Regulatory T cell plasticity: another layer of complexity in atherosclerosis

Hafid Ait-Oufella, Alain Tedgui

Regulatory T cell plasticity: another layer of complexity in atherosclerosis

Hafid Ait-Oufella[1,2]& Alain Tedgui[1]

1- INSERM UMR-S 970, Paris Cardiovascular Research Center – PARCC, Université Paris Descartes, Sorbonne Paris Cité, France

2- AP-HP (Assistance Publique-Hôpitaux de Paris), Hôpital Saint-Antoine, UPMC Université Paris 06, France

Correspondence

Alain Tedgui

UMR-S 970 Paris Cardiovascular Research Center – PARCC

56 rue Leblanc, 75015 Paris, France

Phone: +33 1 53 98 80 06

Fax: + 33 1 53 98 80 52

alain.tedgui@inserm.fr

Total word count: 2155

During the last 2 decades, human and animals studies have clearly documented the crucial role of inflammation in the development and complications of atherosclerosis [1]. 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 $CD3^+$ lymphocytes in human and mouse atherosclerotic lesions. Most of T cells in mouse and human atherosclerotic plaques are $CD4^+$ T-helper(Th) cells expressing the αβ T-cell antigen receptor (TCR).Among $CD4^+$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[1]. 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 transcriptionfactor T-bet and stimulatesthe production of the Th1 prototypical cytokine IFN-γ[2]. Tregshave a central role in the dominant control of immunologicaltolerance and maintenance of immune homeostasis. They were first identified in miceand later in humans[3]. The transcription factor FoxP3 is essential for the generation and the functions ofTregs[4]. 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[5]. Natural or thymic $FoxP3^+$ Tregs acquire regulatory lineage commitment already upon maturation in the thymus [3], whereas adaptive or peripheral $Foxp3^+$ Tregs can be induced from mature $CD4^+$Thcells in the periphery under the influence of different stimulations, especially TGF-β[6]. 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 [7]. 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 [8,9]. 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[10], all concluded to increased vascular inflammation and atherosclerosis in the absence of Tregs. In contrast, adoptive transfer of $CD4^+CD25^{high}$ Treg cells [8]or IL-10 producing Tr-1 cells [11]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 toa specific Th lineage are notexclusively terminally differentiated cells, but that some maintain a certain degree of plasticity. Mature $CD4^+$ T cells can acquire characteristicsof alternative lineages upon antigen restimulation[12]. For example, IL-12can induce IL-10 production by Th1 cells and IFN-γ/IL-10 co-producing T cells with regulatory functions have been reported in peripheral blood of healthy donors[13]. IFN-γ/IL-10 co-producing T cells could also be generated after stimulation of Th17 cells in the presence of IL-12 or IL-27 [14]. Recent findings suggest that $FoxP3^+$Tregsalso show functional and phenotypic plasticity, beingable to secrete proinflammatory cytokines. For instance, Tregs expressing the T-bet and the Th1-associated chemokine receptor CXCR3 canswitch to a Th1 program and accumulate at sites of Th1 inflammatory responses[15].

In this issue of *Circulation Research*, Li et al. report for the first time data showingthat more than40%of $CD4^+$ T cellsin atherosclerotic aorta of *Apoe*$^{-/-}$mice under high fat dietexpress CCR5and exhibit a unique repertoire of molecular and cell surface markers, including positivity for FoxP3,T-bet, and IFN-γ, but are CD25negative.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 issupported by studies employing Tregs with non-functional 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[16]. In their study, Li et al. have shown that $Foxp3^+$CCR5Teff were able to significantly reduce IFN-γ, IL-4, IL-13, Il-17A production by effector T cells,but were unable to suppress effector T cell proliferation.This might be accounted by theinability of CCR5Teff to suppress IL-2 secretion. As the proliferation of $FoxP3^+$CCR5Teff cells themselves in the absence of effector T cells was not reported, it is difficult to classifythis vascular-located T cell population as true effector T cells that shouldproliferate in response to coated anti-CD3 or when co-cultured with mature antigen-presenting cells, whereas regulatory T cellsshould not. IL-2 is required to induce $CD25^{high}$Treg cell proliferation *in vitro*. So-called $FoxP3^+$CCR5 Teff cells displaysome features of previously described Th1-like regulatory population that

