Review

Regulatory T cells in atherosclerosis and strategies to induce the endogenous atheroprotective immune response

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Abstract

Atherosclerosis is a chronic inflammatory disease, in which multiple types of immune cells are involved. Th1 and Th17 cells play a prominent role in induction and progression of local inflammation in the atherosclerotic plaque. Regulatory T cells (Tregs) can be also found in the plaque but their numbers are decreased and function may be impaired. Tregs are the master modulators of the immune system possessing the immunosuppressive capacity to prevent unfavorable immune responses and maintain tolerance to self-antigens. These cells play the atheroprotective role by inhibiting Th1/Th17-mediated proinflammatory response and down-regulating the antigen-presenting function of dendritic cells (DCs). Tregs mediate the immune response through the cell-to-cell contacts and secretion of anti-inflammatory cytokines IL-10 and TGF-β. In addition to the natural CD4+CD25+Foxp3+ Tregs presented in the thymus, there are several subtypes of inducible Tregs that can be induced from naïve CD4+ T cells by tolerogenic DCs in the periphery. Thus, stimulation of the immunosuppressive activity of Tregs and increasing numbers of Tregs and immunocompetent DCs has a great clinical potential in prevention and treatment of atherosclerosis and its vascular complications. A promising strategy to induce the anti-atherogenic immune response is an oral administration of anti-inflammatory immunomodulators capable to activate the intestine immune tolerance by recruiting mucosal tolerogenic DCs and inducing Tregs. Induced Tregs can then migrate to the inflamed vascular sites and reduce atherogenesis.

1. Introduction

Although low-density lipoprotein (LDL) remains the most important risk factor for atherosclerosis, immune and inflammatory mechanisms play significant and non-redundant role in atherogenesis. The presence of leukocytes within atherosclerotic arteries was discovered in the late 1970s [1]. Multiple leukocyte types were then reported in the atherosclerotic plaque [2]. Regulatory T cells (Tregs) were also found in the atherosclerotic plaque. Several subsets of Tregs, which are responsible for maintenance of immunological tolerance and suppressing immune overactivity of effector T cells [3], diminish atherosclerosis development by down-regulation of activated T cell responses [4–6]. These Tregs subsets secrete two major anti-inflammatory cytokines, interleukin-10 (IL-10) and transforming growth factor (TGF-β) capable to reduce proatherogenic inflammatory response in atherosclerosis [7]. Indeed, the balance between effector T cells and Tregs is sufficient to control of atherosclerosis development and progression.

In this review, we characterize Tregs subtypes, their induction, function and role in atherosclerosis. We also consider approaches...
aimed to restore/stimulate Tregs function in atherosclerotic patients and improve dysbalance between Tregs and effector T cells by activating an endogenous immune response.

2. Tregs subsets and their function

2.1. Natural CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Tregs

There are several subpopulations of Tregs including naturally occurring CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Tregs and inducible Tregs subsets [3]. Natural CD4<sup>+</sup>CD25<sup>+</sup> Tregs, which are produced in the thymus, are the key players in suppressing self-reactive immune responses and maintaining dominant self-tolerance [8]. They constitutively express CD25, an α-subunit of the trimERIC IL-2 receptor, at high levels thereby producing the complete high-affinity IL-2Rαβγ complex that is capable to respond to physiologically low concentrations of IL-2 in vivo. This cytokine is critical in the generation and maintenance of CD4<sup>+</sup>CD25<sup>+</sup> Tregs [9]. In CD4<sup>+</sup>CD25<sup>+</sup> Tregs, IL-2 induces expression of Foxp3, a transcription factor that is crucial for their development and function [10] and is currently the most reliable marker for them [11,12]. Foxp3 acts to control the core module of Tregs suppressive function by regulating expression of a number of key molecules such as CTLA-4 and CD25 [13].

Natural CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Tregs can suppress a variety of immune cells including CD4<sup>+</sup> and CD8<sup>+</sup> T cells, B cells, natural killer (NK) cells, NK cells, monocytes, and dendritic cells (DCs) (Fig. 1). A main function of natural CD4<sup>+</sup>CD25<sup>+</sup>Tregs is suppression of activation of naïve T cells, but they can also inhibit activated effector T cells and memory CD4<sup>+</sup> and CD8<sup>+</sup> cells [14]. Natural CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Tregs inhibit immune responses through cell-to-cell contact by suppressing T-cell receptor (TCR)-induced proliferation and IL-2 transcription in target T cells [15] or CTLA-4-mediated down-regulation of CD80/CD86 expression in DCs [16]. In addition, natural CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Tregs can exhibit in vitro potent granzyme B-dependent, partially perforin-independent cytotoxic cells that are capable of specifically killing antigen-presenting B cells [17]. Upon CD3/CD46 activation, Gromman et al. [18] also reported the possibility of human natural CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup>Tregs to express the serine protease granzyme A and kill CD4<sup>+</sup> T cells and other target cells in a perforin-dependent manner.

2.2. Tregs type 1

A subpopulation of inducible Tregs called Tregs type 1 (Tr1) was first described by Groux et al. [19] who induced these cells from T-cell receptor–transgenic mice by repeated stimulation of naïve T cells with ovalbumin and IL-10. This CD4<sup>+</sup> T cell subset exhibits a unique cytokine profile distinct from that of T helper (Th)0, Th1, or Th2 cells. These Tr1 cells primarily produce IL-10 and TGF-β and some IL-5 and interferon-γ (IFN-γ) with little or no IL-2 or IL-4 and proliferate poorly after polyclonal T-cell receptor-mediated activation. Tr1 cells express markers LA<sub>G</sub>3, CD49b, ICOS, PD-1, and LAP [20]. Functional studies on Tr1 cells have indicated that Tr1 cells have immunosuppressive properties and have been shown to prevent the development of Th1-mediated autoimmune diseases [19]. Compared to natural CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Tregs, Tr1 cells do not express Foxp3 [21]. IL-10-producing Tr1 cells are capable to suppress a variety of immune cells including DCs and Th17 cells [22]. Treg-derived IL-10 is important for control of inflammation at environmental interfaces but seems to be dispensable for control of systemic autoimmunity [23]. Along that line, IL-10- or IL-10 receptor-deficient mice do not develop autoimmunity, but are susceptible to colitis in the presence of triggering flora [24].

2.3. Th3 cells

Th3 cells are another subset of inducible Tregs. These cells, which are primary producers of TGF-β, may be induced on periphery by TGF-β. Th3 induction may be enhanced by IL-4 and IL-10 [25]. Carrier et al. [26] developed TGF-β-transgenic mice in which TGF-β was linked to the IL-2 promoter and T cells transiently overexpressed TGF-β upon TCR stimulation but produce little or no IL-2, IL-4, IL-10, IL-13, or IFN-γ. Th3 cells derived from these mice were capable to induce Foxp3 expression in both CD25<sup>+</sup> and CD25<sup>−</sup> T cell populations [26] and rescue IL-2-deficient mice from autoimmunity due to the induction of CD25<sup>−</sup> Tregs in the periphery [27]. Indeed, Th3 cells may play a critical role in inducing and maintaining peripheral tolerance by driving differentiation of Foxp3<sup>+</sup> Tregs in the periphery by secretion TGF-β. TGF-β-derived Foxp3<sup>+</sup>CD25<sup>−</sup>Th3 Tregs represent a different cell lineage from thymic-derived CD25<sup>+</sup> Tregs in the periphery.

