

## Regulatory T Cell Immunity in Atherosclerosis

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### **ABSTRAK**

*Aterosklerosis merupakan suatu proses peradangan kronik, yang melibatkan imunitas alamiah dan adaptif. Respon sel T efektor (Teff) berperan dalam mempercepat terjadinya aterosklerosis. Adapun sel T regulator (Treg), mempunyai peran sebagai komponen pelindung dalam proses aterosklerosis melalui modulasi respon inflamasi yang melibatkan berbagai mekanisme. Dari berbagai studi eksperimental menunjukkan bahwa perubahan keseimbangan antara Treg/Teff dengan polarisasi ke arah Treg dapat menjadi pendekatan terapeutik pada aterosklerosis, meskipun peran Treg dalam studi klinis belum sepenuhnya dapat dijelaskan. Dalam tinjauan pustaka ini, kami akan menelaah berbagai perkembangan dan pemahaman terkini mengenai peran Treg dan Teff dalam studi eksperimental dan klinis aterosklerosis.*

**Kata kunci:** aterosklerosis, sel T regulator, peradangan.

### **ABSTRACT**

*Atherosclerosis is a chronic inflammatory disorder involving innate and adaptive immunity process. Effector T cell (Teff) responses promote atherosclerotic disease, whereas regulatory T cells (Tregs) have been shown to play a protective role against atherosclerosis by down-regulating inflammatory responses which include multiple mechanisms. Compelling experimental data suggest that shifting the Treg/Teff balance toward Tregs may be a possible therapeutic approach for atherosclerotic disease, although the role of Tregs in human atherosclerotic disease has not been fully elucidated. In this review, we discuss recent advances in our understanding of the roles of Tregs and Teffs in experimental atherosclerosis, as well as human coronary artery disease.*

**Keywords:** atherosclerosis, regulatory T cells, inflammation.

### **INTRODUCTION**

Atherosclerotic disease is a major cause of mortality worldwide. Atherosclerosis has been known as a chronic inflammatory disorder involving innate and adaptive immunity process.<sup>1</sup> T cell-mediated immune responses play an important role in atherogenesis.<sup>2</sup> Therefore, specific targeted approach to inflammatory

responses may be effective to improve cardiovascular outcome.

T cells, macrophages, and smooth muscle cells, which generate cytokines, growth factors, and pro-inflammatory mediators are found in atherosclerotic plaques. Although several suspected self-antigens important for atherosclerosis are reported, critical antigens have

not been identified. After antigen presentation, naïve T cells differentiate into effector T cells (Teffs), which may promote atherosclerotic disease. Importantly, regulatory T cell (Treg) expressing CD25 molecule and the transcription element FoxP3 (fork-head box P3) have been shown to play a protective role against atherosclerosis by down-regulating inflammatory responses.<sup>2</sup> In this review, we discuss recent advances in our understanding of the roles of Tregs and Teffs in experimental atherosclerosis, as well as human coronary artery disease.

### **EFFECTOR T CELLS AND ATHEROSCLEROSIS PROGRESSION**

After antigen presentation or activation by macrophages or dendritic T cell (DCs), naïve CD4+ T cells differentiate into Teffs such as T helper type 1 (Th1), T helper type 2 (Th2), and T helper type 17 (Th17) cells, which all critically affect atherogenesis in both humans and mice.<sup>3</sup> Th1 cells are known to promote atherosclerotic disease, whereas the roles of Th2 and Th17-mediated immune responses in atherosclerosis remain controversial.

Th1 cells producing high amounts of interferon (IFN)- $\gamma$  are the most populated pathogenic T cells in atherosclerosis. IFN- $\gamma$  has many detrimental effects in atherosclerosis including promotion of atherosclerotic lesion development and destabilization. IFN- $\gamma$  is reported to initiate activation of monocytes/macrophages and DCs, resulting in the pathogenic Th1 response continuation.<sup>4</sup> Interleukin (IL)-12 produced by monocytes/macrophages also has crucial roles in Th1 differentiation, production of IFN- $\gamma$ , and inhibition of IL-4 and IL-5 production in T cells. Th1 immune responses have been shown to be critical in the development of many inflammatory diseases such as colitis, multiple sclerosis, diabetes, and rheumatoid arthritis.

Th2 cells release IL-4, IL-5, IL-10, and IL-13, and afford support for antibody generation by B cells. IL-4 induces the expression of the transcription factor GATA-3 via STAT-6 activation and stimulates Th2 cell differentiation, leading to up-regulation of IL-4 and IL-5 and inhibition of IFN- $\gamma$ . These responses may oppose pro-atherogenic Th1 immune responses yielding

atheroprotective state. However, the significance of Th2 immune responses in atherosclerosis remains disputable.

