Lymphocyte Migration Into Atherosclerotic Plaque

Jie Li, Klaus Ley

Abstract—Adaptive immunity is involved in the pathogenesis of atherosclerosis, but the recruitment of T and B lymphocytes to atherosclerotic lesions is not as well studied as that of monocytes. In this review, we summarize the current understanding of the role of lymphocyte subsets in the pathogenesis of atherosclerosis and discuss chemokines and chemokine receptors involved in lymphocyte homing to atherosclerotic lesions. We review evidence for involvement of the chemokines CCL5, CCL19, CCL21, CXCL10, and CXCL16 and macrophage migration inhibitory factor in lymphocyte homing in atherosclerosis. Also, we review the role of their receptors CCR5, CCR6, CCR7, CXCR3, CXCR6, and CXCR2/CXCR4 and the role of the L-selectin in mouse models of atherosclerosis. (Arterioscler Thromb Vasc Biol. 2015;35:40-49. DOI: 10.1161/ATVBAHA.114.303227.)

Key Words: atherosclerosis ▶ CC chemokine receptor ▶ lymphocytes

Atherosclerosis is the underlying cause of heart attacks and strokes, which are the leading causes of death and disability in North America.1 Atherosclerosis is a chronic inflammatory disease in the wall of arteries characterized by formation of lipid-rich lesions, called plaques. Within the vessel wall, crystallization of cholesterol and oxidation of low-density lipoprotein (LDL) result in cell-activating proinflammatory signals, which stimulate both innate and adaptive chronic inflammatory responses in the arterial intima.2,3 The innate immune system initiates the recruitment of monocytes from blood and their differentiation to macrophages in the vessel wall. The adaptive immune response enhances and regulates inflammation in mature lesions.4,5

There has been an increasing focus on the role of T-cell-mediated adaptive immunity in the pathogenesis of atherosclerosis because the inflammation in atherosclerosis is modulated by specific immune responses against plaque antigens such as oxidized LDL (ox-LDL).6 This suggests that manipulating the adaptive immune system toward downregulation of the antigen-specific immune response or tolerance may ameliorate plaque progression.

The fundamental importance of chemokines for atherosclerosis is well accepted.7 Chemokines are crucial players in directing movement and activity of leukocytes in homeostasis, immune surveillance, and inflammation. A large number of studies have been done to investigate the role of chemokines and chemokine receptors in the development of atherosclerosis; however, the majority of them were focused on monocytes rather than lymphocytes. In this review, we summarize current knowledge of lymphocyte migration in atherosclerosis observed in the 2 commonly used murine models of atherosclerosis, Apoe−/− mice and Ldr−/− mice (Table).

Lymphocytes in Atherosclerosis

Role of T Cells in Atherosclerosis

The presence of activated T cells in the human atherosclerotic plaque was discovered by Göran Hansson’s laboratory in 1986,8 which provided the first indication that the adaptive immune system is involved in atherosclerosis. Notably, T cells are the second largest leukocyte population in the atherosclerotic aorta after monocyte-derived cells and produce cytokines including interferon (IFN)-γ, interleukin (IL)-2, and IL-17 that modulate local inflammation.9

Th1

The majority of T cells in the atherosclerotic lesions are activated CD4+ effector and memory T cells,10–13 The T-helper (Th1) subset of CD4+ T cells, which preferentially produces IFN-γ and TNF, is both the most proatherogenic and abundant T-cell population in human atherosclerotic plaques.14 Adoptive transfer of T cells from ox-LDL immunized Apoe−/− mice to immunodeficient Apoe−/− mice accelerates atherosclerosis.15 Downregulation of Th1 polarization in Apoe−/− mice dramatically reduces the lesion size.16 IFN-γ secreted by Th1 cells activates dendritic cells and macrophages reinforcing M117 and thus perpetuating the Th1 response.9 Inhibition of IFN-γ by deleting either IFN-γ or its receptor decreases atherosclerosis and alters plaque antigen-specific immune responses,18–20 whereas injection of recombinant IFN-γ increases lesion size.21 IL-12 and IL-18 are key cytokines that promote Th1 differentiation. Exogenous administration of IL-12 and IL-18 accelerates atherosclerosis, whereas genetic deletion or inhibition of these 2 cytokines reduces the disease.22–26 Consistent with the cytokine studies, deficiency of T-bet, which is the key transcription factor determining Th1 lineage, results in significant decrease of atherosclerosis in...


