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## **Regulatory T cell plasticity: another layer of complexity in atherosclerosis**

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During the last 2 decades, human and animals studies have clearly documented the crucial role of inflammation in the development and complications of atherosclerosis<sup>1</sup>. Both innate 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 amounts of CD3<sup>+</sup> lymphocytes in human and mouse atherosclerotic lesions. Most of T cells in mouse and human atherosclerotic plaques are CD4<sup>+</sup> T-helper(Th) cells expressing the  $\alpha\beta$  T-cell antigen receptor (TCR). Among CD4<sup>+</sup>T cells, Th1 cells have been shown to exert proatherogenic effects, whereas regulatory T cells (Tregs) display atheroprotective properties. The role of Th2 and Th17 cells is still debated<sup>1</sup>. Helper T cell subsets are defined by the production of cytokines and/or the expression of characteristic lineage-defining transcription factors. Th1 cells are generated upon priming in the presence of IL-12 that promotes the expression of the transcription factor T-bet and stimulates the production of the Th1 prototypical cytokine IFN- $\gamma$ <sup>2</sup>. Tregs have a central role in the dominant control of immunological tolerance and maintenance of immune homeostasis. They were first identified in mice and later in humans<sup>3</sup>. The transcription factor FoxP3 is essential for the generation and the functions of Tregs<sup>4</sup>. FoxP3 deficiency leads to a multi-organ autoimmune disease as can be observed in the ‘scurfy’ mouse and in human immune dysregulation, polyendocrinopathy, enteropathy X-linked syndrome (IPEX) patients<sup>5</sup>. Natural or thymic FoxP3<sup>+</sup> Tregs acquire regulatory lineage commitment already upon maturation in the thymus<sup>3</sup>, whereas adaptive or peripheral Foxp3<sup>+</sup> Tregs can be induced from mature CD4<sup>+</sup>Th cells in the periphery under the influence of different stimulations, especially TGF- $\beta$ <sup>6</sup>. Other subsets of Tregs that do not express FoxP3 have been described. Tr-1 cells are characterized by the production of large amounts of IL-10, a potent anti-inflammatory and anti-atherosclerotic cytokine, and IL-10-dependent suppression of T cell responses<sup>7</sup>. 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, ICOS or after treatment with CD25-depleting antibodies<sup>8,9</sup>. Other approaches for Treg cell depletion, including antisense-induced Treg cell apoptosis, vaccination of mice against FoxP3, or use of mice expressing the diphtheria toxin receptor under control of the FoxP3 promoter<sup>10</sup>, all concluded to increased vascular inflammation and atherosclerosis in the absence of Tregs. In contrast, adoptive transfer of CD4<sup>+</sup>CD25<sup>high</sup> Treg cells<sup>8</sup> or IL-10 producing Tr-1 cells<sup>11</sup> reduced atherosclerotic lesion development in *ApoE*<sup>-/-</sup> 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<sup>+</sup> T cells can acquire characteristics of alternative lineages upon antigen restimulation<sup>12</sup>. For example, IL-12 can induce IL-10 production by Th1 cells and IFN- $\gamma$ /IL-10 co-producing T cells with regulatory functions have been reported in peripheral blood of healthy donors<sup>13</sup>. IFN- $\gamma$ /IL-10 co-producing T cells could also be generated after stimulation of Th17 cells in the presence of IL-12 or IL-27<sup>14</sup>. Recent findings suggest that FoxP3<sup>+</sup>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 CXCR3 can switch to a Th1 program and accumulate at sites of Th1 inflammatory responses<sup>15</sup>.

In this issue of *Circulation Research*, Li et al. report for the first time data showing that more than 40% of CD4<sup>+</sup> T cells in atherosclerotic aorta of *Apoe*<sup>-/-</sup> 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- $\gamma$ , but are CD25<sup>-</sup>. These so-called « CCR5<sup>+</sup>Teff » cells use CCR5 and its ligand CCL5 to home to the aorta and interact with CD11c<sup>+</sup> 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 employing Tregs with non-functional Foxp3, which demonstrated that not all Foxp3<sup>+</sup> T cells are functional Tregs, and that part of the Treg signature can be induced in the absence of FoxP3<sup>16</sup>. In their study, Li et al. have shown that Foxp3<sup>+</sup>CCR5<sup>+</sup>Teff were able to significantly reduce IFN- $\gamma$ , IL-4, IL-13, IL-17A production by effector T cells, but were unable to suppress effector T cell proliferation. This might be accounted for by the inability of CCR5<sup>+</sup>Teff to suppress IL-2 secretion. As the proliferation of FoxP3<sup>+</sup>CCR5<sup>+</sup>Teff cells themselves in the absence of effector T cells was not reported, it is difficult to classify this vascular-located T cell population as true effector T cells that should proliferate in response to coated anti-CD3 or when co-cultured with mature antigen-presenting cells, whereas regulatory T cells should not. IL-2 is required to induce CD25<sup>high</sup>Treg cell proliferation *in vitro*. So-called FoxP3<sup>+</sup>CCR5<sup>+</sup>Teff cells display some features of previously described Th1-like regulatory population that