TGF-β-deficient mice develop T cell-mediated autoimmunity within several weeks after birth [28]. A similar phenotype is observed in mice lacking TGF-β responsiveness specifically in T cells [28]. These mice showed enhanced Th1 and Th2 responses and immunopathology including colitis; however, these mice were also resistant to the induction of experimental autoimmune encephalitis likely due to impaired Th17 induction [29]. Indeed, the role of TGF-β in Treg-mediated suppression might depend very much on the type of effector cell and the site of the immune response, and TGF-β may even promote proinflammatory Th17 responses. Tregs can produce high amounts of membrane-bound and soluble TGF-β, and blocking TGF-β partially abrogated suppression of T cell proliferation in vitro suggesting that Treg-produced TGF-β controls autoimmunity [30]. Treg-produced TGF-β can induce apoptosis of lymphoid cells including self-reactive effector T cells through cleavage of Bcl-xL with activated caspase-1-like protease [31]. Activated CD4<sup>+</sup> T cells induced by DCs are particularly sensitive to TGF-β [32].

2.4. Foxp3<sup>+</sup> Tregs

Compared to natural CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Tregs, which arise from the thymus and are released into peripheral tissues after thymic–positive selection, inducible Foxp3<sup>+</sup> Tregs are generated via peripheral conversion (after antigen-specific stimulation) from mature, naïve CD4<sup>+</sup>T cells or from “rescued” self-reactive effector T lymphocytes [33]. Foxp3<sup>+</sup> Tregs can be induced in periphery from CD4<sup>+</sup>CD25<sup>−</sup> naïve T cells by IL-2 and TGF-β [34,35]. In synergy with TGF-β, retinoic acid generated in functionally specialized mucosal DCs can induce Foxp3 expression in CD4<sup>+</sup> naïve T cells [36]. Compared to natural CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Tregs whose CpG island within the Foxp3 locus is demethylated, it was reported to be methylated in TGF-β-induced Foxp3<sup>+</sup> Tregs [37]. It seems that the demethylation status is a prerequisite for stable Foxp3 expression and suppressive activity. As a consequence, methylation profile of the Foxp3 promoter would facilitate the distinction of truly committed Tregs [38].

In contrast to natural CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Tregs, TGF-β-induced Foxp3<sup>+</sup> Tregs rapidly lose both Foxp3 expression and suppression activity [39]. IL-4-producing Th2 cells seem to be a major cause for the disappearance of Foxp3<sup>+</sup> Tregs during long culture. IL-4 is an important suppressor of Foxp3 induction, which down-regulates Foxp3 expression in a STAT6-dependent manner [39]. IL-4 activates the Th2 transcription factor Gata3 that blocks Foxp3 transcription by direct binding to the Foxp3 promoter [40]. Foxp3 expression is efficiently suppressed also by transcription factor Pu.1, which is transiently induced during Th2 differentiation [41].

In addition to its effect on Tregs, TGF-β also induces the differentiation of Th17 cells in the presence of a pro-inflammatory cytokine, IL-6 [42]. In a sharp contrast to Tregs, which actively
suppress immune responses, Th17 cells are involved in promoting autoimmune and inflammatory responses. Intriguingly, retinoic acid inhibits Th17 differentiation [42] by reducing the expression of RORγt, a master transcription factor for Th17 cells [43]. TGF-β-induced Foxp3+ Tregs suppress Th17 differentiation by antagonizing RORγt function [44].

Interestingly, Foxp3+ Tregs producing IL-17 were found in humans [45]. Upon T-cell receptor stimulation in the presence of IL-1β, IL-2, IL-21, and IL-23, peripheral CD4+Foxp3+ Tregs can differentiate into IL-17 producers. IL-17-secreting Tregs express Foxp3 and RORγt transcription factors. The biological significance of T cells that display the function of Treg and the opposing function of Th17 is unclear. In normal conditions, these cells can potentially contribute to the antimicrobial innate immune defense while controlling inflammation and autoimmunity at the same time, particularly at mucosal sites. IL-17-producing Tregs could be implicated in controlling Th17/Treg balance, which is altered in cardiovascular pathology including acute coronary syndrome [46] and atherosclerosis [47].

In the presence of IL-6 alone without exogenous addition of TGF-β, natural CD4+CD25+Foxp3+ Tregs can differentiate themselves into IL-17 producers [48,49]. In contrast, TGF-β and IL-2-induced Foxp3+ Tregs are resistant to IL-6-dependent conversion to Th17 cells [50]. Thus, natural and TGF-β-induced Foxp3+ Tregs are not mirror images of each other. These two subsets differ in their principal antigen specificities and in the T-cell receptor signal strength and co-stimulatory requirements needed for their generation [51]. Indeed, natural Foxp3+ Tregs may have undesirable effects through their ability to differentiate into pathogenic Th17 in the presence of IL-6 and/or IL-23 at sites of inflammation. Induced Foxp3+ Tregs thereby might retain suppressive function at inflammatory sites.

3. Protective role of Tregs in cardiovascular pathology

3.1. Interactions between Tregs and DCs in atherosclerosis

Two rodent models of atherosclerosis, apolipoprotein (apo)E-deficient and LDL receptor (LDLR)-deficient mice, were widely used to assess molecular mechanisms of atherogenesis including T-cell mediated proinflammatory responses. Monocytes migrating through the intima from the early atherosclerosis stages may differentiate into macrophages or DCs affected by inflammatory cytokines and chemokines. DCs and DC precursors may also migrate from bloodstream and accumulate in the plaque on chemokines and the adhesion molecule-dependent pathway [52]. Significantly increased levels of DCs were found in atherosclerotic lesions of apoE-deficient mice [53]. DCs are a heterogeneous population of bone marrow-derived immune cells that specialize in capturing, processing and presenting antigens to T lymphocytes in order to induce and control immunity. DCs, after entering the vascular tissue, screen the environment for potential antibodies. In atherosclerotic plaque, immature DCs can capture and process self-antigens derived from dying cells or from neutrophil extracellular traps such as heat shock proteins (HSP)60/65 [54], self-antigenic protein-DNA complexes [55], and others. Oxidized LDL (oxLDL) contribute to induction of a proinflammatory cytokine profile in human DCs leading to DC maturation and differentiation [56]. After capturing an antigen, DCs differentiate into mature DCs capable to induce activation and differentiation naive T cells towards the proinflammatory phenotype [57,58].

Tregs contribute to the maturation, activation, and function of DCs [52]. Tregs inhibit the antigen-presenting function of proatherogenic DCs through CTLA-4-dependent suppression of...
their CD80 or CD86 expression [59]. CTLA-4, an important coinhibitory molecule, is constitutively expressed in natural CD4+CD25+ Tregs. CTLA-4 binds to B7 family molecules such as B7-1 (CD80) and B7-2 (CD86) abundantly expressed on the surface of macrophages and DCs and conveys a negative signal to DCs reducing expression of both costimulatory molecules [60].

