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Review Article

Regulatory T cells as a new therapeutic target for atherosclerosis

Han-xiao OU[1, #], Bing-bing GUO[1, #], Qi LIU[1], Yu-kun LI[1], Zhen YANG[2], Wen-jie FENG[3], Zhong-cheng MO[1, *]

[1]*Clinical Anatomy & Reproductive Medicine Application Institute, Department of Histology and Embryology, University of South China, Hengyang 421001, China;* [2]*2016 Grade Clinical Medicine Class of Medical School, University of South China, Hengyang 421001, China;* [3]*2015 Grade Medical Imaging Class of Medical School, University of South China, Hengyang 421001, China*

Abstract

Atherosclerosis is an autoimmune disease caused by self- and non-self-antigens contributing to excessive activation of T and B cell immune responses. These responses further aggravate vascular inflammation and promote progression of atherosclerosis and vulnerability to plaques via releasing pro-inflammatory cytokines. Regulatory T cells (Tregs) as the major immunoregulatory cells, in particular, induce and maintain immune homeostasis and tolerance by suppressing the immune responses of various cells such as T and B cells, natural killer (NK) cells, monocytes, and dendritic cells (DCs), as well as by secreting inhibitory cytokines interleukin (IL)-10, IL-35 and transcription growth factor β (TGF-β) in both physiological and pathological states. Numerous evidence demonstrates that reduced numbers and dysfunction of Treg may be involveved in atherosclerosis pathogenesis. Increasing or restoring the numbers and improving the immunosuppressive capacity of Tregs may serve as a fundamental immunotherapy to treat atherosclerotic cardiovascular diseases. In this article, we briefly present current knowledge of Treg subsets, summarize the relationship between Tregs and atherosclerosis development, and discuss the possibilities of regulating Tregs for prevention of atherosclerosis pathogenesis and enhancement of plaque stability. Although the exact molecular mechanisms of Treg-mediated protection against atherosclerosis remain to be elucidated, the strategies for targeting the regulation of Tregs may provide specific and significant approaches for the prevention and treatment of atherosclerotic cardiovascular diseases.

Keywords: atherosclerosis; cardiovascular diseases; regulatory T cells; immune regulation; treatment strategy

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Introduction

Atherosclerosis is a chronic inflammatory disease characterized by inflammatory cell activation, abnormal lipid deposition and plaque formation within the intima-media layer of large and medium-sized arteries[1]. Epidemiological studies have revealed that the incidences of atherosclerosis are increasing due to the improvement of living standards. The largest threat in atherosclerosis involves the rupture of instable plaques and thrombosis, which leads to cardiovascular complications such as acute coronary syndrome (ACS) and stroke. Previous studies on the pathological mechanism of atherosclerosis have largely focused on lipid metabolism disorders, inflammatory response cascades, abnormal proliferation of vascular smooth muscle cell (VSMC), endothelial cell (EC) dysfunction and foam cell formation[2]. However, accumulating evidence suggests that the activation of innate and adaptive immune responses is involved in the development and progression of atherosclerosis. As a result, numerous monocytes and T and B cells are recruited to lesion sites, enhancing the inflammatory response and promoting the initiation and progression of atherosclerotic lesions.

Although the most important cells in atherosclerotic lesions are macrophage-derived foam cells, T cells are extensively studied in mouse atherosclerosis models based on their immune and genetic manipulation[3]. Tregs are a specific subpopulation of T cells, and numerous subtypes of Tregs, including $CD4^+CD25^+$ Tregs, type 1 regulatory T cells (Tr1), type 3 helper T cells (Th3), $CD4^+LAP^+$ Tregs, $CD8^+CD28^+$ Tregs, and Tγδ Tregs, have been reported. The most typical Treg is $CD4^+CD25^+$, which is crucial in immune response regulation[4]. $CD8^+CD28^+$ Tregs inhibit activation of naive and effector T cells and antibody production[5]. The function of Tγδ Tregs is similar to that of Tr1, which suppress the proliferation and

# These authors contributed equally to this work.
*To whom correspondence should be addressed.
E-mail zhchmo@hotmail.com
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pro-inflammatory cytokine secretion by naive T cells and regulate mucosal immune tolerance. In addition, these cells also regulate tumor immunity and autoimmunity[4, 6]. Th3 mainly mediates oral tolerance and suppresses the proliferation of Th1 and Th2[4]. $CD4^+LAP^+$ Tregs are associated with various autoimmune diseases, such as atherosclerosis and diabetes, in mouse models[7].

