Emerging Role of IL-17 in Atherosclerosis

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Key Words

Atherosclerosis · IL-17 · Innate and adaptive immunity

Abstract

The IL-23-IL-17 axis is emerging as a critical regulatory system that bridges the innate and adaptive arms of the immune system. Th17 cells have been linked to the pathogenesis of several chronic inflammatory and autoimmune diseases. However, the role of Th17 cells and IL-17 in various stages of atherogenesis remains poorly understood and is only beginning to be elucidated. While IL-17 is a predominantly proinflammatory cytokine, it has a pleiotropic function and it has been implicated both as an instigator in the pathogenesis of several inflammatory disorders as well as being protective in certain inflammatory disease models. Therefore, it is not surprising that the current literature is conflicting on the role of IL-17 during atherosclerotic lesion development. Various approaches have been used by several groups to discern the involvement of IL-17 in atherosclerosis. While one study found that IL-17 is protective against atherosclerosis, several other recent studies have suggested that IL-17 plays a proatherogenic role. Thus, the function of IL-17 remains controversial and awaits more direct studies to address the issue. In this review, we will highlight all the latest studies involving IL-17 and atherosclerosis, including both clinical and experimental research.

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Introduction

Atherosclerosis is a lipid-driven, chronic inflammatory disease of the vessel wall in which both innate and adaptive immune responses play a role [1]. Immune cells and their mediators directly cause the chronic arterial inflammation that is a hallmark of atherosclerosis. Macrophages, T lymphocytes and, to a lesser extent, mast cells contribute to the smoldering inflammatory response in the vessel wall [2, 3]. The array of cytokines implicated in atherosclerosis are strikingly similar to those used by immune effector cells to kill foreign pathogens and defective or diseased host cells [4]. Virtually every major cell lineage used in host defense has been identified in human and/or animal plaques [4–6].

Numerous genetic loss- or gain-of-function studies in animal models and other evidence shows that immune cell types are generally neither bystanders nor a consequence of plaque development, but instead directly participate in the disease. T cells are known to play a critical role during lesion development. Both Th1 and Th2 cells are involved in the process in a delicate interplay between the two cell types [7]. The recent addition of T helper 17 (Th17) cells to the mix has further complicated the roles each cell type might play during lesion development. The IL-23 (interleukin-23)-IL-17 axis is emerging as a critical regulatory system that bridges the innate and adaptive

immune systems. While IL-17 is predominantly a proinflammatory cytokine, it has pleiotropic and environment-specific functions. Indeed IL-17 was found to be both proinflammatory and protective for various inflammatory disease models, depending on the model and the environment it acts in [8–10].

While IL-17 has been reported to be important in the pathogenesis of several inflammatory disorders and has been linked to inflammatory bowel disease [11–13], others have reported that IL-17A mediates a protective effect on T cell-driven intestinal inflammation *in vivo* [8]. Since the Th1 axis plays a critical role in the development of atherosclerosis, and because the Th17 pathway can drive Th1 responses, one would expect that IL-17 would have a proatherogenic function. However, IL-17 has complex and multiple functions, including regulatory functions, and the role of IL-17A in atherosclerosis remains unclear. Recently, several papers have shown conflicting data: one study suggested a protective function for IL-17 in atherosclerosis [14], while several others showed a proatherogenic properties of IL-17 and Th17 T-cells [15, 16]. Here we summarize the current understanding of T lymphocytes, particularly Th17 cells in the development of atherosclerosis.

Progression of Atherosclerosis

The earliest sign of atherosclerosis is endothelial cell dysfunction, activation in response to oxidized lipids in the subendothelium, and expression of VCAM-1 (vascular cell-adhesion molecule 1) [17, 18], which triggers both the adhesion of leukocytes and migration of activated platelets into the endothelium by an increased permeability for plasma lipid components, such as low-density lipoprotein (LDL) [4, 19]. Monocytes and T cells bind to VCAM-1-expressing endothelial cells and migrate into the arterial tissue. The recruited monocytes then differentiate into macrophages and augment expression of many pattern-recognition receptors, including scavenger receptors [20, 21]. The macrophages accumulate cholesterol via scavenger receptors and become foam cells. This leads to intimal fatty-streak lesions. During this time, there is a chronic inflammatory response to these modified lipids [4].

The inflammatory response is mediated by components of the innate immune system, including macrophages and dendritic cells [22, 23] and by components of the adaptive immune system, including T lymphocytes [4, 24]. T cells become activated in response to antigen

and contribute to disease progression by producing proinflammatory mediators, which further amplifies the inflammatory response. The continued cell accumulation and the subsequent apoptosis of plaque cells lead to the formation of a necrotic core and result in the progressive narrowing of the arterial lumen.

The pro- and anti-inflammatory mediators regulate the magnitude of the inflammatory response within the plaque as well as plaque stability and the propensity for thrombus formation by modulating apoptosis, collagen production, and smooth muscle cell content. In the later stages of atherosclerosis, B cells and plasma cells also appear in the deeper layers of the plaque and in the adventitia [25]. Secretion of matrix proteases and cytokines by plaque cells can then trigger thinning of the fibrous cap and its disintegration with plaque erosion or rupture, leading to thrombus formation and vascular occlusion, which underlie coronary syndromes, myocardial infarction, and stroke.

Th1 Immunity in Atherosclerosis

It has been clearly shown that the adaptive immune system affects the development of atherosclerosis [22]. At present, Th1 cytokines are regarded as important proinflammatory regulators during atherosclerosis. Most of the T cells in atherosclerotic plaques are Th1 cells. In apolipoprotein E-deficient (apoE^{-/-}) or LDL receptor-deficient (LDLR^{-/-}) mice in combination with both T and B cell deficiencies, there is a significant reduction in early atherosclerotic lesion development [26, 27]. The principal Th1 cytokine, IFN- γ , is produced by most T cells in the human atherosclerotic plaque [28, 29]. Moreover, adoptive transfer of CD4⁺ T cells from apoE^{-/-} mice into apoE^{-/-} severe combined immunodeficiency mice enhances atherosclerosis [30]. The transplanted cells produced high levels of IFN- γ , suggesting a Th1-related proatherogenic effect. Atherosclerosis is attenuated in both IFN- γ -deficient and IFN- γ R-deficient mice [31–33], and injections of recombinant IFN- γ increase lesion size [34]. While IFN- γ injections lead to a decrease in serum cholesterol, this effect does not protect against the proatherogenic effect of IFN- γ [34].