By interaction with CD80 or CD86, Treg-derived CTLA-4 may induce indoleamine 2,3-dioxygenase (IDO) expression on DC. This enzyme converts tryptophane to kynurenine, a potent immunosuppressive metabolite capable to induce de novo formation of Tregs from naïve T cells in the local environment [61].

Through cell-to-cell contacts with mature DCs, Tregs may also inhibit the antigen-presenting function via the mechanism of trogocytosis (e.g., transfer of membrane fragments and cell surface proteins between cells) of CD80 and CD86 by Tregs [62]. This process, which is mediated by CTLA-4, CD28, and programmed death ligand–1 (PD-1), includes acquisition of CD80 and CD86 from the surface of DCs and leads to enhanced ability of Tregs to suppress naïve CD4+ T-cell proliferation. CTLA-4 is a key factor in the down-regulation of costimulatory molecules CD80 and CD86 expressed on antigen-expressing mature DCs, since Tregs from CTLA-4-deficient mice were not able to inhibit CD80/CD86 expression [63].

Indeed, CTLA-4 engagement of CD80 and CD86 on DCs present a critical mechanism of tolerance induction in vivo [64]. Thus, CTLA-4 can be considered as a potential therapeutic target for atheroslerotic disease in the development of strategies aimed to enhance Treg-mediated suppression function or increase downregulation of effector T cell function [65].

Lymphocyte function-associated antigen 1 (LFA-1), a T-cell adhesion molecule (CD11a/CD18), is necessary for cell-to-cell contact between a Treg and an antigen-presenting DC [59]. Tregs exert the CD80/86-down-regulating effect even in the presence of strong DC-maturing stimuli such as granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor (TNF)-α, IFN-γ, type I INF, and lipopolysaccharide. Indeed, Tregs may successfully prevent proinflammatory stimuli from antigen-presenting DCs to naïve T cells by binding to immature DCs with subsequent LFA-1/CTLA-4-dependent suppression of CD80/CD86 expression in DCs.

DCs can in turn regulate Tregs by their suppression or induction. Weber et al. [66] reported a proatherogenic population of CCL17-produced monocyte-derived CD11c+ DCs, which were able to suppress Tregs. Recently, Choi et al. [67] found in aortic tissue a subtype of monocyte-derived DCs (CD11c<sup>high</sup>MHCII<sup>high</sup>CD11b<sup>−</sup>CD103−) playing the atheroprotective role and capable to induce Tregs in the atheroslerotic plaque.

Tolerogenic DCs that basically have immature phenotype (CD86–CD80–CD40–MHCII<sup>−</sup>) may induce Tregs through cell-to-cell contact with naïve T cells and by secreting cytokines such as TGF-β and IL-10. In the presence of IL-10, peripheral Tregs differentiate into Tregs, and IL-10 production by tolerogenic DCs contributes to the induction of IL-10-producing Tr1. DCs and Tregs can also interact via chemokines CCL17 and CCL22 and their receptors, chemokine receptor (CCR) 4 and 8 [68]. These receptors are expressed on Tregs and may bind their ligands CCL17 and CCL22 secreted by DCs. Enchancement of CCL22 on tolerogenic DCs may induce and recruit Tregs at inflammatory sites such as atherosclerotic plaque. Stimulatory effect of CCL22/CCR4 on induction of Tregs may be mediated by IDO, since mice deficient for CCR4 exhibited markedly reduced expression of this enzyme in mesenteric lymph nodal DCs [69]. These findings indicate that reciprocal interactions between the DCs and Tregs via both B7/CTLA-4 and CCL22/CCR4 mediated by the immunoregulatory enzyme IDO may play a key role in the pathogenesis of inflammatory disease including the induction of inflammation in atherosclerosis. Indeed, induction of tolerogenic DCs in order to enhance or improve atheroprotective effects of Tregs may be considered as a plausible therapeutic strategy for preventing atherosclerosis.

3.2. Influence of Tregs on Th1/Th2 immune responses in atherosclerosis

The majority of pathogenic T cells in atherosclerosis are of the Th1 profile producing high levels of IFN-γ [70]. IFN-γ displays pleiotropic proatherogenic effects including activation of monocytes/macrophages and DCs, inhibiting vascular muscle cells proliferation, down-regulating extracellular matrix (ECM) collagen production and up-regulating matrix metalloproteinases [71]. The proatherogenic activity of IFN-γ was clearly shown in apoE-deficient mice deficient for this cytokine or its receptor [72] and by exogenous administration of IFN-γ to the apoE-deficient mice [73]. The proatherogenic role of IL-12 and IL-18 was demonstrated in the apoE-deficient atherosclerosis mouse model [74]. IL-12 produced by DCs, monocytes and macrophages, is crucial for differentiation of naïve T cells into Th1 cells by stimulating IFN-γ production and down-regulating Th2 type cytokines (IL-4 and IL-5) in T cells. In synergy with IL-12, IL-18 up-regulates expression of IFN-γ [75].

Th2 cells secrete IL-4, IL-5, IL-10, and IL-13 and provide help for antibody production by B cells. Th2 cells are rarely detected within the atherosclerotic lesions. However, in hyperlipidemic conditions, Th1/Th2 balance may be switched towards Th2-bias [76]. The role of the Th2 pathway in the development of atherosclerosis seems to be multifaceted depending on the stage and/or site of the lesion, as well as on the experimental model [77]. In atherosclerosis-resistant mice strains such as C57BL/6 and BALB/c fed on fat diet, IL-4-mediated Th2-bias has been shown to protect against early fatty streak development [78]. In more permissive model such as apoE- or LDL-deficient mice, IL-4 shows the proatherogenic role [79] that could be even more exacerbated by prolonged hypercholesterolemia [80]. As shown in hypercholesterolemic apoE<sup>−/−</sup> mice immunized with oxLDL, other Th2-type cytokines such as IL-5 and IL-33 exert antiatherogenic function through the induction of humoral immunity associated with the production of high levels of IgM-type anti-oxLDL antibodies [81,82]. Along with Th1/Th2 switch, hypercholesterolemia may also stimulate Th3 immune effector responses by inducing TGF-β levels, increasing amounts of TGF-β1/CD4<sup>+</sup> Th3 regulatory cells in lesions, and elevating Th3-dependent IgG2b antibodies to oxLDL [83].