\textbf{Ldlr}^{−/−} mice and shifts the immune response toward Th2.\textsuperscript{27} These data provide evidence for the pathogenic role of Th1 T cells in atherosclerosis.

\textbf{Th2}

Th2 cells are rarely detected in atherosclerotic lesions,\textsuperscript{14} and the role of Th2 cells in atherosclerosis remains controversial. It was thought that Th2 cells are antiatherogenic because shifting the T-cell response from Th1 to Th2 is associated with decreased lesion size in mice.\textsuperscript{28−30} However, the results from studies that delete the Th2 cytokine IL-4 do not support this view. IL-4\textsuperscript{−/−} Apoe\textsuperscript{−/−} mice and irradiated Ldlr\textsuperscript{−/−} mice transplanted with bone marrow from IL-4\textsuperscript{−/−} mice both showed reduced atherosclerosis, suggesting a proatherogenic role of Th2 cells.\textsuperscript{22,23} In another study, neither exogenous delivery nor genetic deficiency of IL-4 significantly influenced the development of atherosclerotic lesions.\textsuperscript{31} Of note, IL-4 is secreted not only by Th2 cells but also by mast cells, basophils, and other cells.\textsuperscript{32,33} Moreover, activated Th2 cells also produce IL-5, IL-9, IL-13, and IL-25. IL-5 plays a protective role in atherosclerosis, probably by its ability to stimulate the synthesis of natural antibodies.\textsuperscript{35,36} IL-13 protects from atherosclerosis, probably by its ability to stimulate the synthesis of natural antibodies.\textsuperscript{35,36} IL-13 protects from atherogenesis and modulates plaque composition by skewing the macrophage phenotype.\textsuperscript{37} The role of IL-9 and IL-25 remains to be elucidated.

\textbf{Th17}

Th17 is a distinct non-Th1/Th2 CD4 T-cell lineage that produces IL-17A and IL-17F. These cells were found to be involved in the pathogenesis of atherosclerosis in recent years, but their role is still unclear.\textsuperscript{38−47} To directly study the role of Th17 in atherosclerosis, neutralizing IL-17 or genetic deletion of IL-17A in mice should provide direct evidence; however, the results are controversial. Neutralizing rat antimouse IL-17A antibodies reduce atherosclerosis in Apoe\textsuperscript{−/−} mice, but there is no evidence that these antibodies actually disrupt IL-17 signaling in the treated mice.\textsuperscript{43} Mouse antimouse IL-17A antibody did not affect atherosclerosis, although the IL-17 signaling was abolished.\textsuperscript{48} This suggests that the protective effects of rat antimouse IL-17A may not be through reduced IL-17 signaling but dependent on responses to the foreign (rat) antibody used. Blockade of IL-17A in Apoe\textsuperscript{−/−} mice by use of adenovirus-produced IL-17 receptor A reduced plaque burden in Apoe\textsuperscript{−/−} mice, but this study also did not show a sustained reduction of IL-17 signaling.\textsuperscript{39} Studies of genetic IL-17 deficiency in Apoe\textsuperscript{−/−} mice from three different laboratories also provide contradictory results.\textsuperscript{44,45,47} Recent evidence from other inflammatory diseases suggest that only a subset of Th17 cells that are IL23R\textsuperscript{high} CCR6\textsuperscript{+} may be proinflammatory and another subset may be regulatory.\textsuperscript{49,50} Focusing on the role of these subsets could potentially resolve the controversy.

