Editorial

Regulatory T-Cell Plasticity
Another Layer of Complexity in Atherosclerosis

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Duri ng the past 2 decades, human and animals studies have clearly documented the crucial role of inflammation in the development and complications of atherosclerosis. Both innate immunity and adaptive immunity are involved in this process. The first evidence that suggested a role of adaptive immunity in atherosclerosis was the widespread detection of the major histocompatibility class II in human atherosclerotic plaques and the presence of a large amount of CD3+ lymphocytes in human and mouse atherosclerotic lesions. Most of the T cells in mouse and human atherosclerotic plaques are CD4+ T-helper (Th) cells expressing the $\alpha\beta$ T-cell antigen receptor. Among CD4+ T cells, Th1 cells have been shown to exert proatherosclerotic effects, whereas regulatory T cells (Tregs) display atheroprotective properties. The role of Th2 and Th17 cells is still debated. Helper T-cell subsets are defined by the production of cytokines or the expression of characteristic lineage-defining transcription factors. Th1 cells are generated on priming in the presence of interleukin (IL)-12 that promotes the expression of the transcription factor T-bet and stimulates the production of IFN-$\gamma$. Th1 cells have a central role in the dominant control of immunologic tolerance and maintenance of immune homeostasis. They were first identified in mice and later in humans. The transcription factor FoxP3 is essential for the generation and the functions of Tregs. FoxP3 deficiency leads to a multiorgan autoimmune disease as can be observed in the scurfy mouse and in humans with immune dysregulation, polyendocrinopathy, enteropathy X–linked syndrome patients. Natural or thymic FoxP3+ Tregs acquire regulatory lineage commitment already on maturation in the thymus, whereas adaptive or peripheral FoxP3+ Tregs can be induced from mature CD4+ Th cells in the periphery under the influence of different stimulations, especially transforming growth factor-β. Other subsets of Tregs that do not express FoxP3 have been described. Type 1 regulatory T cells (Tr-1) are characterized by the production of large amounts of IL-10, a potent anti-inflammatory and antiatherosclerotic cytokine, and IL-10–dependent suppression of T-cell responses. In atherosclerosis, Tregs and Tr-1 cells exert protective effects. Significant acceleration of atherosclerosis has been observed in mice with reduced Treg cell numbers as obtained by invalidation of CD80/86, CD28, and ICOS (inducible T-cell costimulator) or after treatment with CD25-depleting antibodies. Other approaches for Treg cell depletion, including anti-CD25, anti-CD3 antibodies, or treatment with FoxP3 promoter, all concluded to increased vascular inflammation and atherosclerosis in the absence of Tregs. In contrast, adoptive transfer of CD4+CD25high Treg cells or IL-10–producing Tr-1 cells reduced atherosclerotic lesion development in Apoe−/− mice.

Th cells express a lineage-specific transcription factor that controls their generation, effector cytokine production, phenotype, and function and prevents the differentiation to an alternative lineage. Nevertheless, it has become increasingly clear that cells belonging to a specific Th lineage are not exclusively terminally differentiated cells but that some maintain a certain degree of plasticity. Mature CD4+ T cells can acquire characteristics of alternative lineages on antigen restimulation. For example, IL-12 can induce IL-10 production by Th1 cells, and IFN-γ/IL-10 coproducing T cells with regulatory functions have been reported in peripheral blood of healthy donors. IFN-γ/IL-10 coproducing T cells could also be generated after stimulation of Th17 cells in the presence of IL-12 or IL-27. Recent findings suggest that FoxP3+ Tregs also show functional and phenotypic plasticity, being able to secrete proinflammatory cytokines. For instance, Tregs expressing the T-bet and the Th1-associated chemokine receptor CXCRI3 can switch to a Th1 program and accumulate at sites of Th1 inflammatory responses.

In this issue of Circulation Research, Li et al report for the first time data showing that >40% of CD4+ T cells in atherosclerotic aorta of Apoe−/− mice under high fat diet express CCR5 and exhibit a unique repertoire of molecular and cell surface markers, including positivity for FoxP3, T-bet, and IFN-γ, but are CD25 negative. These so-called CCR5Teff cells use CCR5 and its ligand CCL5 to home to the aorta and interact with CD11c+ antigen-presenting cells there.

