Foxp3+CD4+ Regulatory T-Cell Subtypes and Atherosclerosis

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Atherosclerosis is initiated by accumulation of low-density lipoproteins in the intima of large arteries with inflammation largely driven by cells of the innate and adaptive immune system. Early studies indicated the presence of large numbers of T cells in all stages of lesion development—early lesions, fibrous plaques, and more advanced complex lesions, with many activated T cells secreting interferon-γ (IFN-γ).\(^1\) T cells present in mouse and human atherosclerotic lesions include invariant NKT (natural killer T) cells, cytotoxic CD8+ T cells, and CD4+ T cells. CD4+ T cells can be subdivided into multiple subtypes including Th1, Th2, Th17, follicular CD4+ T (Tfh) cells, and regulatory T cells, largely based on their patterns of cytokine secretion and the transcription factors they express. Th1 cells express the transcription factor Tbet, Th2 cells GATA3, Th17 cells RORγt, Tfh cells Bcl6, and the majority of CD4+ regulatory T cells express Foxp3.\(^2\) In atherosclerotic lesions, the majority of proatherogenic CD4+ T cells exhibit a Th1 profile producing high levels of IFN-γ, which activates monocytes, macrophages, and dendritic cells and inhibits the proliferation of vascular smooth muscle cells and their ability to produce collagen.\(^3\) Th1 cells are also proatherogenic,\(^4\) whereas the role of Th2 cells is more complex but can be protective;\(^5\) the significance of Th17 cells in atherosclerosis remains to be elucidated.

CD4+Foxp3+ regulatory T cells (Tregs) are also present in atherosclerotic lesions\(^6\) and have major roles in controlling immunologic tolerance and immune homeostasis. They are derived from the thymus but can also be induced in the periphery and require Foxp3 for their suppressive effects. Foxp3 is highly conserved in humans and mice, and it is Foxp3 protein expression levels rather than Treg numbers that is the major factor influencing the magnitude of Treg-suppressive effects.\(^7\) Active demethylation of the Foxp3 locus largely driven by, for example, hydrogen sulfide is essential for Foxp3 expression, which can be further regulated by multiple factors including transcription factors such as Ets-1, CREB/ATF, Foxo1, and STAT5.\(^8\) Foxp3 protein levels are also subject to regulation by ubiquitin ligases and deubiquitinases, which determine its DNA-binding, protein stability and degradation, and the suppressive capacity of Tregs under inflammatory conditions.\(^8\) The cells are highly migratory and accumulate at sites of inflammation using chemokine receptors such as CCR2, CCR5, CCR6, and CXCR3, which also enable them to home to atherosclerotic lesions suppressing lesion development and progression.\(^9\) They suppress inflammation by multiple mechanisms that include contact inhibition, secreting anti-inflammatory cytokines interleukin (IL)-10, transforming growth factor-β1 and IL-35 or other factors such as the cytotoxin granzyme B.

In addition to exerting suppressive anti-inflammatory effects, Tregs are plastic and can also promote diseases by shifting to a proinflammatory phenotype.\(^10\) In the microenvironment of chronic inflammation and cancer, Tregs express the Th17 cell transcription factor RORγt and secrete IL-17. These CD4+Foxp3+IL-17+ cells are a distinct Treg subtype whose anti-inflammatory properties are compromised.\(^11\)

In this issue of Circulation Research, Butcher et al\(^12\) report that atherosclerosis promotes limited Treg plasticity, resulting in the accumulation of a IFN-γ+CCR5+ Foxp3+ Treg (Th1-Treg) subset exhibiting a Th1-like phenotype within the atherosclerotic aorta, spleen, and mesenteric lymph nodes of hyperlipidemic ApoE\(^{-/-}\) mice and is associated with a reduction in natural Tregs. In contrast to normal Tregs that express high levels of Foxp3, GITR, and CD25 (IL-2Rα) and low levels of CXCR3 and negligible levels of Th1 markers, the Th1-Tregs express high/intermediate levels of IFN-γ together with the Th1 transcription factor Tbet; low levels of CD25, GITR, and CXCR3; and high levels of Foxp3 and CCR5 consistent with development of a Hybrid-Treg in atherosclerotic mice. The marked reduction in CD25 in these Hybrid-Tregs together with coexpression of IFN-γ and Tbet is suggestive of a reduction in suppressor activity despite relatively unaltered Foxp3 levels.\(^13\) The authors provide strong evidence that Treg plasticity of thymus-derived natural Tregs rather than those that develop in the periphery is largely responsible for these Th1-Tregs within the spleen, lymph nodes, and atherosclerotic aortas of hyperlipidemic ApoE\(^{-/-}\) mice and provide evidence that proatherosclerotic conditions are essential for their development, probably mediated by prolonged inflammation (Figure), which has been shown to initiate Treg plasticity in other inflammatory disorders.\(^10\) The authors provide in vitro evidence that responses to a high mixed proinflammatory cytokine environment are responsible for the Treg plasticity, but effects of individual cytokines were not examined. It is possible that a single cytokine such as IL-12 has an important role in promoting Treg plasticity. IL-12 is expressed by dendritic cells in the spleen and lymph nodes under inflammatory conditions and in atherosclerotic lesions and directs development of CD4+ Th1 cells.\(^14\) Also, in colitis, IL-12 alone converts Foxp3+ regulatory T cells into IFN-γ-producing Foxp3+ T cells.\(^15\)