Although cellular immune responses are detrimental to the development of atherosclerosis, all Treg types mediate immunomodulation and protect against atherosclerosis. The processes mainly involve the secretion of inhibitory cytokines, production of immunosuppressive enzymes, inhibition of cell-to-cell contact, metabolic disruption, and suppression of DC maturation and function[8-10]. The inhibitory cytokines secreted by Tregs mainly include IL-10, IL-35, and TGF-β. In addition, Tregs express several characteristic molecules related to their function, including the forkhead box P3 transcription factor (FOXP3), cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), inducible co-stimulator (ICOS), lymphocyte activation gene-3 (LAG-3), CD80/CD86, and CD25. FOXP3 is a regulatory molecule for Treg maturation and maintenance of immunosuppressive functions. CTLA-4 also regulates the suppressive activity of Tregs. ICOS is required for accurate identification by Tregs. ICOS deficiency also inhibits immunosuppressive functions and decreases the numbers of Tregs. Co-stimulatory molecules CD80/CD86 and CD28 are required for Treg development and homeostasis[4, 11]. CD25 is required for Treg survival. Substantial evidence clearly demonstrates that decreased Treg proliferation or dysfunction causes abnormal immune responses and pathologies, such as tumors, infections, autoimmune diseases, and other immune diseases[12, 13].

Over the past decade, the notion that Tregs regulate the development of atherosclerosis by regulating immune activation and inducing immune tolerance in atherosclerotic models and patients has received considerable attention. The focus of this review is to summarize the effects of Tregs in the pathogenesis of atherosclerosis and provide an overview of recent studies on the regulation of Tregs for the treatment of atherosclerotic disease. Understanding the pathogenesis of atherosclerosis and the development of constructive therapeutic strategies to prevent cardiovascular disease (CVD) are important.

The relationship between Tregs and development of atherosclerosis

A number of studies have demonstrated that various functional T cell subsets are closely related to the occurrence of CVD, and effector T cells are positively correlated with atherosclerosis and CVD in human and animals. Tregs are present in atherosclerotic plaques[14]. Additionally, evidence from animal and human studies indicate that decreased Treg numbers are beneficial to atherosclerosis processes. For instance, research reveals no evidence of atherosclerotic lesions in C57BL/6 mice or $ApoE^{-/-}$ mice. However, $ApoE^{-/-}$ mice fed a high-fat diet (HFD) exhibit reduced numbers of Tregs and an increased incidence of atherosclerosis[15]. Moreover, several clinical studies have also reported that plaque vulnerability and the development of ACS are associated with low levels of circulating immunosuppressive Tregs[16, 17] and that pro-inflammatory DC and T effector cell infiltration is increased[18, 19]. Liu *et al* demonstrated that the imbalance of Th17/Tregs also enhanced plaque instability and the occurrence of ACS[20]. Depleting Tregs with anti-CD25 specific antibody accelerates the development of atherosclerotic lesions and increases plaque instability in $ApoE^{-/-}$ mice[21]. In addition, Zhang *et al* observed that Treg apoptosis was increased in non-ST-elevated ACS patients compared with chronic stable angina/chest pain syndrome patients, which may be attributable to decreased anti-apoptotic gene Bcl-2 mRNA and increased pro-apoptotic gene Bak mRNA. Moreover, oxidized low-density lipoprotein (ox-LDL) mediated the reduction in Treg numbers by promoting apoptosis[22, 23].

In addition to the low numbers of Tregs promoting atherosclerotic lesions, numerous studies have demonstrated that dysregulation of Tregs may contribute to the development of atherosclerosis. For example, compared with C57BL/6 mice, the inhibition of effector T cell function by Tregs isolated from $ApoE^{-/-}$ mice was significantly reduced. The same results were validated in ACS patients, thus reflecting defects in the immunosuppressive capacity of Tregs[7]. Atherosclerotic lesions also subsequently affect the plasticity of Tregs. Butcher *et al* found that atherosclerosis promoted the formation of an intermediately plastic Th1/Treg subset with immunosuppressive dysfunction and promoted inflammation and atherogenic T cell responses in $ApoE^{-/-}$ mice[24]. Klingenberg *et al* found that Treg numbers increased in coronary thrombi adjacent to culprit lesions, suggesting that circulating Tregs may migrate to atherosclerotic lesions to regulate local inflammatory responses[25]. Moreover, the deletion of IL-10 increased pro-inflammatory cell recruitment and plaque vulnerability in mice models. Furthermore, compared with stable patients, Treg and IL-10 levels were significantly reduced in unstable patients[26].