As mentioned above, natural CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Tregs are able to suppress a variety of immune cells including Th1 and Th2 lymphocytes. Ait-Oufella et al. [84] showed that endogenous natural CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Tregs may be powerful inhibitors of atherosclerosis in LDLR-deficient mice. Interaction between the costimulatory molecules CD80/CD86 and CD28 is known to be crucial for the generation and homeostasis of Tregs [85]. Using the irradiation/bone marrow transplantation model, Ait-Oufella et al. [84] reported that Treg deficiency due to reconstitution with CD80<sup>−/−</sup>/CD86<sup>−/−</sup> and CD28<sup>−/−</sup> bone marrow led to enhanced atherosclerotic plaque development in mice. Transplantation of CD28<sup>−/−</sup> splenocytes to apoE<sup>−/−</sup>Rag2<sup>−/−</sup> mice deficient for lymphocytes resulted in accelerated atherosclerosis compared to the transplantation of normal splenocytes. Adoptive transfer of natural CD4<sup>+</sup>CD25<sup>+</sup> Foxp3<sup>+</sup> Tregs greatly reduced atherosclerosis in apoE-deficient mice [86]. However, these studies did not provide precise evidence for the atheroprotective role of natural CD4<sup>+</sup>CD25<sup>+</sup> Foxp3<sup>+</sup> Tregs since deficiency for CD80<sup>−/−</sup>/CD86<sup>−/−</sup> and CD28<sup>−/−</sup> may influence function of not only Tregs but also other effector T cells.

A protective role of Tr1 cells against atherosclerosis via suppression of Th1-mediated inflammation was first showed in apoE-deficient mice co-immunized with ovalbumin in complete Freund’s adjuvant and Tr1 cells [4]. The immunization with Tr1
cells resulted in significant reduction in atherosclerotic lesion size, decrease in the production of IFN-γ, and increase in IL-10 production. Overexpression of IL-10 by activated T lymphocytes reduced atherosclerosis in LDLR−/− mice likely due to the anti-inflammatory effect of Tregs [87]. The anti-atherogenic role of Tregs was further supported by Ait-Oufella and co-authors [88] who promoted endogenous adaptive Treg cell response in apoE-deficient mice by treatment with measles virus nucleoprotein, a vaccine with anti-inflammatory properties. The Treg-mediated response markedly inhibited progression of atherosclerosis and accumulation of T lymphocytes and macrophages in lesions.

Interestingly, Tregs were shown to inhibit formation of new plaques on the early atherosclerosis stages whereas their influence on pre-existing lesions is limited by reducing plaque size and inducing a ‘stable’ plaque phenotype [89]. Plaque stabilization shown in atherosclerosis-prone murine strains, is mediated by CD40–CD40L interactions [90]. The CD40–CD40L dyad is known to interact with other co-stimulatory molecules, to activate antigen-presenting cells and to contribute to T-cell priming and B-cell isotype switching. Disrupting the CD40–CD40L co-stimulatory pathway reduces atherosclerosis and induces a stable atherosclerotic plaque phenotype that is low in inflammation and high in fibrosis [91]. Since CD40–CD40L interactions directly promote development of proatherogenic Th1 cells, Treg-mediated suppression of these cells should decrease CD40–CD40L interactions within the plaque and in turn stabilize the plaque. Inhibition of CD40 expression on effector T cells, DCs and B cells may increase Treg population and suppress a humoral immune response in atherosclerosis [92].

3.3. Th17/Treg balance

Th17 cells, a recently identified Th cell population, express a lineage-specific factor RORC (RORγt in mice) and represent a distinct lineage of CD4+ T cells [93]. TGF-β and inflammatory cytokines such as IL-6, IL-21, IL-1β, and IL-23 are required in the generation of Th17 cells. Th17 cells play a sharp proinflammatory role by secretion of inflammatory cytokine IL-17 [94]. In the immune response, Th17 cells are critical for the clearance of extracellular pathogens including Candida and Klebsiella [95]. However, under certain conditions, Th17 cells become a leader in orchestrating inflammatory and autoimmune pathology. By producing two IL-17 isoforms (IL-17A and IL-17F) and other effector molecules such as IL-21, IL-22, GM-CSF, and CCL20, Th17 cells are known to contribute to the pathogenesis of several inflammatory and autoimmune diseases [96].

The pathogenic role of Th17 cells in atherosclerosis was demonstrated in ApoE-deficient mice [47,97,98]. In atherosclerosis and atherosclerotic vascular complications such as acute coronary syndrome, the inflammatory response is accompanied with significant increase in Th17 cell population and elevated production of IL-17 [99,100], which is correlated with the severity and progression of vascular disease [101]. IL-17 is involved in cardiovascular pathology by inducing the mitochondrial-dependent apoptosis of vascular endothelial cells [102] and cooperating with IFN-γ in the induction of proinflammatory responses in vascular smooth muscle cells (VSMCs) [103]. The mechanism by which Th17-derived IL-17A promotes activation of VSMCs may involve NAD(P)H-oxidase dependent generation of reactive oxygen species [104] that in turn induces a proinflammatory signaling [105]. In atherosclerotic disease, the vascular ECM is subjected to the inflammation-driven degradation and remodeling, with induction of collagen II, collagen IV, malondialdehyde-modified laminin, and fragments of other ECM proteins as self-antigens [106]. IL-17 plays a primary role in mediating cellular autoimmunity against ECM structural components in atherosclerotic arteries of both humans and mice [107,108]. In addition, Th17-derived IL-17 promotes activation and expansion of IL-17A/IL-17F-producing neutrophils [99] and IL-17E-secreting B cells [109] in advanced and complicated plaques further enhancing atherosclerotic progression. Assessment of the role of IL-17A and its receptor IL-17A in apoE-deficient mice showed that the IL-17A/IL-17RA axis increases aortic arch inflammation during atherogenesis through the induction of aortic chemokines, and the acceleration of neutrophil and monocyte recruitment to this site [110]. Blockage of IL-17A with anti-IL-17A antibodies attenuated development of atherosclerosis in apoE-deficient mice [111,112]. Altogether, these findings clearly suggest for the proatherogenic role of Th17 and IL-17.

Studies in patients with coronary atherosclerosis reported Th17/Treg functional imbalance associated with significant increase in peripheral Th17 number, Th17-related cytokines (IL-17, IL-6, and IL-23) and transcription factor (RORγt) levels and obvious decrease in Th1 number, Treg-related cytokines (IL-10 and TGF-B1) and transcription factor (Foxp3) levels [46,113]. Indeed, restoring the Th17/Treg balance may be a promising therapeutic approach in the treatment of atherosclerosis and particularly atherosclerotic complications such as acute coronary syndrome and chronic heart failure.

A possible way to improve the Th17/Treg bias is to stimulate TGF-β, which is known to orchestrate differentiation of both Th17 and Tregs in a concentration-dependent manner [114]. At low concentrations, TGF-β synergizes with IL-6 and IL-21 to promote IL-23 receptor expression favoring Th17 cell differentiation. High concentrations of TGF-β repress IL23R expression and favor Foxp3+ Treg cells. RORγt and Foxp3 are co-expressed in naïve CD4+ T cells exposed to TGF-β and in a subset of T cells found in the murine small intestinal lamina propria [44]. TGF-β-induced Foxp3 inhibits RORγt function such as RORγt-induced expression of IL-17A mRNA through their interaction and thereby induces polarization into inducible Tregs [115].

