formation [11].

Apoptosis is a physiological and tightly regulated process that is essential in physiological tissue turnover, including in arteries. Dysregulated apoptosis is associated with the pathogenesis of various vascular diseases, including aneurysms [45–48] (Fig. 1b). As mentioned before, the accumulation of the inflammatory cells in the aortic wall is one of the characteristics of aneurysm. In particular, T lymphocytes can induce Fas-mediated apoptosis in other vascular cells such as SMC. The relationship between inflammation and aneurysm development has been clearly established. The main cell types that are affected by inflammation are predominantly not only SMC but also endothelial cells. In particular, inflammation-generated ROS is affecting endothelial survival and causes dysfunction, which renders these cells important targets for future therapy too [11,49,50]. Far better studied is the fate and function of SMC during AAA. In general, two main processes occur by which SMC contribute to AAA development. First, due to inflammatory triggers, apoptosis of SMC is increased, which leads to the massive loss of these contractile cells and, thus, of dilation of the abdominal aortic wall [45–48]. Second, the delicate balance of synthetic and contractile phenotype of SMC is tilted to a synthetic phenotype. In these phenotypically shifted SMC, the turnover balance of ECM also is tilted toward more degradation (increases of active MMP-2 and MMP-9) than ECM production. This results in the loss of structural

components of the aortic wall, which contributes to dilation too [11,49,51] (Fig. 1b).

Therapeutic Targets for AAA

The development of an effective therapy for AAA has proved a large challenge—the translation of preclinical in vitro and in vivo studies to a better understanding of clinical features of AAA falls short of appropriate models. As mentioned before, most clinical knowledge is gathered from late- and end-stage AAA. Whereas clinically AAA is a slowly developing disease, the current models are acutely induced forms of AAA, although with close similarities in the end stage. Despite the limitations of the current models, they are powerful and suffice to understand large parts of the pathophysiological mechanisms that underlie AAA and to anticipate therapies. Indeed mechanisms implicated in the AAA pathology, which could potentially be targeted by medical therapies, are shown by various studies, such as mast cell degranulation, c-Jun N-terminal kinase (JNK), angiotensin II (Ang-II) blockade, to mention a few (Fig. 2).

Current treatments

For small AAA, that is, AAA that are still developing and focusing to prevent the progression and rupture of AAA, clinical investigations are relevant and could be used to determine the efficacy of interventional treatment. This would result in reduction of the required surgery and limitation of the cardiovascular events. Currently, available evidence from clinical studies in AAA patients and animal studies has shown the beneficial effects of cessation of smoking [52,53], increase of exercise, use of beta-blockers [5,54], doxycycline [55,56], statins [57], angiotensin pathway inhibitors [57–59], antiplatelet drugs [60,61], and mast cell stabilization.

Signaling pathways

Human AAA tissue has increased levels of phosphorylated JNK, which appears to suppress the synthesis and deposition of ECM both in vitro and in vivo [62]. The

in vivo study comprised two animal models for AAA, that is, periaortic application of CaCl_2 in mice and continuous infusion for 4 weeks of Ang-II in apolipoprotein E-deficient ($\text{ApoE}^{-/-}$) mice [63,64]. The development of AAA and its subsequent regression by inhibition of JNK pathways were confirmed in both animal models [62] (Fig. 2).

ECM remodeling

Alternatively, modulation of the excessive ECM degradation during AAA would be therapeutically relevant. Indeed, in MMP-9 knockout mice, the development of AAA was delayed, whereas AAA normally developed in $\text{MMP12}^{-/-}$ mice [65]. Moreover, the administration of the nonselective MMP inhibitor doxycycline also largely retarded AAA development [65]. In another study, Sun et al. [66] used two rodent models for AAA by either infusion of Ang-II in $\text{ApoE}^{-/-}$ mice or by periaortic CaCl_2 application in rats. They found a positive correlation between the number of mast cells and the AAA diameter, both rodent models, which correlated well with their observation in human AAA tissues. Also, the in vivo administration of a mast degranulator, C 48/80, promoted AAA development. In vitro data from their investigation indicated that mast cells directly increased the MMP-9 activity produced by macrophages (Fig. 2).