Interestingly, it is now well accepted that a transcription factor is not the sole requisite signature that determines a specific Th lineage. FoxP3 transduction by itself is not sufficient to completely recapitulate the Treg-transcriptional profile. This conclusion is supported by studies using Tregs with nonfunctional FoxP3, which demonstrated that not all FoxP3+ T cells are functional Tregs and that part of the Treg signature...
can be induced in the absence of FoxP3. In their study, Li et al. have shown that FoxP3+CCR5 T cells were able to significantly reduce IFN-γ, IL-4, IL-13, and IL-17A production by effector T cells but were unable to suppress effector T-cell proliferation (Figure). This might be accounted by the inability of CCR5 T cells to suppress IL-2 secretion. As the proliferation of FoxP3+CCR5 T cells themselves in the absence of effector T cells was not reported, it is difficult to classify this vasculature-located T-cell population as true effector T cells that should proliferate in response to coated anti-CD3 or when cocultured with mature antigen-presenting cells, whereas Tregs should not. IL-2 is required to induce CD25+ Treg cell proliferation in vitro. So-called FoxP3+CCR5 T cells display some features of previously described Th1-like regulatory population that coexpresses FoxP3, T-bet, and IFN-γ. In the context of airway hyper-reactivity, it has been shown that T-bet+FoxP3+ T cells induced by CD8αd-dendritic cells can produce both IL-10 and IFN-γ. In the intestine, the conversion of FoxP3+ Tregs into FoxP3-IFN-γ+ T cells, requiring IL-12 production by antigen-presenting cells, have also been described. However, in both studies, the so-called Th1-like Tregs showed strong suppressive functions. They blocked effector T-cell expansion and protected against organ-specific inflammation. In the study by Li et al., the adoptive transfer of FoxP3+CCR5 T cells into Ccr5−/−Apoe−/− mice was not protective and was even able to accelerate atherosclerosis to the same extent as conventional effector T cells. Therefore, in vitro and in vivo experiments rule out the possibility that CCR5+FoxP3+ T cells are Th1-like Tregs and suggest that these cells display an effector T-cell phenotype. This was supported by comprehensive transcriptome analysis. Effector T-cell population expressing FoxP3 had never been described in mice before but had been previously reported in activated human T cells with unstable expression of FoxP3, which did not acquire suppressive function. Li et al. clearly documented that FoxP3 expression was low in CCR5 T cells, 5- to 6-fold lower than in CD4+CCR5 Tregs. In human, Miyara et al. characterized the Treg cell subsets according to their FoxP3 expression. They identified 3 different populations: CD45RA−FoxP3+ non-Treg, CD45RA FoxP3+ Tregs, and cytokine-secreting CD45RA−FoxP3+ Tregs. Interestingly, the latter population had the same secretory and functional profile as the mouse CCR5 T cells identified by Li et al. They both produced IL-2 and IFN-γ and displayed no suppressive functions.

Li et al. also explored the role of CCR5 on CD4+ T-cell trafficking. Several studies have previously reported the proatherogenic role of CCR5, but most focused on monocytes. Pharmacological inhibition or genetic invalidation of CCR5 significantly reduced monocytes and atherosclerosis. CCR5 blocking prevented macrophage infiltration in the lesions and to a lower extent T-cell infiltration. Li et al. reported that in vitro pharmacological or genetic blocking of the CCL5-CCR5 pathway in T cells reduced their homing into explanted aorta, but in vivo evidence for specific migratory and proatherogenic properties of the newly discovered CCR5+ T cells population would have been more striking. They showed that the adoptive transfer of CCR5 T cells in Ccr5−/−Apoe−/− mice accelerated atherosclerosis. Yet, the same effect was observed after the adoptive transfer of conventional effector T cells that expressed CCR5 at a much lower level. It would have been of great interest to see whether the number of CCR5 T cells that accumulated in the vascular wall was higher than that of conventional effector T cells, which would strongly argue in favor of a powerful migratory property of CCR5 T cells into atherosclerotic lesions. Finally, interestingly, CCR5 T cells

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**Figure.** Almost all of the CCR5 T cells in aortas from Apoe−/− mice coexpress interferon-γ (IFN-γ), T-bet, and low level of FoxP3. These CCR5 T cells are concentrated in the aorta, with some making their way to the para-aortic lymph nodes. They produce high levels of IFN-γ, tumor necrosis factor-α (TNF-α), interleukin (IL)-10, and IL-2 and reduce the secretion of IFN-γ, IL-4, IL-13, IL-6, and IL17A (but not IL-2 or IL-5), by effector CD4+CD25− T cells, but do not suppress the proliferation of the latter. Natural CD4+CD25− FoxP3− Tregs inhibit effector T-cell proliferation and cytokine secretion. TCR indicates T-cell receptor; and TGF, transforming growth factor.
were detected in the aorta and the draining lymph nodes but not in the spleen, suggesting that their mature phenotype was acquired locally. Further studies are required to determine the specific stimulatory pathway, antigen dependent or antigen independent, which was involved in CCR5 expression on CCR5⁺ Teff cells.

Collectively, knowledge gained from the present study by Li et al.²⁶ about CCR5 as the major homing receptor for CD4⁺ T cells into atherosclerotic lesions will help to develop optimal therapies that either undermine proatherosclerotic effectors or enhance the development of stable and anti-atherogenic Tregs for immunotherapy.

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None.

**References**


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