Along with the subset of IL-17 producing inducible peripheral Foxp3+ Tregs, a subgroup of IL-17-producing natural CD4+CD25Foxp3+ Tregs was recently discovered [116]. These cells express high amounts of inducible costimulator (ICOS) and have a unique cytokine secretion profile (IL-10, IL-17, IL-35, TGF-β, and IFN-γ) distinguishable from all other Foxp3+ Tregs. In vitro, ICOS+ Tregs can be induced by 2,4-dinitrofluorobenzene immunization of cultured CD4+CD25+ Tregs. These cell share features of Th17/Th1 cells and Tregs. ICOS+ Tregs were shown to possess a highly suppressive activity to CD8+ T-cell effector responses in a haptenspecific manner in vivo [116]. By secreting IL-35, ICOS-positive Tregs were reported to inhibit Th17-mediated IL-17 production and suppress airway hyperresponsiveness in the mouse model of allergic airways disease [117]. The population of ICOS+ Tregs was found to be decreased in patients with myocardial infarction [118]. Since ICOS+ Tregs have capacity to down-regulate Th17-mediated proinflammatory responses, this Tregs subset is likely to have the atheroprotective role. Immunosuppressive properties of IL-17-producing inducible and natural Tregs should be further assessed in order to explore their promising therapeutic potential in the future.

4. Approaches to enhance Treg-mediated immune regulation in atherosclerosis

Studies in animal models showed the significance of T cell co-stimulatory and co-inhibitory pathways in the regulation of inflammation in atherosclerosis. These pathways are critical in the control of function of naïve and effector T cells and in the generation and function of Tregs. Due to the proinflammatory microenvironment, Tregs function may be markedly down-regulated or impaired and Tregs numbers may be reduced in atherosclerosis [119]. Indeed,
strategies focused on the improvement and/or enhancement of Tregs function and increasing their amount could be considered as a novel therapeutic approach against atherosclerosis.

4.1. Induction of mucosal tolerance

A promising strategy to induce anti-inflammatory effector T cells and Tregs is the induction of oral tolerance, e.g. the specific suppression of cellular and/or humoral immune reactivity to an antigen by prior administration of the antigen by the oral (nasal) route [120]. Antigen-specific immunomodulation by vaccination is an attractive approach to prevent or treat chronic inflammatory diseases including atherosclerosis. By mobilizing protective immune responses in an antigen-specific manner, side effects due to hampered host defense against infections may be avoided.

The mucosal tolerance (with antiatherogenic consequences) may be induced by a variety of agents such as monoclonal antibodies and immunogenic peptides listed in Table 1. To induce an antigen-specific response, investigators used self-antigens or peptides derived from self-antigens usually presented in the atherosclerotic plaque such as oxLDL [121], apoB [122,123], HSP60/65 [124−126], and beta 2-glycoprotein I [127]. Conjugation of a peptide to the subunit B of cholera toxin promotes uptake of an antigen via the nasal and oral mucosa and induction of protective immunity [128].

Natural human LDL is defined as a population of lipoproteins that form lipoprotein particles of 18−28 nm in diameter and can be isolated by ultracentrifugation within a density range of 1.019−1.063 kg/L [129]. In the central lipophilic core, an LDL particle contains up to 170 triglycerides and up to 1600 cholesteryl ester molecules. The core is surrounded by a monolayer comprising ~700 phospholipid molecules and 600 molecules of free cholesterol. A large molecule of apolipoprotein B-100 (apoB-100: 4356 amino acids) is embedded to the outer layer. A total of 2700 molecules of free fatty acids are distributed in the LDL particle, and a half of those are polyunsaturated fatty acids (PUFAs) [130]. Compared to other lipoprotein classes, LDL are enriched (up to 50%) with cholesterol, and increased plasma concentrations of LDL-cholesterol are widely recognized as an established cardiovascular risk factor. Reaching LDL-cholesterol level of less than 100 mg/dL is recommended for lipid-lowering therapy to prevent a high risk of a cardiovascular disease [131].

Oxidation of LDL accumulated in the arterial wall is occurred on early stages of atherosclerosis. There are many possible mechanisms of LDL oxidation including enzymatic action of membrane-associated NAD(P)H oxidase of activated neutrophils, monocytes, and macrophages and 15-lipoxygenase [132] and influence of various reactive oxygen species and radicals such as superoxide anion [133], thyl radicals and superoxide [134], peroxynitrite [135], and by-products of the myeloperoxidase reaction (hypochlorous acid and tyrosyl radical) [136]. Transition metal ions such as Cu²⁺ and Fe³⁺ capable to catalyze LDL oxidation may be used for oxLDL generation in cell-free systems. LDL oxidized by a cell-free system is physiochemically and biologically indistinguishable from LDL oxidized by a cellular system [137].

Oxidation of LDL is a free radical-mediated process resulting in numerous structural changes, all of which depend on a common initiating event, the peroxidation of PUFAs in LDL. Due to the presence of conjugated double bonds, PUFAs are very sensitive to oxidation and could be subsequently oxidized to peroxyl radicals and hydroperoxides [129]. Cholesterol in LDL can be oxidized to oxysterols such as 7-ketocholesterol [138]. In LDL oxidation, the propagation phase is followed by a decomposition or degradation phase, during which there is cleavage of double bonds resulting in the formation of aldehydes. The major aldehydes produced include malondialdehyde (MDA), malondialdehyde-acetaldehyde (MAA), 4-hydroxynonenal, and hexanal, which can cross-link with amino groups on apoB-100 [139,140].

Changes in the protein moiety also occur during the oxidation of LDL. After oxidation, there is an increase in the negative charge on the LDL particle, possibly due to the derivatization of positively charged amino groups through the formation of a Schiff base with aldehydes. Also, after oxidation, apo B-100 undergoes oxidative scission leading to fragmentation [141]. MAA-modified proteins were found in the atherosclerotic plaque. MAA−adducted proteins are involved in the inflammatory reaction that occurs in atherosclerosis [142].

Different classes of modified LDL were shown to differ in their capacity to induce oral tolerance in animal models of atherosclerosis. If the oral administration of oxLDL was able to induce the oral tolerance in LDLR-deficient mice, MDA-LDL did not [117]. The atheroprotective effect of oral administration of oxLDL may be partially explained by the ability of oxLDL to induce tolerogenic DCs that could in turn induce Tregs and inhibit proinflammatory responses [143]. The difference between oxLDL and MDA-LDL in inducing oral tolerance may result from the binding complement anaphylatoxin C3a to MAA-LDL and MDA-LDL [144]. MAA- and LDL-LDL and proteins containing MAA adducts or MAA adducts may bind C3a in vivo and contribute to inflammatory processes involving activation of the complement system in atherosclerosis [142].

4.2. Induction of Tregs by anti-CD3 antibody

Monoclonal antibodies such as anti-IL-2 [145] or anti-CD3 antibodies [6] suitable for induction of mucosal tolerance could possess immunomodulatory properties. The IL-2/anti-IL-2 antibody complex formed with IL-2 and anti-IL-2 neutralizing monoclonal antibody is able to selectively stimulate natural CD4⁺CD25⁺Foxp3⁺ Tregs and greatly attenuate progression of atherosclerosis in apoE-deficient mice [145].