Treg surface molecules are also associated with the development of atherosclerosis. For instance, Jia *et al* found that FOXP3 expression was negatively correlated with the risk of ACS[17]. Tregs isolated from $FOXP3^{-/-}$ mice lost suppressive pathogenic T cell function. Depletion of Tregs (DEREG)/$LDLr^{-/-}$ mice with specific depletion of $FOXP3^+$ Tregs significantly aggravated atherosclerosis development and increased plasma atherogenic lipoprotein levels[27]. Ait-Oufella *et al* demonstrated that CD80/CD86 and CD28 deficiencies in $LDLR^{-/-}$ mice also promoted the development of atherosclerosis[21]. ICOS deficiency decreases Treg numbers and inhibits the immunosuppressive function of Tregs. Peripheral blood $ICOS^+$ Tregs were reduced in myocardial infarction (MI) and stable angina patients[28].

Collectively, these data illustrated that the numbers and immunoregulatory functions of Tregs were closely related to the development of atherosclerosis. Increasing or restoring the numbers and improving the immunosuppressive capacity of Tregs may serve as a fundamental immunotherapy to treat

atherosclerotic cardiovascular diseases.

Mechanism of Treg protection against atherosclerosis

Inhibition of effector T and B cell function

Atherosclerosis involves complicated interplay between different immune cell and cytokine networks. The underlying mechanisms of Treg anti-atherosclerotic effects have been extensively investigated (Figure 1). T cells are first recruited to atheroma and mainly accumulated in unstable plaques. The majority of the atherogenic T cells include effector Th1 and Th17. Multiple studies demonstrate that Th1 promotes the migration of monocytes and T cells into atherosclerotic lesions and activate lesional antigen presenting cells (APCs) by secreting interferon-γ (IFN-γ), which enhances atherosclerosis progression and plaque vulnerability[29, 30]. Th17 plays a vital role in atherogenesis by secreting pro-inflammatory IL-17, IL-6 and chemokines[8, 29]. The function of Th2 remains controversial based on the change of atherosclerotic lesion state, exact site and the differences in experimental models.

Tregs suppress T cell differentiation into Th1 and Th17 subtypes. Interestingly, Tregs suppress Th1 and Th17 immune responses in atherosclerosis by secreting IL-10, IL-35, and TGF-β. IL-10 is produced by Tr1, which reduces atherosclerotic lesions by inhibiting Th1 differentiation and decreasing T cell and macrophage accumulation and cytokine production in atherosclerotic plaques[31]. Consistently, Tr1 reduced immune responses and decreased plaque sizes and inflammation by producing IL-10 and decreasing the production of IFN-γ in $ApoE^{-/-}$ mice[32]. TGF-β attenuated atherosclerosis by inhibiting the recruitment and activation of inflammatory cells in atherosclerotic lesions and increasing the stability of plaques by promoting SMC survival and proliferation and collagen biosynthesis. However, TGF-β deficiency accelerates the development of atherosclerosis and enhances vulnerable plaques in hypercholesterolemic mice[33]. TGF-β overexpression increases plaque stability and prevents the progression of atherosclerosis[34]. IL-35 mediates the prevention of atherosclerosis by suppressing the proliferation of the T cell response, regulating the activation of naive T cells, and inhibiting the production of pro-inflammatory cytokines[35]. Acute myocardial infarction (AMI), unstable angina, and stable angina patients are associated with reduced IL-35 serum levels[36]. In addition, IL-2 is an important cytokine for T cell proliferation. Tregs reduce IL-2 levels, leading to the arrest of T cell proliferation. Therefore, Tregs protect against atherosclerosis by suppressing the function of pro-atherogenic effector T cells.

B cells are very rare within atherosclerotic plaques. However, antibodies produced by B2 cells abundantly bind within atherosclerotic plaques. Moreover, B2 cells aggravate atherogenesis by enhancing pro-inflammatory cytokine activity[29]. IgE was identified in human carotid atherosclerotic plaques, elevating plasma low-density lipoprotein (LDL) and increasing the risk of CVD, and in particular increasing plaque instability[37]. Antibody effector pathways and the classical complement pathway are activated in human atherosclerotic plaques. Transfer of B2 cells into $ApoE^{-/-}$ mice accelerated the progression of atherosclerosis[38]. Selective B cell depletion reduced atherosclerosis development and progression in $ApoE^{-/-}$ mice. Tregs regulate the B cell response by impairing their maturation and inhibiting antibody production and immunoglobulin class switching. Moreover, Tregs modulate survival of B cells by secreting granzymes and perforin[39]. Upon treatment with anti-CD20 antibody, B cell depletion attenuated atherosclerosis by suppressing effector T cell activation in $LDLr^{-/-}$ and $ApoE^{-/-}$ mice[27]. Therefore, regulation of B cell function is also a novel mechanism by which Tregs protect against atherosclerosis.