IL-12 is the principal cytokine promoting Th1 development. IL-12 synergizes with IL-18 for full induction of IFN- γ . Recombinant IL-12 aggravates disease in apoE^{-/-} mice, concomitant with increased IFN- γ expression in the aorta [35]. Indeed, apoE^{-/-} IL-12p40^{-/-} mice exhibit reduced atherosclerotic plaque area [36]. apoE^{-/-} mice

Fig. 1. Complex interactions of CD4⁺ T cell subsets involved in atherosclerosis. SMC = Smooth muscle cell; EC = endothelium cell. * In mice, differentiation of Th17 cells is driven by TGF- β together with IL-6. In humans, Th17 differentiation requires TGF- β and IL-6, enhanced by IL-23, with IL-23 critically required for maintenance of the IL-17-secreting phenotype.

treated with plasmid DNA encoding for IL-18 binding protein [37] and apoE^{-/-} IL-18^{-/-} mice [38] exhibited reduced lesion development, whereas IL-18 treatment of apoE^{-/-} mice accelerated atherosclerosis development [39]. Thus, IL-18 was hypothesized to have proatherogenic effects.

Two major autoantigens, oxidized LDL and heat shock proteins (HSP), have been implicated in the pathogenesis of atherosclerosis [29, 40, 41]. After challenge with HSP, plaque-derived T cells expressed Th1 functions, became cytotoxic, and induced tissue factor production in mac-

rophages [41]. The results of the recent study by Matsuura et al. [42] also indicated that the systemic Th1-immune response against Hp-HSP60 induced by *Helicobacter pylori* infection in the gastric mucosa might promote atherosclerosis because elevated production of Th1-cytokines, such as IFN- γ and IL-12, was observed as well as expression of T-bet mRNA (Th1-polarization marker) [42]. Overall, these results provide convincing elements to incriminate Th1-related responses in the promotion of atherosclerotic plaque development and progression (fig. 1).

Th2 Cells

In contrast, Th2 cells produce mostly IL-4, IL-5, and IL-10, and are associated with eosinophilic inflammatory responses to helminths, ticks, and allergens. The Th2 axis inhibits the formation and differentiation of Th1 cells and vice versa. IL-5 deficiency in LDLR^{-/-} mice leads to enhanced lesion formation in a bone marrow transplantation model [43]. Thus, IL-5 acts in an anti-atherogenic fashion by stimulating the production of protective antibodies. The Th2 cytokine profile in mouse models of atherosclerosis is associated with increased production of 'protective' antioxidantized LDL antibodies [44]. Furthermore, splenectomy in cholesterol-fed apoE^{-/-} mice, which is associated with reduced levels of IgM and Th2-related IgG antioxidantized LDL antibodies, increases atherosclerosis [45]. In addition, promoting Th2 responses in mice with mild hypercholesterolemia results in a reduction of early fatty-streak formation [46]. However, LDLR^{-/-} mice reconstituted with IL-4-deficient bone marrow develop less severe atherosclerosis [47], particularly at the advanced stages of lesion progression [36]. This data indicate that IL-4 may be proatherogenic.

Th1 and Th2: Yin and Yang

The interplay between Th1 and Th2 cell types describes an attractive concept of yin and yang controlling the development of atherosclerosis [48, 49]. On the other hand, IL-12, the prototypical Th1 skewing cytokine commonly produced by cells in the atherosclerotic lesion, can block the effects of the Th2 skewing cytokine, thymic stromal lymphopoietin protein. This protein has been shown to be produced by smooth muscle cells in the lung and, thus, it is possible that the arterial smooth muscle cells might also produce this Th2 skewing cytokine [50].

T Regulatory Cells

Recently, CD4+CD25+ regulatory T cells (Tregs) and Th17 cells have been described as two distinct subsets of Th1 and Th2 cells. Tregs expressing the forkhead/winged helix transcription factor (Foxp3) have an anti-inflammatory role and maintain tolerance to self-components by contact-dependent suppression or releasing anti-inflammatory cytokines [IL-10 and transforming growth

factor (TGF)- β 1]. It has been demonstrated that TGF- β has an antiatherosclerotic effect by using TGF- β neutralizing antibodies [51], genetic deficiency in TGF- β [52], or soluble TGF- β receptors [53] in apoE^{-/-} mice. Reduction in atherosclerosis in apoE^{-/-} mice has also been achieved through adoptive transfer of CD4+CD25+ Tregs [54, 55]. The protective role of IL-10 in [56] atherosclerosis has also been shown in C57BL6 mice, apoE^{-/-} mice, and the LDLR^{-/-} mouse model [57–60]. The balance between Th17 and Tregs may be important in the development/prevention of inflammatory and autoimmune diseases [61] (fig. 1).