The development of Th17 cells, which produce IL-17, the pro-inflammatory mediator, is promoted by growth factor (TGF)- $\beta$  in the presence of IL-6 and IL-23. Several studies support a pro-atherogenic role for Th17 cells,<sup>5,6</sup> whereas some studies demonstrate a protective role for IL-17 in atherosclerosis.<sup>7,8</sup> Further studies are needed to determine the role of this T cell subset in atherosclerosis development and progression.

### **REGULATORY T CELLS PROFILE**

Given that the mammalian immune system protects its host from invading pathogenic microbes and suppresses pathogenic immune responses, discrimination between self and non-self seems to be important for the maintenance of unresponsiveness of the adaptive immune system to self-antigens. Sakaguchi et al first reported that naturally arising Tregs thymus-derived Tregs constitutively express high levels of CD25 (IL-2 receptor  $\alpha$ -chain) molecule, and that depletion of this population elicits autoimmunity similar to the human counterparts.<sup>9</sup> Firm evidence has shown that Tregs expressing the transcription factor Foxp3, which is a master regulator of Treg development and function, and is currently the most reliable molecular marker for them, play a crucial role in dominant suppression of pathogenic immune responses, maintenance of self-tolerance, and immune homeostasis.<sup>10</sup> Several subsets of Tregs including Foxp3+ Tregs, IL-10-producing T regulatory type 1 cells, and TGF- $\beta$ -producing T helper type 3 cells can differentiate from naïve T cells in the periphery under certain conditions, and are called adaptive or induced Tregs which share similar immunological properties with thymus-derived Foxp3+ Tregs.<sup>11</sup>

Tregs have many functions in regulating immune balance. They play crucial roles in governing peripheral immune responses, down-regulating inflammatory reactions, and intercepting autoimmune disorder. Tregs dampen Teff immune responses through several mechanisms. First is through a direct contact

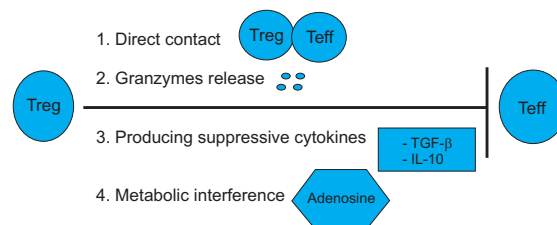
mechanism. A co-inhibitory molecule cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) is constitutively expressed by Tregs. One important mechanism for CTLA-4-dependent suppression is down-modulation of antigen-presenting cell function by reducing their CD80 and CD86 expression<sup>12</sup> or inhibition of CD80/CD86-CD28 co-stimulatory pathways, which may lead to suppression of T-cell activation. Thus, Tregs modulate the immune response through direct contact with other inflammatory cells.

Next mechanism involves killing of inflammatory cells through production of serine proteases family called granzymes. High expression of granzyme-B has been shown in Tregs. Mice lacking granzyme-B show decreased suppressive activity of Tregs.<sup>13</sup> Beside granzyme-B, other factors such as tumor necrosis factor-related apoptosis inducing ligand (TRAIL)/death receptor 5 (DR5) and galectin-1 also have been shown to be involved in inducing Teff apoptosis and dampening its responses by Tregs.<sup>14,15</sup>

The third mechanism is through the production of immunosuppressive cytokines such as transforming growth factor (TGF)- $\beta$ , IL-10, and IL-35. TGF- $\beta$  suppresses immune reactions via promoting Treg generation and suppressive function by inducing Foxp3 expression.<sup>16</sup> Blocking TGF- $\beta$  signaling in T cells aggravates atherosclerosis in atherosclerosis-prone mice, suggesting an indispensable role of this cytokine in the regulation of atherosclerosis.<sup>17</sup> IL-10 deficiency accelerates atherosclerotic plaque development, associated with increased amount and activity of inflammatory cells and pro-inflammatory cytokines.<sup>17</sup> IL-35 is required for Treg-mediated immune suppression<sup>19</sup> and may play a role in preventing atherosclerosis.<sup>20</sup>

The fourth mechanism is through metabolic disturbance of target cells. This mechanism is through IL-2, CD39, and CD73 pathways. Teffs apoptosis may be induced by Tregs via depleting/consuming IL-2.<sup>21</sup> Other metabolic disruptive mechanism is through the expression of CD39 and CD73 ectoenzymes. These ectoenzymes hydrolyze extracellular adenosine triphosphate (ATP) to produce pericellular adenosine.<sup>22,23</sup> Adenosine A2A receptor stimulation not only

suppresses Teff function but also promotes Tregs induction by decreasing IL-6 expression and up-regulating TGF- $\beta$  expression.<sup>24</sup>



**Figure 1.** Tregs suppress Teff via 4 mechanisms. 1) direct contact mechanism; 2) granzymes release; 3) suppressive cytokines production; and 4) metabolic interference results in increased adenosine.