co-expresses FoxP3, T-bet andIFN-γ. In the context of airway hyper-reactivity, it has been shown that T-bet^{+}FoxP-3^{+} T cells induced by CD8α^{+} dendritic cells canproduce both IL-10 and IFN-γ [17]. 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 [18]. 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^{+}CCR5Teff cells into *Ccr5*$^{-/-}$*Apoe*$^{-/-}$micewas not protective, and waseven able to accelerate atherosclerosis to the same extentas "conventional" effector T cells.Therefore,*in vitro* and *in vivo* experiments rule out the possibility that FoxP3^{+}CCR5Teff cells are Th1-like Tregs, and suggest thatthese cellsdisplay 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, whichdid notacquire suppressive function[19].Li et al. clearly documentedthat FoxP3 expression was very low in CCR5Teff cells, 5-6 fold lower thanin CD4^{+}CD25^{high}Tregs. In human, Miyara et al. characterized Treg cellsubsets according to theirFoxP3 expression[20]. They identified 3 different populations: CD45RA^{+}FoxP3^{low} resting Treg cells, CD45RA^{-}FoxP3^{high} activated Treg cells, and cytokine-secreting CD45RA^{-}FoxP3^{low} non-Treg cells. Interestingly, the latter population had the same secretive and functional profileas the mouse CCR5Teff identified by Li et al. They both produced IL-2, IFN-γ and displayed no suppressive functions.

Li et al. alsoexplored the role ofCCR5 onCD4^{+} T cells trafficking.Several studies have previously reported the pro-atherogenic role of CCR5, but most focused on monocytes. Pharmacological inhibition[21]or genetic invalidation ofCCR5significantly reduced monocytosis 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-CCR5pathway in T cellsreduced their homing into explanted aorta, but*in vivo* evidence for specific migratory and pro-atherogenic properties of the newly discoveredCCR5^{+}Teff cell populationwouldhave been more striking.They showed that the adoptive transfer of CCR5Teffcells in *Ccr5*$^{-/-}$*Apoe*$^{-/-}$miceaccelerated atherosclerosis. Yet, the same effect was observed following the adoptive transfer of "conventional" effector T cells that expressed CCR5 at amuch lower level. It would have been of great interest to see whether thenumber of CCR5^{+}Teff cells that accumulatedin the vascular wall was higher than that

of“conventional” effector T cells, which would strongly argue in favor ofa powerfulmigratory propertyof CCR5^{+}Teff into atherosclerotic lesions. Finally,interestingly,CCR5^{+}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 todetermine the specific stimulatory pathway, antigen-dependent or antigen-independent,whichwas involved in CCR5 expression on CCR5^{+} Teff cells.

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

Sources of funding

INSERM

Disclosures

None

Figure Legend

Almost all of the CCR5^{+} T cells in aortas from *Apoe*$^{-/-}$mice coexpress IFN-γ, T-bet and low level of FoxP3. These« CCR5Teff » cells are concentrated in the aorta, with some making their way to the para-aortic lymph nodes. They produce high levels of IFN-γ, TNFα, IL-10 and IL-2, reducethe secretion of IFNγ, 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^{hi}FoxP3^{+}Tregsinhibit effector Tcell proliferation and cytokine secretion.