Mast cells

Pharmacological intervention with inhibitors of mast cell degranulation is expected to attenuate AAA development [66]. Others confirmed that mast cell-deficient ($\text{Kit}^{W-sh}/\text{Kit}^{W-sh}$) mice failed to develop AAA after elastase infusion—a transplantation with bone marrow from wild-type mice, that is, restoration of mast cells regained the susceptibility to AAA formation [67]. Together, these studies emphasize the prominent role of mast cells in AAA development (Fig. 2).

Cell therapy

Cell therapy is a new and noninvasive therapy for different types of (vascular) diseases. Several studies report

FIG. 2. Pathological mechanisms of aneurysm formation and progression and clinical/experimental therapies for abdominal aortic aneurysm. (a) Inhibition of inflammatory responses by JNK-inhibitor, statins, ACE-inhibitors, antiplatelet, etc. (b) JNK-inhibitor, doxycycline, and statins are used to inhibit extracellular matrix (ECM) degradation and proteolytic activities (like MMPs). (c) Inhibition of oxidative stress by statins etc. (d) Antiplatelet therapies usually used to prevent ILT formation and are effective mostly in medium/large aneurysms.

that cell therapy inhibits the development and progression of AAA in experimental models [68–70]. Stem cells are advantageous because, in general, they proliferate well and several types of stem cells can differentiate into cardiovascular cell types, such as endothelial cells and SMC. Whereas the secretion of trophic factors by stem cells, which promote tissue regeneration, renders them suitable candidates for the treatment of AAA too [71]. In general, mesenchymal stem cells (MSC) are present in virtually all organs and tissues of the body, although from a historical perspective most research has been done with MSC from bone marrow. For this review, we focused on MSC, in particular, from adipose tissue, also known as adipose tissue-derived stromal cells (ADSC). Cultured populations of ADSC are heterogeneous by definition, yet do contain F-cfu (fibroblast colony-forming units), whereas every population of ADSC also contains a proportion of clonable self-renewing cells. On the other hand, most cells in an ADSC population will quit proliferating at less than 20 passages. Therefore, we argued it to be more appropriate to indicate the source of ADSC (stroma) than to use the term stem cells for ADSC.

Adipose-Derived Stromal Cells

There is a clear need to improve the classical therapeutic approaches for the treatment of cardiovascular disease. To choose a suitable cell source, a number of criteria such as the availability of the cells, the relative risk of harvesting, the rate of proliferation, and differentiation capacity should be considered.

ADSC are the number one choice candidate cell for the treatment of cardiovascular diseases that require re-education of dysfunctional endothelial cells or SMCs [72,73]. ADSC are easily harvested and are pluripotent, that is, differentiate into different lineages, such as osteoblasts, adipocytes, chondrocytes, and even suggested endothelial cells [74]. Yet, more importantly, ADSC can differentiate into SMC—the predominantly affected cell type in AAA. Besides, ADSC secrete a plethora of trophic factors, such as growth factors and cyto/chemokines, including fibroblast growth factor, insulin-like growth factor (IGF), vascular endothelial growth factor, hepatocyte growth factor, IL-8, monocyte chemoattractant protein 1 (MCP-1), and also indoleamine 2,3-dioxygenase (IDO) and prostaglandin-E2 (PGE-2). The latter two are strong anti-inflammatory factors and contribute to the known anti-inflammatory features of ADSC [75,76]. Moreover, ADSC are potent tissue and matrix remodelers that correlate with their abundant production of ECM components, such as collagen, fibronectin, and elastin [77,78]. Much of the observed protective effects of ADSC for the treatment of cardiovascular disease relate to their secretion of proangiogenic and antiapoptotic factors [79]. Moreover, recent evidence shows that ADSC can modulate remodeling after induction of AAA in experimental animal models. ADSC are relatively easy to expand *in vitro*, so large quantities can be obtained [77,80] without difficulty and at minimal risk, and those features render these suitable candidates for regenerative medicine-based approaches to treat vascular disease. This will be discussed in detail in the following section [77–79,81,82].