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

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In their study, Butcher et al. demonstrate that Th1-Tregs isolated from atherosclerotic mice are unable to suppress proliferation of activated CD4+ T cells in vitro, effects consistent with the low expression of CD25. They explore the role of anti-inflammatory cytokines in suppressing proliferation and demonstrate an absence of IL-10 expression in Th1-Tregs, suggesting that the inability of these Tregs to suppress proliferation is because of the absence of IL-10; transforming growth factor-β was unaltered, and Ebi3 expression that encodes the IL-27β chain of IL-35 and IL-27 was increased, suggesting a partial compensatory response to the lack of IL-10. IL-10 is known to act directly on CD4+ T cells, inhibiting their proliferation and production of proinflammatory cytokines and suppresses atherosclerosis. To confirm that Th1-Tregs lack the ability to suppress atherosclerosis, they focus on miR-146a expression in Tregs. MiR-146a is crucial for Treg suppressor functions. It controls Treg-mediated regulation of IFN-γ by targeting stat1, and in its absence, the heightened stat1 activity results in Tregs secreting IFN-γ. They demonstrate using adoptive transfers that ApoE-/- mice receiving Tregs deficient in miR-146a exhibit larger atherosclerotic lesions. Lesion CD4+ T-cell numbers and IFN-γ+ Th1 cells were also increased, effects consistent with the lack of ability of Th1-Tregs to suppress T-cell proliferation. They also demonstrate that an inflammatory cytokine environment in vitro promotes formation of Th1-Tregs, confirming earlier observations. Although the effects on atherosclerosis were substantial, given that Tregs also inhibit NK cell, NKT cell, and CD8+ T-cell activities, it would have been interesting to also assess effects on apoptosis and necrotic core formation in the lesions; large necrotic cores are an important characteristic of vulnerable lesions and may contribute to plaque rupture. To more clearly define the regulatory characteristics of Th1-Tregs and their similarities to CD4+ Th1 cells and Tregs, the authors performed single-cell RNA-seq analyses. They find that Th1-Tregs express lower levels of activated immunosuppressive genes than natural Tregs while also simultaneously expressing activated Th1-lineage genes, consistent with these Hybrid-Tregs expressing the Th1 transcription factor. This study raises an important question in relation to Treg plasticity and atherosclerosis. Might other additional Hybrid-Tregs that differ in levels of Foxp3 or inflammatory cytokine expression generated during development of atherosclerosis also influence lesion progression? IL-17+ Tregs have also been reported, as have IFN-γ+ Tregs that differ from those described by Butcher et al. in that they possess suppressor activity and are derived from induced Tregs. Recently, CD4+Foxp3+Tbet+CCR5+ T cells have been identified in...
atherosclerotic mice and based on expression of Foxp3 in these cells could also be classified as Hybrid-Tregs. Similar to the Th1-Tregs described by Butcher et al., these cells also express CCR5, Tbet, and IFN-γ and do not suppress T-cell proliferation. However, unlike the Th1-Tregs, these cells seem to be much more Th1-like, expressing not only IFN-γ but also other proinflammatory Th1 cytokines such as tumor necrosis factor-α and IL-10, which are not expressed by Th1-Tregs. Expression of Foxp3 is greatly reduced in these cells. Adoptive transfer of these CD4+Foxp3+Tbet+CCR5+ T cells (CCR5Teff cells) increases atherosclerosis, indicating a proatherogenic role in atherosclerosis. Given that in atherosclerosis Treg plasticity can result in Tregs that cannot suppress atherosclerosis or even promote atherosclerosis, it is tempting to speculate that additional Hybrid-Treg variants may develop at different stages of atherosclerosis, in particular, in more advanced atherosclerosis. Tregs plasticity during prolonged and advanced atherosclerosis might even result in the generation of highly autoaggressive lymphocytes that no longer express Foxp3. In unstable human plaques, Foxp3+ Treg numbers are greatly reduced compared with stable plaques. Whether Treg plasticity contributes to such reductions and greatly accelerates vulnerable plaque development remains to be determined.

In conclusion, the study of Butcher et al. provide strong evidence for natural Treg plasticity that results in development of a Hybrid-Treg with characteristics that include low CD25 and CLTA4 expression, greatly impaired suppressor activity, and an inability to inhibit atherosclerosis (Figure). Expression of a constitutively active form of the kinase Akt has recently been shown to also dampen expression of these Treg signature genes with reductions in CD25, CLTA4, and Foxp3, raising the possibility that the Akt pathway may be important in regulating Treg plasticity. Whether targeting this pathway results in inhibition of natural Treg plasticity and prevents loss of suppressor activity and the inability of Hybrid-Tregs to prevent development of atherosclerosis will require additional studies.

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None.

References


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