Th17 Axis

Recently a subset of CD4 T cells has been categorized into the Th17 lineage, which is distinct from the Th1 and Th2 lineages [62, 63]. Th17 cells differentiate from naive CD4+ T cells by TGF- β 1, and the inflammatory cytokine IL-6. IL-21 produced by Th17 cells promotes Th17 cell commitment in an autocrine manner, whereas IL-23 maintains and expands these cells in vivo. The transcription factors ROR γ t and ROR α can be induced by IL-6, IL-21, and IL-23 through Stat3 alone with TGF- β 1 [64–70]. Th17 cell development is controlled by transcription factors ROR γ t (human ortholog is RORC) and ROR α . It has been reported that LPS-stimulated dendritic cells are potent inducers of Th17 development [71]. In addition to IL-6 and TGF- β 1, inflammatory cytokines such as IL-1 and tumor necrosis factor (TNF) are contributors to murine Th17 differentiation. Although differentiation of Th17 cells in vitro can occur in the absence of IL-1, IL-1RI^{-/-} mice have impaired Th17 cell differentiation and reduced incidence of EAE associated with failure to induce autoantigen-specific Th17 cells [72]. It has been demonstrated that the activator protein 1 transcription factor Batf controls Th17 differentiation [73]. Batf^(-/-) mice have normal Th1 and Th2 differentiation, but show a defect in Th17 differentiation, and are resistant to experimental autoimmune encephalomyelitis. Batf^(-/-) T cells fail to express ROR γ t and IL-21 [66–68, 70].

Members of IL-17 Family Ligands

IL-17 and its related proteins have been cloned, grouped, and designated as the IL-17 cytokine family (IL-17A–F) [10, 63]. IL-17, which is primarily produced by

Th17 cells, coordinates local tissue inflammation via induced release of proinflammatory cytokines and neutrophil-mobilizing chemokines from various cell types, including epithelial cells [9]. IL-17 is involved in the pathogenesis of several autoimmune diseases such as rheumatoid arthritis, inflammatory bowel disease, and asthma. This cytokine may also contribute to host defense during microbial infections of the lung [74]. However, IL-23-mediated IL-17A has also been associated with a protective role in T cell-mediated intestinal inflammation [8]. Therefore, its effect on the surrounding tissues and local immune system may be environment-specific and probably depends on the overall immunological state at the time in specific disease models.

Thus far, six IL-17 family ligands (IL-17A through IL-17F) have been identified. All members of the IL-17 family share a similar protein structure characterized by four highly conserved cysteine residues, but they have no sequence similarity to any other known cytokines [75, 76]. IL-17E, more commonly called IL-25, has different biologic functions, but shares structural features with other IL-17 family members [77, 78]. IL-17A and IL-17F (which are 55% homologous) are both produced by Th17 cells and are the most characterized to date. The IL-17A and IL-17F genes are located adjacent to each other, suggesting the genes encoding IL-17A and IL-17F are the result of a duplication event. Moreover, the IL-17A and IL-17F transcription factor ROR γ t exhibits a similar expression pattern [79–81]. Both IL-17A and IL-17F act as homodimers, but an IL-17A/F heterodimer has been described recently [82–84]. Since IL-17A and IL-17F share common features, they may have a similar regulatory effect. They also induce common transducing pathways through a complex composed of the IL-17RA and IL-17RC receptors [85, 86]. Unlike IL-17A and IL-17F, IL-17E is involved in promoting Th2 immune responses [63]. Furthermore, IL-17E is known to play an important role during allergic airway hyper-reactivity reactions [87].

The IL-17 receptor (IL-17R) subfamily includes IL-17RA, IL-17RB, IL-17RC, IL-17RD, and IL-17RE reviewed in [10, 13]. IL-17RD is also known to have a similar expression as fibroblast growth factor receptor (SEF), as it was initially described as having the same expression pattern as the fibroblast growth factor receptor during zebrafish development [19]. The IL-17A receptor is ubiquitously expressed and the cytokine leads to pleiotropic activities, including induction of TNF- α , IL-1, and MCP-1, as well as adhesion molecules like intercellular adhesion molecule 1 (ICAM-1) [88].

IL-17/Th17 and Inflammation and Atherosclerosis

Th17 cells, characterized by production of the inflammatory cytokine IL-17, were shown to promote chronic inflammatory as well as autoimmune diseases, including experimental models of arthritis, autoimmune encephalitis, and colitis [89, 90]. While the role of IL-17 has been studied extensively in several inflammatory and autoimmune disorders, its role in atherosclerosis development remains controversial. Previously, only indirect clues existed for the potential role of IL-17 in atherogenesis. LDLR/IL-6 double KO mice, which exhibit a decrease of IL-17 levels [91], were found to have a modest reduction in atherosclerotic lesion development, suggesting a potential role for Th17 in the promotion of atherogenesis. Most importantly, a recent study demonstrated the concomitant presence of IL-17 and IFN- γ in clinical specimens of coronary atherosclerosis by showing the presence of IL-17/IFN- γ dual-producing T cells within coronary plaques and a synergistic effect of IL-17 and IFN- γ on elicitation of proinflammatory cytokine and chemokine production by cultured human vascular smooth muscle cells [92]. Another study reported that patients with acute coronary syndrome have significantly increased peripheral Th17 cell numbers, circulating Th17-related cytokine levels (IL-17, IL-6, and IL-23), increased expression of the transcription factor (ROR γ t), and a decrease in Treg numbers. Additionally, Treg-related cytokines (IL-10 and TGF- β 1), and the transcription factor Foxp3 were all reduced as compared with patients with stable angina and controls [93].

In contrast to these studies, Taleb et al. [14] recently published loss of suppressor of cytokine signaling-3 (SOCS-3) in mouse T cells increases both IL-17A and IL-10 production, inducing an anti-inflammatory macrophage phenotype which results in a reduction in lesion development and vascular inflammation. This suggests that IL-17 may have a protective role in atherogenesis. However, it was unclear as to whether IL-10 or IL-17 was leading to the suppressor phenotype, as IL-10 is a very potent regulatory cytokine which is atheroprotective. Additionally, these investigators have also shown that *in vivo* administration of rIL-17 to LDLR^{-/-} mice resulted in reduced endothelial VCAM-1 expression, as well as reduced vascular T cell infiltration and atherosclerotic lesion development [14]. These investigators concluded that endogenous expression of SOCS3 in T cells interrupts a major regulatory pathway in atherosclerosis through inhibition of IL-17 production and that IL-17 functions as an atheroprotective cytokine [14].