Immunomodulation by ADSC

Histopathological analysis of human AAA tissues showed that the normal lamellar architecture was altered in comparison to normal aorta and that these changes were associated with invasive inflammation. Following accumulation of the inflammatory cells in the aortic adventitia, T cells and macrophages produce MMP followed by degradation of ECM and dilation of the aortic wall subsequently. In addition, this local inflammation causes the phenotypic shift and/or death of VSMC and leads to more production of MMP and associated degradation of ECM. Downregulation or better modulation of inflammation is one of the prime goals in the treatment of AAA and can be achieved by using doxycycline, as an MMP-9 inhibitor, or other anti-inflammatory medications. Recently published data show that ADSC reduce inflammation both *in vitro* and *in vivo*. Gonzalez-Rey and others showed that human ADSC modulate the behavior of T lymphocytes and the inflammatory responses in patients with rheumatoid arthritis [83,84]. In a coculture system, human ADSC inhibited both the proliferation of T lymphocytes and their production of inflammatory cytokines. However, this phenomenon required both IL-10-producing T cells and monocytes [84]. Part of the immunomodulatory characteristics of human ADSC relate to suppression of the numbers of circulating T lymphocytes (Th17) [85]. In addition, ADSC-secreted PGE-2 suppresses the activity and function of T lymphocytes [86,87]. Finally, clinical evidence suggests that ADSC influence immune tolerance. In a murine model for Crohn's disease, an autoimmune chronic inflammatory bowel disease, ADSC shifted the immune balance from T helper-1 to more tolerant T lymphocytes. This depended on the upregulation of the anti-inflammatory cytokine IL-10, which was induced in regulatory T lymphocytes by ADSC [88,89]. Importantly, chronic ulcer in Crohn's patients could be cured by treatment with ADSC [88,89]. Taken together, this not only suggests ADSC as a promising moiety for the treatment of autoimmune diseases but also to modulate numbers and function of T lymphocytes in AAA, in which lack of regulatory T lymphocytes causes T helper-1 lymphocytes to take the upper hand [90–92]. Both attraction and activation of immune cells should be better regulated in AAA. In that respect, Hashizume et al. [70] demonstrated the anti-inflammatory potential of MSCs through downregulation of IL-6 and MCP-1 and reduction in the MMP activity [70]. As previously mentioned, macrophages are key players in AAA formation and development. Two archetypal forms of macrophages exist, that is, M1 or classically activated proinflammatory macrophages and M2 or alternatively anti-inflammatory and wound healing type of macrophages [93,94]. With regard to the treatment of AAA, the presence and action of M2 macrophages are desired over M1 macrophages. In particular, while M1 macrophages contribute to and sustain the ongoing chronic deleterious inflammation. Interestingly, the trophic factors secreted by MSC re-educate macrophages and repolarize their phenotype from M1 to M2 [95–98]. On the other hand, MSC attract and direct the macrophage wound healing function *in vivo* [99,100]. In addition, MSC-secreted IDO not only redirects T-lymphocyte function but also repolarizes macrophages to M2 phenotype [101].

Adutler-Lieber et al. investigated the influence of cardiac ADSC on macrophages and they showed that cardiac ADSC directly regulate the phenotype of human macrophages in favor of anti-inflammatory subtype 2 (M2), confirming the anti-inflammatory role of ADSC [102] (Fig. 3).

ADSC Differentiation Potential to SMC and Their Paracrine Effects

The tremendous accumulative loss of SMC in AAA requires an adequate source of replacement cells. In principal, either pre-existing SMC from, for example, the saphenous vein could be used or alternatively a suitable source and type of stem cell could be used and differentiated to SMC. Either of these methods is confronted with significant challenges. Currently available methods such as isolating cells from biopsy samples are associated with limitations, including a low number of cells, decreased cell contractility, and a substantial amount of time required to expand to the number of desired cells. The latter poses a threat to the elderly patient who is at a higher risk, but has cells that feature a low(er) proliferative capacity. Stem cells are less sensitive to the influence of aging and loss of function due to wear and tear. As previously mentioned, ADSC can differentiate to different lineages, including functional SMC [103–105], thus rendering these cells promising for replacement of the arterial media [11]. The role of SMC in AAA is complex: on the one hand, ROS-induced apoptosis

decimates SMC by the numbers, whereas the remaining SMC are overly excessively engaged in the degradation of the remaining ECM. Thus, the challenge for ADSC is to both replace the lost SMC, while the remaining SMC need to be re-educated or at least their balance between synthetic and contractile phenotype needs to be restored (Fig. 3). As mentioned in the previous paragraph, this likely requires tuning of the ongoing inflammation by readjusting present T lymphocytes and macrophages [102]. Yet, it can also be achieved through directly influencing of SMC by ADSC-secreted factors. In non-AAA animal models, such as for kidney ischemia/reperfusion injury and nerve injury model, ADSC could suppress apoptosis in vivo [83,106]. In vitro, the potent antioxidant features of ADSC protected human dermal fibroblasts from the effects of exposure to oxidative stress [107].