Modulation of dendritic cell function and maturation

DCs are specific immune cells that mediate antigen-specific immunity and tolerance and promote T cell activation. Multiple lines of evidence have demonstrated that DCs were significantly increased in atherosclerosis-prone vessels, promoted inflammatory processes within atherosclerotic lesions, and increased plaque instability[29, 40]. These findings suggest that an inverse correlation between DCs and Tregs exists in atherosclerotic lesions. Tregs inhibit DC function and maturation by producing IL-10 and TGF-β. Other surface molecules expressed by Tregs also affect the function of DCs, such as CTLA-4 and LAG-3. Tregs downregulate CD80/CD86 expression in human and murine DCs. CTLA-4 binds to CD80/CD86, blocking the ability of DCs to activate naive T cells and inducing immune tolerance of APCs[41]. Several studies demonstrate that increased CTLA-4 mRNA levels were associated with increased Tregs and prevented atherosclerosis[11, 42-44]. LAG-3 binds with DC surface CD4, which suppresses maturation and the immune-stimulatory capacity of DCs[45].

Inhibition of macrophage inflammation and decreased plaque vulnerability

In the 1960s, macrophages were identified as the key immune cells in atherosclerotic plaques that maintain a chronic inflammation state by modulating inflammatory mediator, adhesion molecule and chemokine factor secretion and reactive oxygen and nitrogen species production[46, 47]. Macrophages are heterogeneous cells within the atherosclerotic plaques that switch phenotypes depending on microenvironment changes. During the development of atherosclerosis, macrophages switch from the "classical" M1 phenotype to the "alternative" M2 phenotype. M1 phenotype macrophages exhibit pro-inflammatory properties and express pro-inflammatory cytokines, including IFN-γ and IL-1β, and produce proteolytic enzymes, resulting in atherosclerosis development by amplifying the inflammation response and enhancing plaque destabilization by degrading the extracellular matrix. In contrast, M2 phenotype macrophages exhibit anti-inflammatory properties and secrete TGF-β and IL-10[48]. Tregs promote the transformation of M1 macrophages to M2 macrophages by releasing IL-10, which is beneficial in preventing the development of atherosclerosis[49]. Tregs and monocyte co-cultures induce M2 phenotype production. Moreover, upon stimulation with lipopolysaccharides (LPS), the secretion of pro-inflammatory cytokines by Treg-treated monocytes was significantly reduced. The unstable