Preclinical studies including nasal or oral administration of anti-CD3 antibodies showed their high efficacy in induction of Tregs and suppression of autoimmune and inflammatory pathology in mice [146,147]. Based on the results achieved in animal experiments, a humanized anti-CD3 monoclonal antibody was orally administered to healthy human individuals [148]. At present, this antibody (otelixizumab) is clinically tested at Phase III for the prevention of type 1 diabetes [149]. The therapy with this antibody is typically safe and biologically active since it induces many favorable immunologic effects such as suppressed Th1 and Th17 responses, induced immunosuppressive Tregs, increased production of anti-inflammatory cytokines (IL-10 and TGF-β), and decreased DC-mediated proinflammatory cytokine (IL-6 and IL-23) secretion [82]. Indeed, oral administration of anti-CD3 antibody may be considered as a promising strategy to induce Tregs-mediated anti-inflammatory response in order to reduce atherosclerosis development and progression.

In atherosclerosis mice, oral administration of anti-CD3 antibody resulted in obvious beneficial effects including reduced plaque development when administered before a high-cholesterol diet and markedly decreased lesion progression in animals with already established atherosclerosis [150]. Treatment with anti-CD3 antibody was shown to induce CD4⁺CD25⁺Foxp3⁺ Tregs (Th3) and CD4⁺CD25⁻LAP⁺ Tregs, likely in gut-associated lymphoid tissues [151]. The latter Tregs subclass expresses surface latency-associated peptide (LAP), the amino-terminal domain of the TGF-β precursor peptide, which remains noncovalently associated with the TGF-β peptide after cleavage forming the latent TGF-β complex [152].

It seems that CD4⁺CD25⁻LAP⁺ Tregs may prevent atherosclerosis [6] but have a limited capacity to attenuate the established
### Table 1

Examples of therapeutics used for stimulation of anti-inflammatory immunosuppressive function of Tregs and increasing their population in atherosclerotic vascular disease.

<table>
<thead>
<tr>
<th>Agent and treatment regimen</th>
<th>Experimental model</th>
<th>Tregs population</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statin (simvastatin): 50 mg/kg daily for 6 weeks</td>
<td>ApoE-deficient mice</td>
<td>Foxp3+ Tregs</td>
<td>Increase in Tregs numbers in plaques; 2–2.5-fold increase in expression of Foxp3, IL-10, and TGF-β mRNA in plaques; 1.5–2-fold increase in protein levels of Foxp3, IL-10, and TGF-β in plaques; 2.5–3.5-fold decrease in circulating blood levels of IFN-γ and IL-17</td>
<td>[162]</td>
</tr>
<tr>
<td>ApoB-CTB fusion protein (peptide p210 3136–3155 fused with the cholera toxin B subunit): 15 μg intranasally twice a week for 12 weeks</td>
<td>ApoE-deficient mice</td>
<td>Tr1</td>
<td>Reduction in aortic lesion size by 35%; 2-fold increase in amounts of IL-10 producing Tr1 cells; 11-fold increase in expression of IL-10 mRNA in plaques</td>
<td>[122]</td>
</tr>
<tr>
<td>HSP60: HSP60 peptide 253–268: orally 4 times in 8 days before starting fat-rich diet</td>
<td>LDLR-deficient mice</td>
<td>CD4+CD25+Foxp3+ Tregs</td>
<td>Reduction in aortic lesion size by 27%; reduction in lesion size in the carotid arteries by 80%; 1.3–1.5-fold increase in amounts of CD4+CD25+Foxp3+ Tregs in blood, spleen, and mesenteric lymph nodes by day 14; 2–3-fold increase in production of TGF-β and IL-10 in plaques by day 14</td>
<td>[126]</td>
</tr>
<tr>
<td>oxDL: 100 μg by intraperitoneal injection before starting fat-rich diet</td>
<td>LDLR-deficient mice</td>
<td>CD4+CD25+Foxp3+ Tregs</td>
<td>Reduction in initiation rate of atherosclerosis by 30–71%; Reduction in progression rate of atherosclerosis by 45%; 1.8-fold increase in amounts of CD4+CD25+Foxp3+ Tregs in spleen, and mesenteric lymph nodes by day 14; 3.2-fold increase in production of TGF-β in plaques by day 14 Reduction in aortic lesion size by 48% (4 weeks post-administration); 1.6–2.3-fold increase in amounts of Foxp3+Tregs and LAP+ Tregs; 4.5-fold increase in production of TGF-β in plaques</td>
<td>[121]</td>
</tr>
<tr>
<td>FTY720</td>
<td>ApoE-deficient mice</td>
<td>Foxp3+Tregs</td>
<td>Reduction in aortic lesion size by 33% (10 weeks post-administration); 1.2-fold increase in amounts of CD4+CD25+Foxp3+ Tregs in spleen and mesenteric lymph nodes; 2.2-fold increase in production of TGF-β in plaques 4-fold decrease in production of IFN-γ in plaques</td>
<td>[6]</td>
</tr>
</tbody>
</table>

atherosclerosis [145]. However, up-regulated secretion of TGF-β by CD4+ CD25+ LAP+ Tregs may induce other TGF-β-dependent Tregs subsets in other lymphoid organs. In anti-CD3-treated mice, a significant increase in amounts of induced Foxp3+ Tregs was observed in mesenteric lymph nodes [6] and spleen [151]. Induced Tregs may migrate from lymphoid organs to atherosclerotic plaques and dampen local immune responses resulting in the attenuation of atherosclerotic lesion formation in already developed atherosclerosis. Alternately, CD4+CD25+Foxp3+ Tregs may be directly induced by anti-CD3 antibody [153].

#### 4.3. Induction of tolerogenic DCs by 1,25-dihydroxyvitamin D3

Tolerogenic DCs may be induced by oral administration of vitamin D, or its hormonally active form calcitriol (1,25-dihydroxyvitamin D). This hormone plays a central role in calcium metabolism and bone formation but also exerts immunomodulatory (immunosuppressive) function. Calcitriol exhibits anti-inflammatory properties by inhibiting Th1 cell differentiation through the down-regulation of IL-6 and IL-23 [154] and inhibiting differentiation and maturation of DCs [155]. Therefore, calcitriol-treated immature DCs became tolerogenic and can promote Foxp3+ Tregs, Tr1, and Th3 cells via production of IL-10 and TGF-β [156]. Finally, 1,25-dihydroxyvitamin D3 can directly modulate differentiation of Foxp3+ Tregs [157,158]. In a recent double-blind, placebo-controlled trial, Bock et al. [159] showed that intake of calcitriol at high dose (140,000 IU monthly) for 3 months significantly increased the frequency of Tregs in healthy volunteers.