Interestingly, several more recent studies by various investigators have now shown additional, and in some cases more direct, evidence for the proatherogenic role of IL-17. Xie et al. [94] demonstrated that apoE^{-/-} mice express a significant increase in Th17-related cytokines (IL-17 and IL-6) and transcription factor (RORγt) levels and a concomitant decrease in Treg numbers, Treg-related cytokines (TGF-β1), and transcription factor (Foxp3) levels as compared with age-matched C57BL/6J mice. This indicates that a Th17/Treg functional imbalance exists during atherogenesis in apoE^{-/-} mice [94]. Another recent study found that different IL-17 family members (IL-17A, IL-17F, and IL-17E) are expressed in human atherosclerotic lesions [95]. The major source of proinflammatory cytokines, IL-17A and IL-17F, were not T cells (Th17), but mast cells and neutrophils [95]. Furthermore, the anti-inflammatory cytokine IL-17E was found to be expressed in normal and atherosclerotic vessels, and might play a role in regulating inflammatory processes in the vessel wall [95]. In another recent study investigating the apoE/IL18 double KO mice, investigators have shown that the exacerbated atherosclerotic lesion formation correlated with increased Th17 cells, IL-23-producing vascular smooth muscle cells and macrophages, and a thin fibrous cap in lesions, a morphology indicative of unstable plaques prone to rupture [96].

The most direct evidence of the proatherogenic role of IL-17 comes from four very recent studies. van Es et al. [15] conducted bone marrow chimera studies and transplanted irradiated LDLR-deficient recipient mice with IL-17R-deficient bone marrow. They observed that Western-type diet-induced atherosclerotic lesions were reduced by 46% in the aortic root and plaque in the recipient mice.

Erbel et al. [16] administered in vivo IL-17A-blocking antibody in apoE^{-/-} mice, and found that functional blockade of IL-17A reduced atherosclerotic lesion development and decreased plaque vulnerability, cellular infiltration, and tissue activation in apoE-deficient mice. They concluded that the involvement of IL-17 in proatherogenesis appears to be via proinflammatory changes at multiple levels such as cell adhesion, extravasation, cell activation, T cell (co)stimulation/proliferation, and Ag presentation in the inflammatory cascade of atherosclerosis [16]. A limitation of this study is that blocking IL-17A will not prevent the formation of Th17 cells, or other IL-17 producing cell types, which may also have effects aside from IL-17A production [63, 97, 98].

In the most recent Keystone Symposia (Advances in Molecular Mechanisms of Atherosclerosis, February

2010), Smith et al. [99] showed that IL-17A⁺ T cells were significantly increased in the aortas and surrounding adventitia, spleen, and lamina propria of aged apoE^{-/-} mice compared to the control C57Bl6 mice. The IL-17A⁺ cells were characterized as CD45⁺CD3⁺ T cells and were predominantly Th17 and γδ⁺ T cells. Blockade of IL-17A in apoE^{-/-} mice using adenovirus-produced soluble IL-17R-reduced plaque in treated apoE^{-/-} mice fed a Western diet resulted in significant reduction of atherosclerotic lesions [99].

Nagarajan et al. [100] also demonstrated that apoE/Fcγ chain double KO mice had a 50% reduction of aortic lesion compared to apoE^{-/-} mice, and the double KO mice had high IFN-γ and IL-17, but lower IL-10 and TGF-β expression. In addition to all these studies, we have shown in the same Keystone Meetings that IL-17A^{-/-} mice (on a C57/Bl6 background) develop significantly smaller atherosclerotic lesion size and less lipid deposition in the aorta and aortic sinus following 12 weeks of a high-fat diet (cholate) compared to wild-type C57Bl/6 mice [101].

Concluding Remarks

Although the precise role of IL-17 in atherosclerosis remains controversial, recent studies have begun to provide more direct evidence that IL-17 seems to be predominantly proatherogenic. Nevertheless, IL-17 clearly has pleiotropic and environment-specific protective and regulatory functions. Hence, the role of IL-17 in various stages of lesion development are still unknown and probably complex. It will therefore be important to further investigate this intersection between more traditional immunology and vascular biology, and to address and resolve questions raised by conflicting studies. Additionally, the effects of other isoforms of IL-17 (e.g. IL-17F or IL-17E) have not even begun to be investigated. This research area will continue to provide many opportunities for investigations that will significantly expand our understanding of how specific immune cells and general immune paradigms, such as Th1, Th2, and Th17, influence the development of atheroma and its clinical sequelae.

Acknowledgements

The authors are supported by grants from NIH HL66436 and AI 067995 to M.A. and AHA 09BGIA2060145 to S.C.