ADSC and ECM Production

The disturbed wall hemostasis and loss of structure in the aortic wall following inflammation caused by the loss of SMC and degradation of elastin/collagen fibers are the main pathophysiological features of aneurysm formation. Thus, the restoration of the physiological ECM of the aortic wall is a main therapeutic challenge. Together with the modulation of the inflammation and re-education and replenishment of the SMC, ECM remodeling is an important third therapeutic goal in the treatment of AAA. Elastin and collagen (mostly

FIG. 3. Hypothetical mode of action of adipose tissue-derived stromal cells (ADSC) in AAA treatment. We hypothesize that ADSC can treat AAA. ADSC are well known as immunomodulatory cells. They inhibit the activation of T lymphocytes and repolarize the phenotype of M1 macrophages to M2. ADSC themselves can differentiate to functional SMC-like cells, but inhibit SMC apoptosis. In addition, ADSC produce essential ECM components such as collagen, elastin, and laminin. All of these characteristics, as summarized in Fig. 3, are promising for AAA treatment. Color images available online at www.liebertpub.com/scd

type I and III) fibers are the main components of a large vessel structure. Degradation of these fibers results in aneurysm formation. ADSC produce and process a number of important vascular ECM components (Fig. 3). Remarkably, mechanical stimulation is an important physical stimulus for ADSC and regulates the synthesis of collagen and elastin synthesis and their processing [78]. Thus, ADSC can help rebuild the architecture of the aortic wall through deposition of ECM. In fact, this is exploited for the generation of replacement vessels, as other studies have shown that ADSC-secreted ECM is suitable as a scaffold for tissue engineering purposes. Production of ECM components by ADSC can influence SMC phenotype and survival, depending on the ECM component and activated pathways [108–111]. Various ECM productions, differentiation capacity of ADSC make them into a suitable cell source for tissue engineering [78]. Yet, in a rat model for AAA, ADSC promoted the secretion of elastin by SMC in the affected wall [112].

Current Cell Therapy Status in AAA

As previously described, a cell therapy approach in diseases, such as aneurysm, could be used to directly or indirectly target affected cells. The indirect approach (paracrine effect) is mostly used to influence the microenvironment, immune cells, inflammation, and to stimulate/rescue resident cells. In AAA, the suppression of the ongoing inflammation, the rescue of SMC from apoptosis, and assisting SMC to be more ECM-producing cells instead of MMP-producing cells are the main goals. In a rat xenograft model, administered VSMC inhibited AAA in a paracrine manner [68,69,113]. In this model, the paracrine action reduced inflammation and proteolysis and resulted in an improved homeostasis of the arterial wall [68,69,113]. In a similar model, Losy et al. [114] showed the advantage of cell therapy through endovascular therapy of VSMC in an animal model of AAA. The endovascular delivery of SMC halted the progression of AAA progression through interference with the TGF β -1 pathway. However, the animal model used on these experiments, that is, the xenograft rat model, is not the optimal model to study AAA. The isolated SMC from the tissue of the xenograft rat model are also not comparable to the SMC isolated from proper AAA tissues [114] (Table 1).

Only limited studies have targeted the inflammation in AAA with MSC [70,115]. In vitro cocultures, bone marrow-derived MSC suppressed the expression of MMP-2, MMP-9, and TNF- α in macrophages [70]. In coculture of MSC and SMC, the expression of elastin expression by SMC was increased [70]. This shows that MSC can target and re-educate both culprit cell types in AAA, that is, macrophages and SMC. Ex vivo, bone marrow-derived MSC decreased the MMP-2 activity in SMC, whereas the elastin content was preserved. In the Ang-II infusion *ApoE*^{-/-} mice model for AAA, implantation of MSC caused a decrease in the wall of MMP, IL-6, MCP-1, and TNF- α and an increase of IGF-1 and TIMP-1 [70]. Together, this suggests that also in vivo, MSC can readjust the tissue homeostasis of the aortic wall. Moreover, in an elastase-perfused model for AAA in *IL-23*^{-/-} or *IL-17*^{-/-} mice, the administration of human placental MSC promoted a shift to regulatory T lymphocytes involving IL-17 production that correlated with a delay in

the progression of AAA [115] (Table 1). It remains to be confirmed that ADSC can perform similar to BM-MSC in the above pathology models.