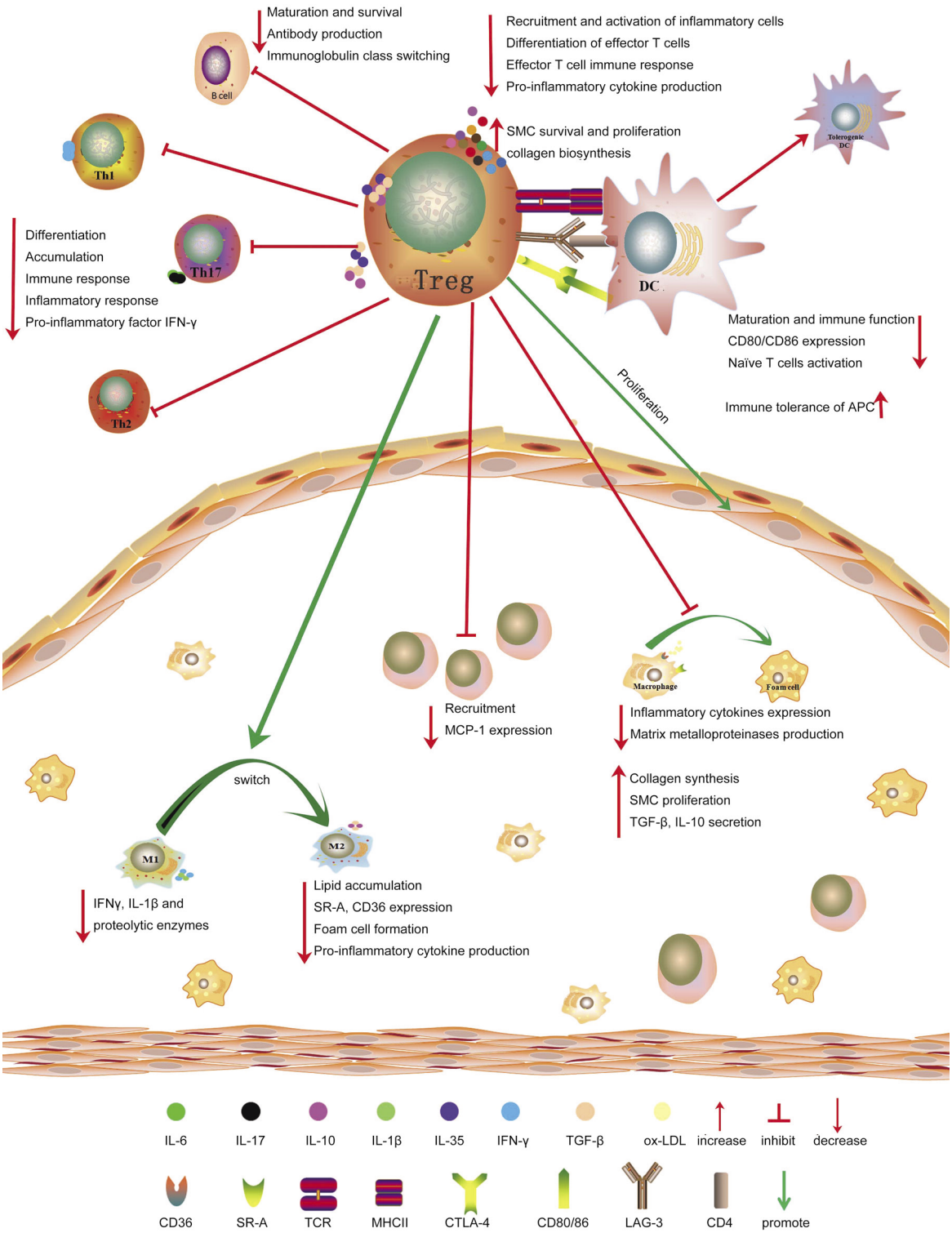

Figure 1. The mechanism of Tregs in the protection against atherosclerosis. Through secretion of IL-10, IL-35, and TGF-β, Tregs decrease inflammatory cell recruitment and activation, inhibit effector T cells differentiation and inflammation, and promote SMC survival and proliferation and collagen biosynthesis. Accordingly, IL-10 induces the M1 macrophage switch to the M2 phenotype, which attenuates atherosclerosis by decreasing IFN-γ, IL-1β, and proteolytic enzyme production and promoting collagen synthesis and SMC proliferation. Moreover, Tregs inhibit T cell differentiation into Th1, Th2, and Th17 subtypes and suppress the immune and inflammatory responses by inhibiting pro-inflammatory cytokine IFN-γ production. Tregs also suppress B cell maturation, antibody production and immunoglobulin class switching and modulate B cell survival by secreting granzymes and perforin. Among other pathways, Tregs inhibit the function and maturation of DCs, induce immune tolerance of APCs, and suppress naive T cell activation by CTLA-4-, TGF-β- and IL-10-mediated mechanisms. Tregs enhance plaque stability by inhibiting monocyte recruitment and promoting SMC proliferation. Furthermore, Tregs inhibit lipid and foam cell formation by decreasing the expression of CD36 and SR-A and reduce plasma VLDL, LDL, and cholesterol levels.

plaques are attributed to lipid-filled necrotic cores, thin fibrous caps, and low ratios of SMCs to macrophages. Activated M2 macrophages enhance plaque stability by promoting collagen synthesis and SMC proliferation. These findings suggest that Tregs enhance lesion stability by inducing M2 macrophages. Tregs reduce the risk of plaque rupture by increasing SMC and collagen content and reducing pro-inflammatory cytokine release and matrix metalloproteinase (MMP) production in atherosclerotic lesions in ApoE$^{-/-}$ mice[50].

Regulation of cholesterol metabolism and foam cell formation

Chronically high blood lipid levels activate vascular immune responses, promote the differentiation of monocytes to macrophages and phagocytosis of lipids, and ultimately promote the formation of macrophage-derived foam cells and atherosclerotic plaques[51-53]. The ox-LDL is digested by macrophages and serves as an antigen peptide presented to T cells, resulting in adaptive immune system activation and cytotoxic Th1 immune response initiation[44]. Therefore, regulation of macrophage cholesterol influx and efflux is of great importance in the prevention of atherosclerosis[51]. Numerous studies demonstrated that the development of atherosclerosis can be prevented by promoting the expression of cholesterol transporter-related protein ATP binding cassette transporters A1/G1 (ABCA1/G1) and Scavenger receptor type B class I (SR-BI) and enhancing the efflux of cholesterol in macrophages[52-54]. Studies have demonstrated that Tregs inhibit lipid accumulation in peritoneal macrophages by downregulating the expression of scavenger receptor class A (SR-A) and CD36, which inhibits foam cell formation[49]. Monocyte chemotactic factor-1 (MCP-1) mediates the migration of bone marrow-derived mononuclear cells to peripheral blood. Subramanian *et al* found that Tregs may impede monocyte recruitment into lesions by inhibiting MCP-1 expression, which also exhibits a negative effect on macrophage-derived foam cell formation and accumulation[55]. In addition, IL-10 reduces very low-density lipoprotein (VLDL) and LDL levels in LDLr$^{-/-}$ mice. DEREG mice exhibit increased plasma cholesterol and VLDL levels[27]. Induction of Treg proliferation in atherosclerotic mice reduces plasma cholesterol levels[56].