References

- Hansson GK, Libby P, Schonbeck U, Yan ZQ: Innate and adaptive immunity in the pathogenesis of atherosclerosis. *Circ Res* 2002;91: 281–291.
- van der Wal AC, Das PK, Bentz van de Berg D, van der Loos CM, Becker AE: Atherosclerotic lesions in humans. In situ immunophenotypic analysis suggesting an immune mediated response. *Lab Invest* 1989;61:166–170.
- Kovanen PT, Kaartinen M, Paavonen T: Infiltrates of activated mast cells at the site of coronary atheromatous erosion or rupture in myocardial infarction. *Circulation* 1995;92: 1084–1088.
- Hansson GK: Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 2005;352:1685–1695.
- Vanderlaan PA, Reardon CA: Thematic review series: the immune system and atherogenesis. The unusual suspects: an overview of the minor leukocyte populations in atherosclerosis. *J Lipid Res* 2005;46:829–838.
- Wick G, Knoflach M, Xu Q: Autoimmune and inflammatory mechanisms in atherosclerosis. *Annu Rev Immunol* 2004;22:361–403.
- Coffman RL: Origins of the t(h)1-t(h)2 model: a personal perspective. *Nat Immunol* 2006;7:539–541.
- O'Connor W Jr, Kamanaka M, Booth CJ, Town T, Nakae S, Iwakura Y, Kolls JK, Flavell RA: A protective function for interleukin 17A in T cell-mediated intestinal inflammation. *Nat Immunol* 2009;10:603–609.
- Kolls JK, Linden A: Interleukin-17 family members and inflammation. *Immunity* 2004;21:467–476.
- Moseley TA, Haudenschild DR, Rose L, Reddi AH: Interleukin-17 family and IL-17 receptors. *Cytokine Growth Factor Rev* 2003; 14:155–174.
- Niess JH, Leithauser F, Adler G, Reimann J: Commensal gut flora drives the expansion of proinflammatory CD4 T cells in the colonic lamina propria under normal and inflammatory conditions. *J Immunol* 2008;180: 559–568.
- Ivanov, IL, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV, Tanoue T, Imaoka A, Itoh K, Takeda K, Umesaki Y, Honda K, Littman DR: Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 2009;139:485–498.
- Kullberg MC, Jankovic D, Feng CG, Hue S, Gorelick PL, McKenzie BS, Cua DJ, Powrie F, Cheever AW, Maloy KJ, Sher A: IL-23 plays a key role in *Helicobacter hepaticus*-induced T cell-dependent colitis. *J Exp Med* 2006;203: 2485–2494.
- Taleb S, Romain M, Ramkhalawon B, Uyttenhove C, Pasterkamp G, Herbin O, Esposito B, Perez N, Yasukawa H, Van Snick J, Yoshimura A, Tedgui A, Mallat Z: Loss of SOCS3 expression in T cells reveals a regulatory role for interleukin-17 in atherosclerosis. *J Exp Med* 2009;206:2067–2077.
- van Es T, van Puijvelde GH, Ramos OH, Segers FM, Joosten LA, van den Berg WB, Michon IM, de Vos P, van Berkel TJ, Kuiper J: Attenuated atherosclerosis upon IL-17R signaling disruption in LDLr deficient mice. *Biochem Biophys Res Commun* 2009;388: 261–265.
- Erbel C, Chen L, Bea F, Wangler S, Celik S, Lasitschka F, Wang Y, Bockler D, Katus HA, Dengler TJ: Inhibition of IL-17A attenuates atherosclerotic lesion development in apoE-deficient mice. *J Immunol* 2009;183:8167–8175.
- Cybulsky MI, Gimbrone MA Jr: Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. *Science* 1991;251:788–791.
- Nakashima Y, Raines EW, Plump AS, Breslow JL, Ross R: Upregulation of VCAM-1 and ICAM-1 at atherosclerosis-prone sites on the endothelium in the ApoE-deficient mouse. *Arterioscler Thromb Vasc Biol* 1998;18:842–851.
- Ross R: Atherosclerosis – an inflammatory disease. *N Engl J Med* 1999;340:115–126.
- Smith JD, Trojan E, Ginsberg M, Grigaux C, Tian J, Miyata M: Decreased atherosclerosis in mice deficient in both macrophage colony-stimulating factor (op) and apolipoprotein E. *Proc Natl Acad Sci USA* 1995;92: 8264–8268.
- Edfeldt K, Swedenborg J, Hansson GK, Yan ZQ: Expression of toll-like receptors in human atherosclerotic lesions: a possible pathway for plaque activation. *Circulation* 2002; 105:1158–1161.
- Binder CJ, Chang MK, Shaw PX, Miller YI, Hartvigsen K, Dewan A, Witztum JL: Innate and acquired immunity in atherogenesis. *Nat Med* 2002;8:1218–1226.
- Yan ZQ, Hansson GK: Innate immunity, macrophage activation, and atherosclerosis. *Immunol Rev* 2007;219:187–203.
- Hansson GK: Immune mechanisms in atherosclerosis. *Arterioscler Thromb Vasc Biol* 2001;21:1876–1890.
- Houtkamp MA, van Der Wal AC, de Boer OJ, van Der Loos CM, de Boer PA, Moorman AF, Becker AE: Interleukin-15 expression in atherosclerotic plaques: An alternative pathway for T-cell activation in atherosclerosis? *Arterioscler Thromb Vasc Biol* 2001;21:1208–1213.
- Dansky HM, Charlton SA, Harper MM, Smith JD: T and B lymphocytes play a minor role in atherosclerotic plaque formation in the apolipoprotein E-deficient mouse. *Proc Natl Acad Sci USA* 1997;94:4642–4646.
- Daugherty A, Pure E, Delfel-Butteiger D, Chen S, Leferovich J, Roselaar SE, Rader DJ: The effects of total lymphocyte deficiency on the extent of atherosclerosis in apolipoprotein E^{-/-} mice. *J Clin Invest* 1997;100:1575–1580.
- Hansson GK, Holm J, Jonasson L: Detection of activated T lymphocytes in the human atherosclerotic plaque. *Am J Pathol* 1989; 135:169–175.
- Stemme S, Faber B, Holm J, Wiklund O, Witztum JL, Hansson GK: T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. *Proc Natl Acad Sci USA* 1995;92:3893–3897.
- Zhou X, Nicoletti A, Elhage R, Hansson GK: Transfer of CD4(+) T cells aggravates atherosclerosis in immunodeficient apolipoprotein E knockout mice. *Circulation* 2000;102: 2919–2922.
- Buono C, Come CE, Stavrakis G, Maguire GF, Connelly PW, Lichtman AH: Influence of interferon-gamma on the extent and phenotype of diet-induced atherosclerosis in the LDLr-deficient mouse. *Arterioscler Thromb Vasc Biol* 2003;23:454–460.
- Gupta S, Pablo AM, Jiang X, Wang N, Tall AR, Schindler C: IFN-gamma potentiates atherosclerosis in ApoE knockout mice. *J Clin Invest* 1997;99:2752–2761.
- Whitman SC, Ravisankar P, Daugherty A: IFN-gamma deficiency exerts gender-specific effects on atherogenesis in apolipoprotein E^{-/-} mice. *J Interferon Cytokine Res* 2002;22:661–670.
- Whitman SC, Ravisankar P, Elam H, Daugherty A: Exogenous interferon-gamma enhances atherosclerosis in apolipoprotein E^{-/-} mice. *Am J Pathol* 2000;157:1819–1824.
- Lee TS, Yen HC, Pan CC, Chau LY: The role of interleukin 12 in the development of atherosclerosis in ApoE-deficient mice. *Arterioscler Thromb Vasc Biol* 1999;19:734–742.
- Davenport P, Tipping PG: The role of interleukin-4 and interleukin-12 in the progression of atherosclerosis in apolipoprotein E-deficient mice. *Am J Pathol* 2003;163: 1117–1125.
- Mallat Z, Corbaz A, Scoazec A, Graber P, Alouani S, Esposito B, Humbert Y, Chvatchko Y, Tedgui A: Interleukin-18/interleukin-18 binding protein signaling modulates atherosclerotic lesion development and stability. *Circ Res* 2001;89:E41–E45.
- Elhage R, Jawien J, Rudling M, Ljunggren HG, Takeda K, Akira S, Bayard F, Hansson GK: Reduced atherosclerosis in interleukin-18 deficient apolipoprotein E-knockout mice. *Cardiovasc Res* 2003;59:234–240.
- Whitman SC, Ravisankar P, Daugherty A: Interleukin-18 enhances atherosclerosis in apolipoprotein E(-/-) mice through release of interferon-gamma. *Circ Res* 2002;90:E34–E38.