References

1. Ait-Oufella H, Sage AP, Mallat Z, Tedgui A. Adaptive (T and B cells) immunity and control by dendritic cells in atherosclerosis. *Circ Res*. 2014;114:1640-1660.
2. Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell*. 2000;100:655-669.
3. Sakaguchi S. Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. *Nat Immunol.* 2005;6:345-352.
4. Fontenot JD, Rasmussen JP, Williams LM, Dooley JL, Farr AG, Rudensky AY. Regulatory T cell lineage specification by the forkhead transcription factor foxp3. *Immunity*. 2005;22:329-341.
5. Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, Kelly TE, Saulsbury FT, Chance PF, Ochs HD. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat Genet*. 2001;27:20-21.
6. Fantini MC, Becker C, Tubbe I, Nikolaev A, Lehr HA, Galle P, Neurath MF. Transforming growth factor beta induced FoxP3+ regulatory T cells suppress Th1 mediated experimental colitis. *Gut*. 2006;55:671-680.
7. Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, Roncarolo MG. A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature*. 1997;389:737-742.
8. Ait-Oufella H, Salomon BL, Potteaux S, et al. Natural regulatory T cells control the development of atherosclerosis in mice. Nat Med. 2006;12:178-180.
9. Gotsman I, Grabie N, Gupta R, Dacosta R, MacConmara M, Lederer J, Sukhova G, Witztum JL, Sharpe AH, Lichtman AH. Impaired regulatory T-cell response and enhanced atherosclerosis in the absence of inducible costimulatory molecule. *Circulation*. 2006;114:2047-2055.
10. Klingenberg R, Gerdes N, Badeau RM, et al. Depletion of FOXP3+ regulatory T cells promotes hypercholesterolemia and atherosclerosis. *J Clin Invest*. 2013:123:1323-1334.
11. Mallat Z, Gojova A, Brun V, Esposito B, Fournier N, Cottrez F, Tedgui A, Groux H. Induction of a regulatory T cell type 1 response reduces the development of atherosclerosis in apolipoprotein E-knockout mice. *Circulation*. 2003;108:1232-1237.
12. Geginat J, Paroni M, Maglie S, Alfen JS, Kastirr I, Gruarin P, De Simone M, Pagani M, Abrignani S. Plasticity of human CD4 T cell subsets. *Front Immunol*. 2014;5:630.
13. Haringer B, Lozza L, Steckel B, Geginat J. Identification and characterization of IL-10/IFN-gamma-producing effector-like T cells with regulatory function in human blood. *J Exp Med*. 2009:206:1009-1017.
14. Heinemann C, Heink S, Petermann F, Vasanthakumar A, Rothhammer V, Doorduijn E, Mitsdoerffer M, Sie C, Prazeres da Costa O, Buch T, Hemmer B, Oukka M, Kallies A, Korn T. IL-27 and IL-12 oppose pro-inflammatory IL-23 in CD4+ T cells by inducing Blimp1. *Nat Commun.* 2014;5:3770.
15. Koch MA, Tucker-Heard G, Perdue NR, Killebrew JR, Urdahl KB, Campbell DJ. The transcription factor T-bet controls regulatory T cell homeostasis and function during type 1 inflammation. *Nat Immuno*l. 2009;10:595-602.
16. Ohkura N, Sakaguchi S. Treg cells acquire new directions, cytokines navigate. *Immunity*. 2012:37:443-444.
17. Stock P, Akbari O, Berry G, Freeman GJ, Dekruyff RH, Umetsu DT. Induction of T helper type 1-like regulatory cells that express Foxp3 and protect against airway hyper-reactivity. *Nat Immunol.* 2004;5:1149-1156.

18. Feng T, Cao AT, Weaver CT, Elson CO, Cong Y. Interleukin-12 converts Foxp3+ regulatory T cells to interferon-gamma-producing Foxp3+ T cells that inhibit colitis. *Gastroenterology*. 2011;140:2031-2043.
19. d'Hennezel E, Yurchenko E, Sgouroudis E, Hay V, Piccirillo CA. Single-cell analysis of the human T regulatory population uncovers functional heterogeneity and instability within FOXP3+ cells. J *Immunol*. 2011;186:6788-6797.
20. Miyara M, Yoshioka Y, Kitoh A, et al. Functional delineation and differentiation dynamics of human CD4+ T cells expressing the FoxP3 transcription factor. *Immunity*. 2009;30:899-911.
21. Combadiere C, Potteaux S, Rodero M, Simon T, Pezard A, Esposito B, Merval R, Proudfoot A, Tedgui A, Mallat Z. Combined inhibition of CCL2, CX3CR1 and CCR5 abrogates Ly6C(hi) and Ly6C(lo) monocytosis and almost abolishes atherosclerosis in hypercholesterolemic mice. *Circulation*. 2008;117:1649-1657.