\textbf{Regulatory T Cells}

Regulatory T cells (Tregs) express the high-affinity IL-2 receptor CD25 and the transcription factor Foxp3. They play a protective role in the progression of atherosclerosis. Recent studies strongly suggest that the Treg-mediated immune tolerance is hampered in atherosclerosis. In human atherosclerotic lesions, the number of Foxp3-positive cells is much

\begin{table}[h]
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\begin{tabular}{|c|c|c|}
\hline
\textbf{Mouse Model} & \textbf{Effect on Atherosclerosis} & \textbf{Effect on Cell Content} \\
\hline
\textbf{Cc57L1-Ldlr}^{−/−} & ↑ Lesion size in aorta and aorta root & ↑ T cells in the lesion, blood, bone marrow, and spleen\textsuperscript{90} \\
\textbf{Cc57L1-Apoe}^{−/−} & ↓ Lesion size in aorta and aorta root & ↑ CD3 and CD4 T cells in aorta root\textsuperscript{90} \\
\textbf{Cc57L1-Ldlr}^{−/−} & ↓ Lesion size in aorta root and thoracoabdominal aorta & ↑ CD3 T cells in aorta root\textsuperscript{102} \\
\textbf{Ccr1}^{−/−} bone marrow transfer to Ldlr\textsuperscript{−/−} mice & ↑ Lesion size in thoracic aorta & ↑ CD3 T cells in thoracic aorta\textsuperscript{103} \\
\textbf{Ccr5}^{−/−} Apoe\textsuperscript{−/−} & ↓ Plaque area in aorta root and thoracoabdominal aorta & ↓ CD3 T cell in aorta root\textsuperscript{103} \\
\textbf{Ccr5}^{−/−} reperfused myocardial infarction & ↑ Inflammatory cytokine and chemokine in infarcted heart & ↑ CD4+Foxp3+ Treg recruitment in infarcted heart\textsuperscript{106} \\
\textbf{Ccr5} antagonist in Ldlr\textsuperscript{−/−} mice & ↓ Atherosclerosis in aortic root and carotid arteries & ↓ CD3 T-cell count in the plaque\textsuperscript{108} \\
\textbf{Ccl5} antagonist in Ldlr\textsuperscript{−/−} mice & ↓ Lesion size in aorta and aorta root & ↓ CD4 T cell in aorta root\textsuperscript{107} \\
\textbf{Cxc12}^{−/−} Apoe\textsuperscript{−/−} & ↓ Lesion size in thoracoabdominal aorta & ↓ CD4 T cell in the lesion ↑ Treg in aorta root\textsuperscript{112} \\
\textbf{CxcR3} antagonist in Ldlr\textsuperscript{−/−} mice & ↓ Lesion in aorta and aorta root & ↑ CD4+ CD62L\textsuperscript{high} T cell and CD4+ CD25\textsuperscript{+} T cells in the draining LNs from the aortic arch ↑ TGF-β in aorta root & ↑ Foxp3, CD25, CTLA-4 mRNA in the plaque\textsuperscript{115} \\
\textbf{Cxc110}^{−/−} Apoe\textsuperscript{−/−} & ↓ Lesion area in aorta arch & ↑ CD4 T cell in plaque \\
\textbf{Cxc6R}^{−/−} Apoe\textsuperscript{−/−} & ↓ Lesion size in aorta and aorta root & ↑ Cxcr6 T cell and IFN-γ in aorta\textsuperscript{119} \\
\textbf{Cxc110}^{−/−} Ldlr\textsuperscript{−/−} & ↑ Lesion area in aorta arch & No effect on T cell in aorta arch\textsuperscript{21} \\
\textbf{MIF blocking antibody in Apoe}^{−/−} mice & ↓ Plaque area in aorta root & ↓ CD3 T cell in aorta root\textsuperscript{25} \\
\textbf{L-selectin}^{−/−} lymphocytes transfer to Apoe\textsuperscript{−/−} mice & N.D. & ↓ L-selectin\textsuperscript{−/−} B cell and T cell in aorta\textsuperscript{65} \\
\textbf{Id3}^{−/−} Apoe\textsuperscript{−/−} & ↑ Lesion area in aorta & ↓ B cell in aorta\textsuperscript{78} \\
\hline
\end{tabular}
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lower (1%–4% of total T cells) than that in other inflamed tissue (∼25% of total T cells are Foxp3+).51 Patients with coronary artery disease have reduced numbers of Tregs in peripheral blood with reduced immune-suppressive capacity in vitro.52–54 In Ldlr−/− mice, the number of Tregs in the aorta decreases and the ratio of effector T cell/Treg greatly increases as the disease progresses.55