- 40 Xu Q, Kleindienst R, Waitz W, Dietrich H, Wick G: Increased expression of heat shock protein 65 coincides with a population of infiltrating T lymphocytes in atherosclerotic lesions of rabbits specifically responding to heat shock protein 65. *J Clin Invest* 1993;91:2693–2702.
- 41 Benagiano M, D'Elia MM, Amedei A, Azzurri A, van der Zee R, Ciervo A, Rombola G, Romagnani S, Cassone A, Del Prete G: Human 60-kDa heat shock protein is a target autoantigen of T cells derived from atherosclerotic plaques. *J Immunol* 2005;174:6509–6517.
- 42 Matsuura E, Kobayashi K, Matsunami Y, Shen L, Quan N, Makarova M, Suchkov SV, Ayada K, Oguma K, Lopez LR: Autoimmunity, infectious immunity, and atherosclerosis. *J Clin Immunol* 2009;29:714–721.
- 43 Binder CJ, Hartvigsen K, Chang MK, Miller M, Broide D, Palinski W, Curtiss LK, Corr M, Witztum JL: IL-5 links adaptive and natural immunity specific for epitopes of oxidized LDL and protects from atherosclerosis. *J Clin Invest* 2004;114:427–437.
- 44 Zhou X, Paulsson G, Stemme S, Hansson GK: Hypercholesterolemia is associated with a T helper (Th) 1/Th2 switch of the autoimmune response in atherosclerotic apo E-knockout mice. *J Clin Invest* 1998;101:1717–1725.
- 45 Caligiuri G, Nicoletti A, Poirier B, Hansson GK: Protective immunity against atherosclerosis carried by B cells of hypercholesterolemic mice. *J Clin Invest* 2002;109:745–753.
- 46 Huber SA, Sakkinen P, David C, Newell MK, Tracy RP: T helper-cell phenotype regulates atherosclerosis in mice under conditions of mild hypercholesterolemia. *Circulation* 2001;103:2610–2616.
- 47 King VL, Szilvassy SJ, Daugherty A: Interleukin-4 deficiency decreases atherosclerotic lesion formation in a site-specific manner in female LDL receptor^{-/-} mice. *Arterioscler Thromb Vasc Biol* 2002;22:456–461.
- 48 Daugherty A, Rateri DL: T lymphocytes in atherosclerosis: the yin-yang of Th1 and Th2 influence on lesion formation. *Circ Res* 2002;90:1039–1040.
- 49 Abbas AK, Murphy KM, Sher A: Functional diversity of helper T lymphocytes. *Nature* 1996;383:787–793.
- 50 He R, Oyoshi MK, Garibyan L, Kumar L, Ziegler SF, Geha RS: TSLP acts on infiltrating effector T cells to drive allergic skin inflammation. *Proc Natl Acad Sci USA* 2008;105:11875–11880.
- 51 Mallat Z, Gojova A, Marchiol-Fournigault C, Esposito B, Kamate C, Merval R, Fradelizi D, Tedgui A: Inhibition of transforming growth factor-beta signaling accelerates atherosclerosis and induces an unstable plaque phenotype in mice. *Circ Res* 2001;89:930–934.
- 52 Grainger DJ, Mosedale DE, Metcalfe JC, Bottinger EP: Dietary fat and reduced levels of TGFbeta1 act synergistically to promote activation of the vascular endothelium and formation of lipid lesions. *J Cell Sci* 2000;113:2355–2361.
- 53 Lutgens E, Gijbels M, Smook M, Heeringa P, Gotwals P, Kotliansky VE, Daemen MJ: Transforming growth factor-beta mediates balance between inflammation and fibrosis during plaque progression. *Arterioscler Thromb Vasc Biol* 2002;22:975–982.
- 54 Mor A, Planer D, Luboshits G, Afek A, Metzger S, Chajek-Shaul T, Keren G, George J: Role of naturally occurring CD4⁺ CD25⁺ regulatory T cells in experimental atherosclerosis. *Arterioscler Thromb Vasc Biol* 2007;27:893–900.
- 55 Ait-Oufella H, Salomon BL, Potteaux S, Robertson AK, Gourdy P, Zoll J, Merval R, Esposito B, Cohen JL, Fisson S, Flavell RA, Hansson GK, Klatzmann D, Tedgui A, Mallat Z: Natural regulatory T cells control the development of atherosclerosis in mice. *Nat Med* 2006;12:178–180.
- 56 Sakaguchi S, Ono M, Setoguchi R, Yagi H, Hori S, Fehervari Z, Shimizu J, Takahashi T, Nomura T: Foxp3⁺ CD25⁺ CD4⁺ natural regulatory T cells in dominant self-tolerance and autoimmune disease. *Immunol Rev* 2006;212:8–27.