co-expresses FoxP3, T-bet and IFN- $\gamma$ . In the context of airway hyper-reactivity, it has been shown that T-bet<sup>+</sup>FoxP3<sup>+</sup> T cells induced by CD8 $\alpha$ <sup>+</sup> dendritic cells can produce both IL-10 and IFN- $\gamma$ <sup>17</sup>. In the intestine, the conversion of FoxP3<sup>+</sup>Tregs into FoxP3<sup>+</sup>IFN- $\gamma$ <sup>+</sup> T cells, requiring IL-12 production by antigen-presenting cells, have also been described<sup>18</sup>. 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<sup>+</sup>CCR5<sup>+</sup>Teff cells into *Ccr5*<sup>-/-</sup>*Apoe*<sup>-/-</sup> 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 FoxP3<sup>+</sup>CCR5<sup>+</sup>Teff 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 mouse before, but had been previously reported in activated human T cells with unstable expression of FoxP3, which did not acquire suppressive function<sup>19</sup>. Li et al. clearly documented that FoxP3 expression was very low in CCR5<sup>+</sup>Teff cells, 5-6 fold lower than in CD4<sup>+</sup>CD25<sup>high</sup>Tregs. In human, Miyara et al. characterized Treg cell subsets according to their FoxP3 expression<sup>20</sup>. They identified 3 different populations: CD45RA<sup>+</sup>FoxP3<sup>low</sup> resting Treg cells, CD45RA<sup>-</sup>FoxP3<sup>high</sup> activated Treg cells, and cytokine-secreting CD45RA<sup>-</sup>FoxP3<sup>low</sup> non-Treg cells. Interestingly, the latter population had the same secretory and functional profile as the mouse CCR5<sup>+</sup>Teff identified by Li et al. They both produced IL-2, IFN- $\gamma$  and displayed no suppressive functions.

Li et al. also explored the role of CCR5 on CD4<sup>+</sup> T cells trafficking. Several studies have previously reported the pro-atherogenic role of CCR5, but most focused on monocytes. Pharmacological inhibition<sup>21</sup> or genetic invalidation of CCR5 significantly reduced monocyte infiltration 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 pro-atherogenic properties of the newly discovered CCR5<sup>+</sup>Teff cell population would have been more striking. They showed that the adoptive transfer of CCR5<sup>+</sup>Teff cells in *Ccr5*<sup>-/-</sup>*Apoe*<sup>-/-</sup> mice accelerated atherosclerosis. Yet, the same effect was observed following 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<sup>+</sup>Teff 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<sup>+</sup>Teff into atherosclerotic lesions. Finally, interestingly, CCR5<sup>+</sup>Teff cells 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<sup>+</sup> Teff cells.

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

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### **Disclosures**

None

### **Figure Legend**

Almost all of the CCR5<sup>+</sup> T cells in aortas from *Apoe*<sup>-/-</sup> mice coexpress IFN- $\gamma$ , T-bet and low level of FoxP3. These « CCR5<sup>+</sup>Teff » cells are concentrated in the aorta, with some making their way to the para-aortic lymph nodes. They produce high levels of IFN- $\gamma$ , TNF $\alpha$ , IL-10 and IL-2, reduce the secretion of IFN $\gamma$ , IL-4, IL-13, IL-6 and IL17A (but not IL-2 or IL-5), by effector CD4<sup>+</sup>CD25<sup>-</sup>T cells, but do not suppress the proliferation of the latter. Natural CD4<sup>+</sup>CD25<sup>hi</sup>FoxP3<sup>+</sup>Tregs inhibit effector T cell proliferation and cytokine secretion.

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