Strategies for Tregs in the prevention and treatment of atherosclerosis

As the main cause of death worldwide, atherosclerotic plaque rupture seriously affects the health of the population and causes enormous financial burden. Over recent years, statin lipid-lowering drugs are the most common drugs used for the treatment of atherosclerotic-related diseases. Although these drugs are effective in the prevention and treatment of atherosclerosis, they have numerous undesirable effects[57]. Safer and more effective treatment strategies are urgently needed. Newly developed regulatory Treg biology has expanded the horizons for the treatment of autoimmune diseases and the prevention of graft versus host disease (GVHD)[12, 13]. The development of atherosclerosis and plaque instability is mainly caused by an imbalance between effector T cells and Tregs, triggering a cascade of inflammatory reactions. Therefore, targeted treatment of atherosclerosis by modulating immunosuppressive properties and increasing the numbers of Tregs may result in significant progress, as shown in Table 1.

Adoptive transfer and induced expansion of Tregs

As mentioned above, the decrease in Tregs leads to the development of atherosclerosis; thus, a potential therapeutic target involves an increase in Tregs. Recent studies have confirmed that adoptive transfer or expansion of Tregs markedly prevented the development of CVD in animal models. For example, the application of IL-2/anti-IL-2 complex-induced Treg expansion suppressed effector T cell function, reduced initial atherosclerotic lesion formation, decreased blood lipid levels, and enhanced lesion stability in LDLr$^{-/-}$ mice[58]. The administration of clonal Tregs reduces the development of atherosclerotic plaques and inflammation by reducing the atherogenic immune response and inducing IL-10 production in ApoE$^{-/-}$ mice[59]. The development of atherosclerosis may be prevented based on adoptive transfer of CD4$^+$CD25$^+$ Tregs in ApoE$^{-/-}$ mice[60]. Moreover, trials of adoptive transfer of Tregs have been employed for GVHD, transplantation, and autoimmunity therapy, and effective results were presented with no significant adverse effects[61]. Bone marrow-derived mesenchymal stem cells (BM-MSCs) exhibit immunosuppressive properties by inhibiting the activation of immune cells, such as T, B, and NK cells. Administration of BM-MSCs decreased atherosclerotic plaque size by inducing Treg expansion; improving the immunosuppressive ability of Tregs; inhibiting effector T cell proliferation; promoting anti-inflammatory cytokine TGF-β and IL-10 expression; decreasing pro-inflammatory cytokine IFN-γ, MMP-1, and hypersensitivity C reactive protein (hs-CRP) expression; and inhibiting foam cell formation by downregulation CD36 and SR-A expression in ApoE$^{-/-}$ mice[62].

To some extent, atherosclerosis can be regarded as an autoimmune disease caused by autoimmune reactions to self-proteins, such as ox-LDL, apolipoprotein B (ApoB), and heat shock protein 60 (HSP60)[63]. Moreover, according to the identification of the causative antigen generation of antigen-specific Tregs has been studied in autoimmune diseases, such as rheumatoid arthritis[12]. Therefore, administration of atherosclerosis-relevant antigens, including ox-LDL, HSP60, and ApoB100, induced antigen-specific Tregs and tolerogenic DCs, which exhibit atheroprotective effects in mice[11, 64]. For example, adoptive transfer of HSP60-specific Tregs inhibits atherosclerosis formation in ApoE-deficient mice[65]. Nasal ox-LDL- or HSP60-induced mucosal tolerance attenuated atherosclerosis by increasing Tregs and TGF-β or IL-10 in ApoE$^{-/-}$ mice[66]. Adoptively transferred ox-LDL or ApoB100 also induced immune tolerance of DCs and inhibited atherosclerosis development by suppressing the T cell response, reducing plaque macrophage accumulation, and increasing collagen content. Recent studies have demonstrate that continuous subcutaneous injection of adjuvant-free ApoB100 peptides prevent the progression of atherosclerosis by inducing a specific Treg response in ApoE$^{-/-}$ mice[67]. Interestingly, ApoB100- and

Table 1. Targeted therapy of atherosclerosis by regulating regulatory T cells.