- 57 Mallat Z, Besnard S, Duriez M, Deleuze V, Emmanuel F, Bureau MF, Soubrier F, Esposito B, Duez H, Fievet C, Staels B, Duverger N, Scherman D, Tedgui A: Protective role of interleukin-10 in atherosclerosis. *Circ Res* 1999;85:e17–e24.
- 58 Pinderski Oslund LJ, Hedrick CC, Olvera T, Hagenbaugh A, Territo M, Berliner JA, Fyfe AI: Interleukin-10 blocks atherosclerotic events in vitro and in vivo. *Arterioscler Thromb Vasc Biol* 1999;19:2847–2853.
- 59 Caligiuri G, Rudling M, Ollivier V, Jacob MP, Michel JB, Hansson GK, Nicoletti A: Interleukin-10 deficiency increases atherosclerosis, thrombosis, and low-density lipoproteins in apolipoprotein e knockout mice. *Mol Med* 2003;9:10–17.
- 60 Von Der Thüsen JH, Kuiper J, Fekkes ML, De Vos P, Van Berkel TJ, Biessen EA: Attenuation of atherogenesis by systemic and local adenovirus-mediated gene transfer of interleukin-10 in LDLR^{-/-} mice. *FASEB J* 2001;15:2730–2732.
- 61 Homey B: After Th1/Th2 now comes Treg/Th17: Significance of T helper cells in immune response organization (in German). *Hautarzt* 2006;57:730–732.
- 62 Stockinger B, Veldhoen M: Differentiation and function of Th17 T cells. *Curr Opin Immunol* 2007;19:281–286.
- 63 Weaver CT, Hatton RD, Mangan PR, Harrington LE: IL-17 family cytokines and the expanding diversity of effector T cell lineages. *Annu Rev Immunol* 2007;25:821–852.
- 64 Chen Z, Laurence A, Kanno Y, Pacher-Zavitsin M, Zhu BM, Tato C, Yoshimura A, Henninghausen L, O'Shea JJ: Selective regulatory function of SOCS3 in the formation of IL-17-secreting T cells. *Proc Natl Acad Sci USA* 2006;103:8137–8142.
- 65 Dong C: Th17 cells in development: an updated view of their molecular identity and genetic programming. *Nat Rev Immunol* 2008;8:337–348.
- 66 Korn T, Bettelli E, Gao W, Awasthi A, Jager A, Strom TB, Oukka M, Kuchroo VK: IL-21 initiates an alternative pathway to induce proinflammatory T(h)17 cells. *Nature* 2007;448:484–487.
- 67 Nurieva R, Yang XO, Martinez G, Zhang Y, Panopoulos AD, Ma L, Schluns K, Tian Q, Watowich SS, Jetten AM, Dong C: Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. *Nature* 2007;448:480–483.
- 68 Wei L, Laurence A, Elias KM, O'Shea JJ: IL-21 is produced by Th17 cells and drives IL-17 production in a STAT3-dependent manner. *J Biol Chem* 2007;282:34605–34610.
- 69 Yang L, Anderson DE, Baecher-Allan C, Hastings WD, Bettelli E, Oukka M, Kuchroo VK, Hafler DA: IL-21 and TGF-beta are required for differentiation of human T(h)17 cells. *Nature* 2008;454:350–352.
- 70 Zhou L, Ivanov II, Spolski R, Min R, Shenderov K, Egawa T, Levy DE, Leonard WJ, Littman DR: IL-6 programs T(h)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nat Immunol* 2007;8:967–974.
- 71 Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B: TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity* 2006;24:179–189.
- 72 Sutton C, Brereton C, Keogh B, Mills KH, Lavelle EC: A crucial role for interleukin (IL)-1 in the induction of IL-17-producing T cells that mediate autoimmune encephalomyelitis. *J Exp Med* 2006;203:1685–1691.
- 73 Schraml BU, Hildner K, Ise W, Lee WL, Smith WA, Solomon B, Sahota G, Sim J, Mukasa R, Cemerski S, Hatton RD, Stormo GD, Weaver CT, Russell JH, Murphy TL, Murphy KM: The AP-1 transcription factor Batf controls T(H)17 differentiation. *Nature* 2009;460:405–409.
- 74 Tesmer LA, Lundy SK, Sarkar S, Fox DA: Th17 cells in human disease. *Immunol Rev* 2008;223:87–113.
- 75 Huang SH, Frydas S, Kempuraj D, Barbacane RC, Grilli A, Boucher W, Letourneau R, Madhappan B, Papadopolou N, Verna N, De Lutiis MA, Iezzi T, Riccioni G, Theoharides TC, Conti P: Interleukin-17 and the interleukin-17 family member network. *Allergy Asthma Proc* 2004;25:17–21.