The atheroprotective effect of 1,25-dihydroxyvitamin D3 was demonstrated in apoE-deficient mice treated daily with 200 ng of calcitriol for 12 weeks [160]. Atherosclerotic lesion formation was significantly attenuated, with decreasing monocyte/macrophage accumulation in lesions and reducing numbers of mature CD11c+CD86+ DCs in the mesenteric lymph nodes, spleen, and intestine. In the plaque, Foxp3+ Tregs counts were elevated and their immunosuppressive capacity was increased in association with stimulated IL-10 production and inhibited IL-12 expression. Calcitriol increased mRNA levels of CCL22 and its receptor CCR4 in DCs suggesting that tolerogenic DCs release Tregs-attracting chemokines and draw Tregs to bind to CCR4 on the surface of DCs [161].

#### 4.4. Statins

Tregs and DCs can be quantitatively and qualitatively modulated by small molecule drugs possessing immunomodulatory features. For example, statins (lipid-lowering agents) exert pleiotropic effects on atherosclerosis including the modulation of inflammatory responses. In atorvastatin-treated apoE-deficient mice, population of Foxp3+ Tregs was increased and Tregs +Th2/Th1+Th17 balance was improved [162]. A short-term (4-week) therapy with atorvastatin at daily dose of 20 mg significantly inhibited the immunostimulatory function of DCs in patients with abdominal aortic aneurism [162]. In hyperlipidemic subjects treated with simvastatin or pravastatin, numbers of natural CD4+CD25+Foxp3+ Tregs were increased [163].

Atorvastatin was shown to be capable to induce CD4+CD25+ Foxp3+Tregs from human peripheral CD4+CD25–Foxp3– T cells and enhance their functional suppressive properties. A stimulatory effect of atorvastatin on induction of Tregs, Foxp3 expression, and TGF-β production was observed in patients with ST-segment elevation myocardial infarction [164]. Simvastatin promoted an accumulation of Tregs, subsequently decreased Th1 and Th17 response and modulated the Th1/Th2 balance toward a Th2 phenotype in the atherosclerotic plaques, and this result may be
an additional mechanism of simvastatin in stabilizing vulnerable plaques [165].

4.5. Lysosphingolipid sphingosine 1-phosphate analogs

The biologically active lysosphingolipid sphingosine 1-phosphate (SIP) is an important lipid mediator generated from phospholipids on cell activation [166]. A large body of evidence has documented the pleiotropic effects of SIP in various cell types and organs including the immune system. SIP was demonstrated to interfere with proliferation, migration, and cytokine secretion by lymphocytes and to prevent their recirculation from lymphoid compartments to peripheral sites of antigen presentation [167].

FTY720, a synthetic SIP analog, possesses immunosuppressory properties via inhibiting T lymphocyte proliferation, down-regulating secretion of proinflammatory cytokines IL-6, IFN-γ, and TNF-α, and stimulating the anti-inflammatory path of activation of macrophages towards the M2 phenotype [168]. FTY720 was shown to inhibit atherosclerosis in LDLR-null [168] and apoE-deficient mice [169]. Oral administration of this sphingolipid analog to apoE-deficient mice was shown to result in significant stimulation of CD4+CD25–LAP+ and CD4+CD25–/–Foxp3+ Tregs and suppression of Th1 immune response [170]. Recently, FTY720 (Fingolimod) was approved as a new therapeutic drug for multiple sclerosis in more than 50 countries [171]. Hopefully, due to the marked capacity of Fingolimod to inhibit the early development of atherosclerosis in mice via induction of a Tregs response and inhibition of effector T responses, this sphingolipid analog will be clinically tested soon in patients with coronary artery disease.

4.6. Steroids

Glucocorticoids such as hydrocortisone and their synthetic analogs such as dexamethasone, methylprednisolone, and prednisone possess potent immunosuppressive and anti-inflammatory properties. Treatment with intranasal steroid was shown to inhibit nasal inflammation and induce increased amounts of Foxp3+ Tregs and anti-inflammatory cytokine (IL-10) in nasal polyps [172]. Methylprednisolone treatment resulted in significant increase in a population of highly suppressive DRhighCD45RA− Tregs in transplanted patients [173]. Similarly, dexamethasone-treated monocytes differentiate into tolerogenic IL-10–producing DCs capable to induce IL-10–producing Tregs and restore immune tolerance [174,175].

In contrast, aldosterone, a mineralocorticoid hormone, may support inflammation, fibrosis, and vascular remodeling, e.g. proatherogenic pathogenic mechanisms. As a component of the renin–angiotensin–aldosterone system, aldosterone at high concentrations may contribute to the vascular damage through the induction of hypertension, oxidative stress, and vascular inflammation [176]. Human VSMCs exposed to aldosterone present an increase in type I and III collagen, IL-16, and CTLA-4 expression, molecules associated with fibrosis, inflammation, and vascular calcification [177]. Adipocyte transfer of natural CD4+CD25−Foxp3+ Tregs leads to suppression of aortic and renal vascular inflammation, reduction of macrophage and T-cell infiltration into the vascular wall and other improvements in mice with aldosterone-mediated vascular injury [178].

These findings may suggest for potential beneficial effects of treatment of coronary artery disease with glucocorticoids. However, these hormones are usually used in therapy of severe proinflammatory diseases such as eczema, psoriasis, asthma, and arthritis or as immunosuppressive agents in transplantation due to the systemic inhibiting effect on the immune system. Glucocorticoids are unlikely to be efficient for treatment of chronic diseases with persisting low-dose inflammation such as atherosclerosis since long-term administration of these steroids significantly increases risk of side effects including weight gain, mood swings, sleep disruptions, dizziness, glaucoma, lowering bone density, and an overall weakened immune system [179]. In addition, treatment of apoE-deficient mice with dexamethasone was found to increase hyperlipidemia through mechanisms related to the increase in the synthesis of triglyceride-rich lipoproteins [180].

4.7. Non-steroid anti-inflammatory drugs (NSAIDs)

Most of NSAIDs are non-selective inhibitors of both forms of cyclooxygenase (COX), COX-1 and COX-2, an enzyme involved in the biosynthesis of prostanoids. The pharmacological inhibition of COX provides relief from the symptoms of inflammation and pain. Aspirin, a broadly used NSAID, may mediate immune regulation in a wider manner including mechanisms, which are not limited by the only COX inhibition. For example, aspirin is able to induce tolerogenic DCs from immature DCs through the suppression of NFκB–dependent signaling, reduced expression of pro-stimulatory molecules (CD80, CD86, ICOS), and induction of immunoglobulin-like transcript-3, a B-cell immune receptor preventing activation of B lymphocytes [181]. Indeed, tolerogenic DCs can then induce differentiation of naïve T cells to CD4+CD25+Foxp3+ Tregs and support expansion of natural CD4+CD25+Foxp3+ Tregs [182]. New aspirin derivatives such as ASC14 (a hydrogen sulfide–releasing aspirin) may possess advanced anti-inflammatory properties in preventing atherosclerosis and thrombosis via several pathways including suppression of a fraktalkine–dependent macrophage activation and migration [183], blocking platelet aggregation, and down-regulating fibrinogen receptor activation [184]. Aspirin continues therefore to be of interest due to its wide effects on immune regulation including the capacity to induce tolerogenic DCs at therapeutic concentrations in humans.