Approaches for regulating Tregs		Effects of regulation Tregs
Adoptive transfer Tregs		Tregs expansion and inhibition of effector T cells function
Atherosclerosis relevant antigens (ox-LDL, HSP60, ApoB100)		Induction of antigen specific Tregs and tolerogenic DCs
Pharmacological approaches	Rapamycin	Tregs expansion and T effector cell depletion
	Mycophenolate mofetil	Inhibition of macrophages, DCs, T cells, and NKs activation and increase in the number of Tregs
	Vitamin D3	Induction of Tregs and tolerogenic DCs
	FTY720 (fingolimod)	Tregs expansion
	Pioglitazone	Regulation of the balance between effector T cells and Tregs
	Cholesterol lowering drugs	Modulation of the ratio of Treg/effector T cell and promotion of TGFβ and IL-10
	Amygdalin (vitamin B17)	Induction of Tregs expansion and increase in IL-10 and TGF-β expression
Application of antibodies and cytokines	IL-2	Selective Tregs expansion
	Anti-CD3 antibody	Induction of Tregs expansion, reducing $CD4^+$ T cells and lowering plasma cholesterol
	Integrin αvβ8	Modulation of Tregs function and inhibition of effector T cell function
	G-CSF	Induction of Tregs expansion and increase in IL-10 expression
Physical therapy	UVB irradiation	Improvement of the function of Tregs and regulation of effector T-cell responses

HSP60-associated auto-antigens have been defined and evaluated as therapeutic vaccines in animal models[63]. Notably, an ApoB100 vaccination has been developed for first-in-human clinical trials[64]. These data imply that regulating the number of Tregs may serve as a powerful therapeutic approach in the treatment of CVD patients. Moreover, the expansion of Tregs induced by atherosclerosis-associated vaccines may open a new path for the treatment of atherosclerosis.

Strategies for pharmacological regulation of Treg function

Recent studies have demonstrated that numerous drugs regulating Tregs have achieved significant efficacy in the treatment of atherosclerosis in animal models. For instance, mycophenolate mofetil has a strong immunosuppressive effect and inhibits the proliferation of T cells by interfering with DNA synthesis. Recent research demonstrated that mycophenolate mofetil can be used in the treatment of atherosclerotic diseases by reducing the activation of macrophages, DCs, T cells, and NKs; increasing the number of Tregs; reducing the production of MMP and cathepsins; and promoting the expression of lipid metabolism-associated genes, such as ApoE, peroxisome proliferator activated receptor (PPAR), ABCA1/G1[64, 68]. Mammalian target of rapamycin (mTOR) signaling regulates Treg differentiation and function. Rapamycin, an inhibitor of mTOR, exhibits immunosuppressive activity, induces Treg expansion and depletes T effector cells, implying that it could be employed as a new immunotherapy in T cell-mediated CVD[69]. Orally activated vitamin D3 is beneficial in the treatment of CVD by increasing Treg levels, inducing tolerogenic DCs, decreasing IL-12 expression, and increasing IL-10 expression in mice[70]. Consistent with these findings, oral FTY720 (fingolimod) ameliorates atherosclerosis by expanding Tregs, inhibiting effector T responses, and increasing TGF-β expression in $ApoE^{-/-}$ mice[71]. Pioglitazone, a PPARγ agonist, ameliorates atherosclerosis by regulating the balance of Th1/Th2 cells, enhancing Treg response, and increasing the number of SMC and collagen content in ApoE-deficient mice[72]. Diet-induced hypercholesterolemia decreases the Treg/effector T cell ratio in atherosclerotic plaques, and this ration normalizes after diet reversal, suggesting that cholesterol-lowering therapies may reduce atherosclerosis by modulating the ratio of Tregs/effector T cells[73]. Meng *et al* demonstrated that simvastatin increased Treg levels and promoted TGF-β, IL-10, and FOXP3 expression in the atherosclerotic plaques of $ApoE^{-/-}$ mice. Atorvastatin also induces Treg expansion and promotes FOXP3 expression in humans but not in C57BL/6 mice. Moreover, simvastatin treatment in ACS patients also promotes Treg expansion, which is consistent with observations in animal studies[74]. Amygdalin, which is also referred to as vitamin B17, attenuates the development of atherosclerosis through regulation of lipid metabolism by decreasing plasma total cholesterol (TC), triglyceride (TG), and LDL levels; inducing Treg expansion and upregulating IL-10 and TGF-β expression in $ApoE^{-/-}$ mice[75]. Therefore, pharmacological regulation of the numbers and immunosuppressive activity of Tregs may provide valuable treatment options for atherosclerotic diseases.