## PROTECTIVE ROLES OF TREGS IN ATHEROSCLEROSIS

Atherosclerosis is a chronic inflammatory disorder with many complex immunological properties interactions. Recent experimental evidence has indicated that Tregs inhibit atherosclerosis development or progression and induce regression of established atherosclerotic plaque by dampening Teff responses.<sup>25</sup> Treg-mediated immune suppression includes multiple mechanisms described before.

Several subsets of Tregs have been shown to play a crucial role in the suppression of atherosclerosis.<sup>26-29</sup> Ait-Oufella et al.<sup>26</sup> have shown that CD4+CD25+ Treg deficiency due to reconstitution with CD80-/-/CD86-/- or CD28-/- bone marrow is associated with a significant increase in atherosclerotic lesions in low-density lipoprotein receptor-deficient mice, indicating that endogenous CD4+CD25+ Tregs play a protective role in atherogenesis in mice.<sup>26</sup> Although Tregs are considered to have constitutively high expression of CD25 molecule,<sup>9</sup> Teffs also express this molecule upon activation. The DEREK (depletion of regulatory T cells) mouse expressing a diphtheria toxin receptor under the control of the foxp3 gene locus is a quite useful tool to analyze the precise role of Foxp3+ Tregs in atherosclerosis, because diphtheria toxin injection to this mouse can induce selective and efficient depletion of Foxp3+ Tregs.<sup>30</sup> Klingenberg et al. used bone

marrow transplantation model and demonstrated that depletion of Foxp3<sup>+</sup> Tregs leads to a substantial increase of atherosclerosis in low-density lipoprotein receptor-deficient mice, which is associated to an increase in plasma cholesterol levels with an atherogenic lipoprotein profile.<sup>28</sup> Further studies will be required to elucidate the precise role of Foxp3<sup>+</sup> Tregs in the modulation of lipoprotein metabolism.

Therapeutic approaches such as the use of FcR-non-binding anti-CD3 monoclonal antibody,<sup>29,31</sup> IL-2/anti-IL-2 monoclonal antibody complex,<sup>32</sup> their combination,<sup>33</sup> and active form of vitamin D3 have been shown to be effective for preventing atherosclerosis through induction of Tregs.<sup>34</sup> Abdominal aortic aneurysm associated with atherosclerosis is prevented by shifting the Treg/Teff balance toward Tregs.<sup>35</sup> Intravenous administration of anti-CD3 monoclonal antibody, in addition to lowering plasma cholesterol, is demonstrated to induce rapid regression of established atherosclerosis in atherosclerosis-prone mice by enhancing a Treg-mediated immune response.<sup>36</sup> We recently reported that ultraviolet B exposure prevents atherosclerosis by enhancing a regulatory immune response and suppressing Teff responses in hypercholesterolemic mice.<sup>37</sup> We also demonstrated that CTLA-4, one of essential molecules for Treg-mediated immune suppression, regulates atherosclerosis by suppressing pro-atherogenic immune responses and could be an attractive therapeutic target for atherosclerosis.<sup>38</sup> Thus, therapeutic intervention aimed at inhibiting a Teff-mediated immune response or enhancing a Treg-mediated immune response may be a valuable approach for preventing atherosclerosis.

In addition to a possible protective role for Tregs in experimental atherosclerosis, many clinical studies have shown their importance in human atherosclerotic disease. Several recent studies have reported reduced peripheral Treg numbers in patients with acute coronary syndrome compared with healthy controls or patients with stable angina, suggesting that decreased numbers of Tregs may be responsible for the pathogenic inflammation in acute coronary syndrome.<sup>39-42</sup> However, only a few reports examined peripheral

Treg levels in patients with stable coronary plaques. We recently reported that patients with coronary artery disease have reduced Treg/Teff ratio compared with healthy controls, suggesting potential importance of Tregs in human coronary artery disease.<sup>43</sup> Prospective studies are required to ascertain whether reduced Treg levels promote atherosclerosis in humans.

## CONCLUSION

Atherosclerosis is a chronic inflammatory disorder, in which Teffs responses have been shown to aggravate atherosclerosis. On the other hand, Tregs play a protective role against atherosclerosis. Tregs maintain immunological balance and regulates Teff immune responses through several mechanisms. Recent experimental evidence suggests that Tregs can act as a paramount factor in the inflammation series of atherosclerosis. Thus, Treg-targeted therapy could be a possible approach to improve cardiovascular outcome. However, recent experimental studies have shown that under inflammatory conditions such as atherosclerosis, Tregs become dysfunctional and differentiate into proinflammatory Th1-like cells, which may contribute to promotion of arterial inflammation and atherosclerosis development.<sup>44,45</sup> The plasticity and stability of Tregs remain controversial issues and additional studies are required. Moreover, further clinical studies are needed to elucidate Treg function and roles in human atherosclerotic disease.

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