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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 TNF-beta. 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

*Abbreviations:* LDL, low density lipoprotein; Tregs, regulatory T cells; IL, interleukin; TGF-β, transforming growth factor beta; Foxp3, forkhead box 3; IL-2R, interleukin-2 receptor; CTLA-4, cytotoxic T-lymphocyte antigen 4; NK, natural killer; DCs, dendritic cells; TCR, T-cell receptor; Tr1, Tregs type 1; Th, T helper cell; Bcl-xL, B-cell lymphoma-extra large; RORγt, RAR-related orphan receptor gamma; apoE, apolipoprotein E; LDLR, LDL receptor; oxLDL, oxidized LDL; Scid, severe combined immunodeficiency syndrome; CCL, C-C motif chemokine ligand; IFN, interferon; VSMC, vascular smooth muscle cell; HSP, heat shock protein; IDO, indoleamine 2,3-dioxigenase; PDL-1, programmed death ligand 1; LFA-1, lymphocyte function-associated antigen 1; GM-CSF, granulocyte-macrophage colony-stimulating factor; TNF-α, tumor necrosis factor alpha; CCR, C-C motif chemokine receptor; ECM, extracellular matrix; Ig, immunoglobulin; Rag2, recombination activating gene 2; ICOS, inducible costimulator; LAP, latency-associated peptide; S1P, lysosphingolipid sphingosine 1-phosphate; STAT6, signal transducer and activator of transcription 6; COX, cyclooxygenase; NSAID, non-steroid anti-inflammatory drug; DMARD, disease-modifying anti-rheumatic drug; MTX, methotrexate; RA, rheumatoid arthritis.

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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 $CD4^+CD25^+Foxp3^+$ Tregs

There are several subpopulations of Tregs including naturally occurring $CD4^+CD25^+Foxp3^+$ Tregs and inducible Tregs subsets [3]. Natural $CD4^+CD25^+$ Tregs, which are produced in the thymus, are the key players in suppressing self-reactive immune responses and mantaining dominant self-tolerance [8]. They constitutevely 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 maintainance of $CD4^+CD25^+$ Tregs [9]. In $CD4^+CD25^+$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 $CD4^+CD25^+Foxp3^+$ Tregs can suppress a variety of immune cells including $CD4^+$ and $CD8^+$ T cells, B cells, natural killer (NK) cells, NKT cells, monocytes, and dendritic cells (DCs) (Fig. 1). A main function of natural $CD4^+CD25^+$Tregs is suppression of activation of naïve T cells, but they can also inhibit activated effector T cells and memory $CD4^+$ and $CD8^+$ cells [14]. Natural $CD4^+CD25^+Foxp3^+$ 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 $CD4^+CD25^+Foxp3^+$ 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, Grossman et al. [18] also reported the ability of human natural $CD4^+CD25^+Foxp3^+$Tregs to express the serine protease granzyme A and kill $CD4^+$ 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+ 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 LAG3, 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 $CD4^+CD25^+Foxp3^+$ 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 $CD25^+$ and $CD25^-$ T cell populations [26] and rescue IL-2-deficient mice from autoimmunity due to the induction of $CD25^-$ Tregs in the periphery [27]. Indeed, Th3 cells may play a critical role in inducing and maintaining peripheral tolerance by driving differentiation of $Foxp3^+$ Tregs in the periphery by secretion TGF-β. TGF-β-derived $Foxp3^+CD25^{+/-}$ Th3 Tregs represent a different cell lineage from thymic-derived $CD25^+$ 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+ T cells induced by DCs are particularly sensitive to TGF-β [32].

### 2.4. Foxp3+ Tregs

Compared to natural $CD4^+CD25^+Foxp3^+$ Tregs, which arise from the thymus and are released into peripheral tissues after thymic-positive selection, inducible $Foxp3^+$ Tregs are generated *via* peripheral conversion (after antigen-specific stimulation) from mature, naïve $CD4^+$T cells or from "rescued" self-reactive effector T lymphocytes [33]. $Foxp3^+$ Tregs can be induced in periphery from $CD4^+CD25^-$ 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 $CD4^+$ naïve T cells [36]. Compared to natural $CD4^+CD25^+Foxp3^+$ Tregs whose CpG island within the Foxp3 locus is demethylated, it was reported to be methylated in TGF-β-induced $Foxp3^+$ 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 $CD4^+CD25^+Foxp3^+$ Tregs, TGF-β-induced $Foxp3^+$ 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 $Foxp3^+$ 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



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