Application of antibodies and cytokines to regulate Tregs

In addition to pharmacological approaches, the treatment of atherosclerosis with antibodies and cytokines has drawn increasing attention. IL-2 promotes the proliferation and differentiation of effector T cells and Tregs. However, low-dose IL-2 is effective in treating atherosclerosis via selective expansion of Tregs with significant sensitivity to IL-2. This method has been used in the clinical treatment of systemic lupus erythematosus[37, 76]. Oral or intravenous administration of anti-CD3 antibody suppresses atherogenesis and atherosclerotic plaque formation by inducing Treg expansion and reducing $CD4^+$ T cells in mice[77]. Treatment with anti-CD3 antibody

and IL-2 complex also inhibits atherosclerosis[78]. Integrin αvβ8 mediates TGF-β activation. Thus, manipulation of integrin αvβ8 may modulate Treg function to suppress effector T cell-mediated atherosclerotic diseases[79]. Granulocyte Colony-Stimulating Factor (G-CSF) modulates immunity and improves immune-related diseases in animals. G-CSF also increases the numbers of Tregs and IL-10 levels and reduces IFN-γ levels in $ApoE^{-/-}$ mice[80]. Physical therapy can also play a protective role in atherosclerosis. For example, ultraviolet B irradiation attenuates the progression of atherosclerosis in atherosclerosis-prone mice by enhancing the function of Tregs and regulating effector T-cell response[81].

Limitations of Tregs in atherosclerosis treatment

This review provides new insights into atherosclerotic immunopathogenesis and summarizes new therapeutic approaches related to the modulation of Tregs. Despite extensive evidence for the beneficial effects of Tregs in the treatment of animal immune diseases, there are limitations to the effects and safety related to the transfer or induction of Tregs in patients with CVD. First, transfer of Tregs is limited by low yields of amplification and purification *in vitro*. Second, insufficient evidence on the duration of the effects of transferred Tregs is available. Moreover, induced Tregs exhibit short survival durations after infusion, and Tregs are prone to loss of phenotype during *in vitro* manipulation and following reinfusion, which limits long-term treatment efficacy. For example, several studies demonstrated that induced Tregs may lose FOXP3 expression, the loss of which is accompanied by decreasing immunosuppressive ability and transformation to atherosclerotic T cells due to plasticity and instability in unique environments[64, 82]. Third, the persistence of Tregs infusion may increase the risk of cancer and infection. Fourth, clinical studies are required to examine whether the therapeutic effects achieved in animal experiments are applicable to humans. Remarkably, because human atherosclerosis is a dynamic and complex disease, the same therapeutic strategies may have conflicting effects at different stages of atherosclerosis progression. For example, enhancing TGF-β function is not suitable for long-term treatment of CVD given its multiple targets and pleiotropic effects[83]. Whether IL-10 supplementation can be successfully applied to the treatment of atherosclerosis is unclear because IL-10 potentially increases the risk of cancer and lupus erythematosus[84]. Investigations into the precise therapeutic effects of TGF-β and IL-10 in atherosclerosis are warranted.

Several studies have demonstrated that atherosclerosis-associated antigen-induced antigen-specific Tregs reduce atherosclerosis, which can also cause antigen-dependent inflammatory responses and further aggravate the development of atherosclerosis[33, 85]. Immune suppressive drugs, including corticosteroids and cytotoxic drugs, are effective in the treatment of atherosclerosis in animal models. However, it is not clear whether these therapies would also be beneficial in patients with cardiovascular disease. Additionally, various side effects, such as dyslipidemia, osteoporosis, growth suppression, immunosuppression, and recurrent infection, should be noted[86].

Therefore, the development of safe and effective Treg-based therapies for the treatment of human atherosclerosis is challenging and meaningful. Investigations into atherosclerosis treatments should not only determine the effects of therapy on increasing Treg numbers but also enhance and stabilize Treg immune suppression functions in future clinical trials. Given recent studies on the generation of antigen-specific Treg cells via disease-associated antigens, the development of atherosclerosis-related vaccines based on atherosclerotic-associated antigens is an attractive option. It is encouraging that vaccine studies have shifted from animal experiments to clinical trials, which will lead to decisive outcomes in the treatment of atherosclerosis.

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