In different xenograft rodent models, human MSC were highly effective to treat. The endovascular administration of human bone marrow-derived MSC in rats significantly reduced the expansion of the vessels dilation, whereas MMP-9 was reduced as was the influx of macrophages [116]. Simultaneously, the MMP activity was reduced due to an increased TIMP-1 expression. Moreover, the restructuring of the aortic wall in this rat model for AAA was markedly improved as observed by the formation of neo-aortic tissue that was rich in SM- α -active positive cells surrounded by newly formed collagen and elastin networks. These new tissues were covered by luminal endothelial cells [116]. Even systemic administration of MSC could alleviate symptoms of AAA. Human bone marrow MSC injected intravenously in Ang-II-infused *ApoE*^{-/-} mice caused a decreased aortic diameter, lower intrawall MMP activity, and less macrophage infiltration compared with controls [117]. Moreover, MSC treatment caused a decreased expression of IL-1 β , IL-6, and MCP-1, whereas TIMP-2 and IGF-1 were both increased [117]. In another xenografting approach, murine muscle-derived stem cells differentiated in vitro to VSMC-like progenitor cells (VSMC-PC) by PDGF-BB were implanted into the rat elastase-induced AAA model. VSMC-PC-treated groups had a decreased rate of aneurysm formation that coincided with a decreased MMP expression and activity [118] (Table 1).

Rodents and man differ greatly; thus, large animal models for AAA are required. They only sparsely exist; one model comprises the administration of two spaced patches of Dacron within the wall of the abdominal aorta in pigs to induce AAA [119]. Treatment of the resulting aneurysmal sacculus with ADSC, administered with fibrin glue, showed that the ADSC were retained for at least 3 weeks. Interestingly, the overall inflammation was reduced in ADSC-treated animals compared with controls, which suggests the efficacy of ADSC in a large animal model [119] (Table 1).

Current clinical and predominantly preclinical data show that MSC and ADSC (as a potent source of MSC) act as multitasking therapeutic cells that adjust all three main aspects of AAA: (1) inflammation, (2) SMC loss and phenotypic shift, and (3) remodeling of the ECM (Fig. 3).

Discussion

Accumulated data from various in vitro and in vivo studies have shown promising efficacy of stem cell therapy for the treatment of AAA [68–70,114,115,118–120]. The translation of these data to stem cell-based clinical trials is within reach in the coming years and depends not only on a better mechanistic understanding but also on additional issues such as routing and dosing. Determination of the optimal stem cell source to isolate and generate pure population is the first challenge. Availability, proliferation capacity, trophic factor production, and differentiation potential to SMC-like cells are the main characteristics for optimal stem cells in regenerative medicine. It is important to recognize (1) the best clinically relevant administration route with the lowest risk of further damage or even rupture and (2) safe animal product-free culture conditions and