Other NSAID classes may also possess anti-atherosclerotic properties through COX and acetyl-CoA carboxylase inhibition, lipid-lowering effects, antioxidant activity, and suppressing inflammation [185,186]. Compared to non-specific COX inhibitors, coxibs (rofecoxib, celecoxib, and valdecoxib) that are selectively block COX-2 activity are advantageous in therapy of arthritis and other painful inflammatory syndromes but become associated with increased risk myocardial infarction and stroke as shown in many studies. COX–2–specific inhibitors enhance Th1 type immune response associated with increased production of inflammatory cytokines, unstable plaque phenotype, and acute atherosclerosis complications [187]. By disrupting the physiological balance between thromboxane and prostacyclin, coxibs increase atherosclerosis, thrombogenesis, and the risk of cardiovascular complications. In addition, through uncoupling mitochondrial oxidative phosphorylation, coxibs may induce production of reactive oxygen species and increase cardiovascular risk [188]. COX–2–specific inhibitors suppress IDO production and Tregs, and this may be beneficial for disruption of tumor-induced tolerance but not for prevention of atherogenesis [189].

4.8. Disease-modifying anti-rheumatic drugs (DMARDs)

DMARDs is a category of unrelated drugs defined due to their ability to dampen the development of rheumatoid arthritis (RA) and other autoimmune proinflammatory diseases. These include tetracycline antibiotics (doxycycline, minocycline), DNA synthesis blockers (methotrexate, azathioprine, leflunomide), TNF-α-specific monoclonal antibodies (infliximab, adalimumab, golimumab), non-antibody inhibitors of IL-1 and TNF-α (chloroquine, hydroxychloroquine, sulfasalazine) and some other agents. In many studies, methotrexate (MTX), a folate metabolism inhibitor, showed a potent atheroprotective effect by stimulating reverse
cholesterol transport, cholesterol oxidation [190], down-regulation of proinflammatory genes (TNF-α, IL-1β, CXCL-2, VAP-1, etc.), and up-regulation of anti-inflammatory cytokine TNF-β1 [191].

Compared to other DMARDs such as sulfasalazine and leflunomide, MTX did not inhibit suppressive function of CD4+CD25+ Tregs and Foxp3 expression [192]. In a murine model of collagen-induced arthritis, co-treatment with MTX and etanercept resulted in marked suppression of proinflammatory T cell subsets and their production of proinflammatory cytokines and restoring natural CD4+CD25+Foxp3+ Tregs numbers in the thymus and spleen [193]. TNF-α is a pleiotropic cytokine, which can have proinflammatory or immunosuppressive effects depending on the context, duration of exposure and disease state. In concert with IL-2, TNF-α selectively activates Tregs resulting in proliferation, up-regulation of Foxp3 expression and increases in their suppressive activity [194]. However, in rheumatoid arthritis and other proinflammatory environments, this cytokine down-regulates the function of both natural CD4+CD25+ Tregs and TGFβ1-induced CD4+CD25+ Tregs [195]. Accordingly, specific inhibition of TNF-α with monoclonal antibodies restores Tregs immunosuppressive function and increases numbers of natural CD4+CD25+Foxp3+ Tregs in patients with autoimmune and inflammatory pathology [196–198].

The higher mortality rate among rheumatoid arthritis patients in comparison with the general population is largely attributable to cardiovascular disease, particularly coronary atherosclerosis. Overall, the immunosuppressive capacity of TNF-α inhibitory antibodies, MTX, and other DMARDs has beneficial effects on the cardiovascular system of patients affected with rheumatoid arthritis [199–201]. Longer exposure to low doses of corticosteroids increases cardiovascular risk while treatment with anti-TNF agents, despite some inconsistency, delays and even reverses the progression of endothelial dysfunction and atherosclerosis [199].

5. Conclusion

In addition to Tregs, tolerogenic DCs have a critical role in the regulation of T cell response in atherosclerosis. Modulation of intestinal immune system by inducing both Tregs and tolerogenic DCs might be a therapeutic approach for the prevention of atherosclerosis.

Oral immune modulation by drugs and therapeutic agents possessing immunoregulatory activities is the simplest way to induce gut-associated immune tolerance. Because the intestinal immune system must protect our body from invading pathogens and food antigens, intestinal DCs are crucial for determining inflammatory or tolerogenic immune responses. The presence of the specific CD103+CD11b+ DCs subset in lamina propria capable to capture antigens and induce Tregs through the production of TGF-β and retinoic acid [202,203] is a key component in the induction of intestinal immune tolerance. On the other hand, there are other subpopulations of mucosal DCs that are reside in the gut-associated lymphoid tissue and that are able to induce inflammatory response [204]. Thus, the careful analysis of the mechanisms of how different subtypes of intestinal DCs respond to various antigens and determining the response toward either immunity or tolerance should contribute to the efficiency of the tolerance-inducing therapy.

To facilitate assessment of a role of each DC subtype including aortic DC populations, advanced approaches may be used such as generating mice with selectively labeled DC subsets by a reporter gene such as green fluorescent protein or obtaining mice strains each lacking a specific DC subset. These murine models may set the stage for future work on, for example, the mechanistic function of convenient DCs and monocyte-derived DCs in the aorta, both during steady state and during the development of atherosclerosis.

Such a strategy became to use. For example, Koltsva et al. [58] reported the development of apoE-deficient mice with CD11c+ DCs specifically labeled with yellow fluorescent protein. Using this model, a live-cell imaging of dynamic interactions between DCs and T cell in atherosclerotic aorta was obtained. Using an anti-mouse plasmacytoid dendritic cell antigen 1 (PDCA-1) antibody, Macritchie et al. [205] specifically depleted plasmacytoid plasmacytoid DCs in apoE-deficient mice resulted in significant reduction of atherosclerosis progression and more stable plaque phenotype.

Similar advanced approaches can be implemented for evaluating a role of each Treg subset in atherosclerosis. Regarding Tregs, a special attention should be paid for further characterization and studying immune regulatory function (in the atherosclerosis context) of recently discovered subtypes of Tregs such as IL17-producing inducible Foxp3+ Tregs and IL-17-producing natural CD4+CD25+Foxp3+ Tregs, since these Tregs can share features of TH2/TH17 and TH1/Treg cells. The oral administration of immunomodulatory molecules seems to be the optimal way of inducing a physiologically relevant Tregs-dependent anti-atherogenic response because intravenous injection of immune modulators such as anti-CD3 antibody and superagonist anti-CD28 antibody may activate excessive immune responses [206].

The newest potential strategy of enhancing endogenous immune responses is the direct transplantation of autologous Tregs expanded ex vivo. At present, this cell therapy approach is under consideration to be implemented for treatment of autoimmune or graft-versus-host disease (clinical trials NCT01624077, NCT010651716, and NCT00675831). If Tregs-based cell therapy will be beneficial for treatment of such inflammatory diseases, it becomes possible to use this approach for atherosclerosis treatment in humans. However, multiple issues including biosafety, biocompatibility, homing ability, and functional activity should be investigated before the clinical approval of this technique.

Conflict of interests

The authors have no conflicts of interest to declare.

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References