- 76 Gaffen SL, Kramer JM, Yu JJ, Shen F: The IL-17 cytokine family. *Vitam Horm* 2006;74:255–282.
- 77 Lee J, Ho WH, Maruoka M, Corpuz RT, Baldwin DT, Foster JS, Goddard AD, Yansura DG, Vandlen RL, Wood WI, Gurney AL: IL-17E, a novel proinflammatory ligand for the IL-17 receptor homolog IL-17Rh1. *J Biol Chem* 2001;276:1660–1664.
- 78 Fort MM, Cheung J, Yen D, Li J, Zurawski SM, Lo S, Menon S, Clifford T, Hunte B, Lesley R, Muchamuel T, Hurst SD, Zurawski G, Leach MW, Gorman DM, Rennick DM: IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. *Immunity* 2001;15:985–995.
- 79 Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, Cua DJ, Littman DR: The orphan nuclear receptor ROR γ mat directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell* 2006;126:1121–1133.
- 80 Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, McClanahan T, Kastelein RA, Cua DJ: IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med* 2005;201:233–240.
- 81 Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, Weaver CT: Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol* 2005;6:1123–1132.
- 82 Liang SC, Long AJ, Bennett F, Whitters MJ, Karim R, Collins M, Goldman SJ, Dunussi-Joannopoulos K, Williams CM, Wright JF, Fouser LA: An IL-17F/A heterodimer protein is produced by mouse Th17 cells and induces airway neutrophil recruitment. *J Immunol* 2007;179:7791–7799.
- 83 Wright JF, Guo Y, Quazi A, Luxenberg DP, Bennett F, Ross JF, Qiu Y, Whitters MJ, Tomkinson KN, Dunussi-Joannopoulos K, Carreno BM, Collins M, Wolfman NM: Identification of an interleukin 17F/17A heterodimer in activated human CD4+ T cells. *J Biol Chem* 2007;282:13447–13455.
- 84 Chang SH, Dong C: A novel heterodimeric cytokine consisting of IL-17 and IL-17F regulates inflammatory responses. *Cell Res* 2007;17:435–440.
- 85 Toy D, Kugler D, Wolfson M, Vanden Bos T, Gurgel J, Derry J, Tocker J, Peschon J: Cutting edge: interleukin 17 signals through a heteromeric receptor complex. *J Immunol* 2006;177:36–39.
- 86 Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, Hatton RD, Wahl SM, Schoeb TR, Weaver CT: Transforming growth factor-beta induces development of the T(h)17 lineage. *Nature* 2006;441:231–234.
- 87 Tamachi T, Maezawa Y, Ikeda K, Kagami S, Hatano M, Seto Y, Suto A, Suzuki K, Watanabe N, Saito Y, Tokuhisa T, Iwamoto I, Nakajima H: IL-25 enhances allergic airway inflammation by amplifying a Th2 cell-dependent pathway in mice. *J Allergy Clin Immunol* 2006;118:606–614.
- 88 Jovanovic DV, Di Battista JA, Martel-Pelletier J, Jolicoeur FC, He Y, Zhang M, Mineau F, Pelletier JP: IL-17 stimulates the production and expression of proinflammatory cytokines, IL-beta and TNF-alpha, by human macrophages. *J Immunol* 1998;160:3513–3521.
- 89 Ouyang W, Kolls JK, Zheng Y: The biological functions of T helper 17 cell effector cytokines in inflammation. *Immunity* 2008;28:454–467.
- 90 Dong C: Diversification of T-helper-cell lineages: finding the family root of IL-17-producing cells. *Nat Rev Immunol* 2006;6:329–333.
- 91 Song L, Schindler C: IL-6 and the acute phase response in murine atherosclerosis. *Atherosclerosis* 2004;177:43–51.
- 92 Eid RE, Rao DA, Zhou J, Lo SF, Ranjbaran H, Gallo A, Sokol SI, Pfau S, Pober JS, Tellides G: Interleukin-17 and interferon-gamma are produced concomitantly by human coronary artery-infiltrating T cells and act synergistically on vascular smooth muscle cells. *Circulation* 2009;119:1424–1432.
- 93 Cheng X, Yu X, Ding YJ, Fu QQ, Xie JJ, Tang TT, Yao R, Chen Y, Liao YH: The Th17/Treg imbalance in patients with acute coronary syndrome. *Clin Immunol* 2008;127:89–97.
- 94 Xie JJ, Wang J, Tang TT, Chen J, Gao XL, Yuan J, Zhou ZH, Liao MY, Yao R, Yu X, Wang D, Cheng Y, Liao YH, Cheng X: The Th17/Treg functional imbalance during atherogenesis in ApoE(-/-) mice. *Cytokine* 2009;49:185–193.
- 95 de Boer OJ, van der Meer JJ, Teeling P, van der Loos CM, Idu MM, van Maldegem F, Aten J, van der Wal AC: Differential expression of interleukin-17 family cytokines in intact and complicated human atherosclerotic plaques. *J Pathol* 2009;220:499–508.
- 96 Pejnovic N, Vratimos A, Lee SH, Popadic D, Takeda K, Akira S, Chan WL: Increased atherosclerotic lesions and Th17 in interleukin-18 deficient apolipoprotein E-knockout mice fed high-fat diet. *Mol Immunol* 2009;47:37–45.
- 97 Smith E, Stark MA, Zarbock A, Burcin TL, Bruce AC, Vaswani D, Foley P, Ley K: IL-17a inhibits the expansion of IL-17A-producing T cells in mice through 'short-loop' inhibition via IL-17 receptor. *J Immunol* 2008;181:1357–1364.
- 98 Yang Y, Weiner J, Liu Y, Smith AJ, Huss DJ, Winger R, Peng H, Cravens PD, Racke MK, Lovett-Racke AE: T-bet is essential for encephalitogenicity of both Th1 and Th17 cells. *J Exp Med* 2009;206:1549–1564.
- 99 Smith E, Prasad KMR, Butcher M, Dobrian A, Kolls JK, Ley K, Galkina E: Blockade of IL-17A results in reduced atherosclerosis in apoE-deficient mice. *Circulation* 2010;121:1746–1755.
- 100 Nagarajan S: Fc gamma chain deficiency on hematopoietic cells ameliorates atherosclerosis in apoE-knockout mice by blocking Th17 responses. *Keystone Symposia Advances in Molecular Mechanisms of Atherosclerosis, Banff, 2010*, pp 88.
- 101 Chen S, Shimada K, Crother T, Zhang W, Huang G, Arditi M: The role of nod/rip2 signaling and IL-17 in chlamydia pneumoniae (cpn)-mediated acceleration of atherosclerosis in hypercholesterolemic mice. *Keystone Symposia Advances in Molecular Mechanisms of Atherosclerosis, Banff, 2010*, pp 76.