TABLE 1. SUMMARY OF THE STEM CELL THERAPY STATUS IN ABDOMINAL AORTIC ANEURYSM

AAA model	Cell type	Route of administration	Outcome	References
Xenograft	VSMC	Endoluminally	AAA formation prevented - Decreased monocyte/macrophage infiltration - A shift of the proteolytic-antiproteolytic balance	69
Xenograft	VSMC	Endoluminally	Aneurysm diameter stabilization - Increase in TGF- β 1 infiltration - Paracrine secretion of TGF- β 1 by VSMC	114
Xenograft	VSMC	Endovascular	Aortic diameter and elastin content stabilization - Decreased monocyte/macrophage infiltration - Decreased MMP activity and increased TIMP activity. Aneurysms were rich in collagen and lined with an endothelium instead of a thrombus in controls	68
Ang-II infusion in <i>ApoE</i> ^{-/-}	BM-MSc	Aortic adventitial surface	Decreased AAA diameter - Suppressed activities of MMP-2 - Preservation of elastin content - IL-1 β , IL-6, and MCP-1 down-regulated - TIMP-2 and IGF-1 upregulated	70
Elastase-perfusion	Placenta-MSc	Intravenous	Attenuated AAA formation and IL-17 production	115
Dacron patches	ADSC	Endovascular (into the aneurysmal sac)	Lower inflammation reaction - retention of stem cells for 3 weeks postimplantation	119
Xenograft	BM-MSc	Endovascular	Decreased AAA diameter expansion - Decreased MMP-9 expression and macrophage infiltration - Increased TIMP-1. Formation of a neo-aortic tissue rich in SM- α -active positive cells surrounded by collagen and elastin network covered by luminal endothelial cells	116
Ang-II infusion in <i>ApoE</i> ^{-/-}	BM-MSc	Intravenous	Decreased AAA diameter - Decreased macrophage infiltration - Suppressed activities of MMP-2 and MMP-9 - Preservation of elastin content - IL-1 β , IL-6, and MCP-1 decreased - Increased TIMP-2 and IGF-1	117
Elastase-induced	MDSC	Endovascular	Decreased rate of aneurysm formation - MMP expression at the genetic, protein, and enzymatic level was decreased	118
CaCl ₂ exposure	ADSC	Through common carotid artery	Inhibition of MMP-2 activity - Reconstruction of elastin fibers	112

AAA, abdominal aortic aneurysm; ADSC, adipose tissue-derived stromal cells; MMP, matrix metalloproteinase; MSC, mesenchymal stem cell; VSMC, vascular smooth muscle cells.

pretreatment of stem cells are the main challenge for AAA clinical therapy. Although various studies support the beneficial effects of stem cells in AAA and several other cardiovascular diseases, those effects should be verified.

The impact of stem cells on the remodeling of ECM, inflammation, and diseased SMC during the treatment of AAA, in particular, the stabilization or even reduction of AAA symptoms requires further investigation [78,84,87,121–125] (Fig. 3). Adequate monitoring and visualization of disease parameters such as SMC numbers and quality are not possible with the current measurement systems, but require attention. The retention and function of administered stem cells in the aortic wall are a big challenge, in particular, in clinical trials in which individual stem cells cannot be traced as yet. Finding the best way for stem cell application to improve retention without any negative influence on their function or improve their differentiation/function toward AAA therapy is

required. The use of guiding biodegradable biomaterials for delivery has not been discussed in this review, because it was beyond its scope, yet retention and function of stem cells can be easily tuned using smart biomaterials. In any stem cell-based approach for AAA treatment, MSC or ADSC likely act along two ways (1) direct effect, cell-cell and/or differentiation to target cell types and (2) indirect through paracrine signaling (Fig. 3). ECM assembly, stabilization of elastin components in aortic tissue, attenuated VSMC phenotype and function, and modulating macrophage phenotype and T-lymphocyte activity are the most favorable outcomes for AAA therapy. However, to understand the influence of the AAA microenvironment by local signals, such as provided by inflammation on the behavior of stem cells, is essential to reach the optimal stem cell therapeutic efficacy, for instance, trophic factor production and differentiation, among others. This information could be partially obtained

by well-established in vitro and ex vivo models. Additional information could be achieved through human AAA specimen analysis; however, those investigations lack the initial phase of AAA. In addition, since stem cell application in human, at first place, without any confirmation is not feasible, stem cells' direct or paracrine effect can be investigated in organ culture systems. Further development of appropriate animal models of AAA is important for the effective and systemic evaluation of AAA studies and therapies. Finally, besides all stem cell criteria, the development of feasible and clinically applicable stem cell delivery technique(s) is a challenging area. Local delivery, stem cell retention, efficiency, and a minimally invasive isolation procedure are the main criteria of stem cell therapy. Endovascular and/or perivascular approaches could be considered. Nevertheless, endovascular delivery has been shown to be limited by tissue uptake. Perivascular application seems the ideal site for cell delivery, since AAA is known as aortic media disease. As Hashizume et al. reported in 2011, periadventitial application of stem cells demonstrated effective results on AAA progression [70]. Nonetheless, stem cell retention and functionality in the AAA microenvironments need to be improved (Fig. 3).

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