Various treatments targeting Tregs suggest a direct protective role of Tregs. Genetic deletion of Foxp3 (the transcription factor determine Treg differentiation),56 CD25 neutralizing antibody,57 and vaccination against Foxp3 58 all show significant exacerbation of atherosclerosis. Adoptive transfer of Tregs purified from wild-type (WT) mice into Apoe−/− mice ameliorates atherosclerosis in Apoe−/− mice.59 Treatments that target the Treg cytokines IL-10 and transforming growth factor with genetic deletion or neutralizing antibodies exacerbate atherosclerosis.60–62

**Natural Killer T Cells**

Natural Killer T (NKT) cells express a highly restricted T-cell receptor (TCR) repertoire and respond to CD1d-restricted lipid ligands rather than major histocompatibility complex (MHC) presented antigens. NKT cells have been found in human atherosclerotic plaques.63,64 They are a minor cell population accounting for ∼0.3% to 2% of T cells in the human plaque.65

Recent studies suggest that NKT cells are likely to be proatherogenic. NKT cell–deficient Apoe−/− mice (Cd1d−/−, Apoe−/−) exhibited a 25% decrease in lesion size compared with Apoe−/− mice. Administration of α-galactosylceramide, a glycolipid that activates NKT cells via CD1d, induced a higher antibody,57 and vaccination against Foxp3 58 all show significant exacerbation of atherosclerosis. Adoptive transfer of Tregs purified from wild-type (WT) mice into Apoe−/− mice ameliorates atherosclerosis in Apoe−/− mice.59 Treatments that target the Treg cytokines IL-10 and transforming growth factor with genetic deletion or neutralizing antibodies exacerbate atherosclerosis.60–62

**Role of B Cells in Atherosclerosis**

B cells are present in atherosclerotic lesions at lower frequency than T cells.73 The role of B cells in atherosclerosis is still debated. Early studies with splenectomy suggested a protective role of B cells.76 Splenectomized Apoe−/− mice showed exacerbated atherosclerosis and adoptive transfer of splenic B cells from Apoe−/− mice rescued these mice from the proatherogenic effect. In addition, adoptive transfer of B cells from Apoe−/− mice attenuated atherosclerosis in non-splenectomized mice. Consistent with these findings, Ldlr−/− mice transplanted with bone marrow from B-cell–deficient μMT mice showed increased atherosclerosis. Also, B cells from Apoe−/− mice adoptively transferred to B-cell–deficient Apoe−/− μMT mice attenuated the disease. In contrast, recent studies show that depleting B cells with anti-CD20 antibody decreases atherosclerosis.79,80 In these experiments, antibody-producing plasma cells remain unaffected by depletion.

**B-1 Cells**

B cells can be divided into 2 lineages, B-1 and B-2. B-1 cells secrete natural antibodies that are predominantly IgM and IgA. The atheroprotective role of B cells may be related to observations that some natural antibodies are atheroprotective. Ldlr−/− mice lacking soluble IgM develop larger atherosclerotic lesions. Clinically, the concentration of IgM reactive to ox-LDL has an inverse relation to carotid artery atherosclerosis. A possible mechanism might be that ox-LDL–specific autoantibodies bind to ox-LDL, preventing it from being taken up by macrophages and consequently preventing foam cell formation.

**B-2 Cells**

B-2 cells produce (mainly) IgG antibodies in a CD4 T-cell–dependent manner after isotype switching and affinity maturation. B-2 cells may exacerbate atherosclerosis by producing pathogenic IgG antibodies. IgG antibodies reactive to oxidation-specific epitopes have been detected in both in the plasma and vascular lesions of patients with Apoe−/− mice.72 CD8 T cells respond to antigen presented by MHC-I. Unlike CD8-deficient mice, MHC-I deficient mice on high-fat diet developed increased atherosclerosis.73 It has been reported that CD8 T cells promote the development of vulnerable atherosclerotic plaques by perforin-mediated granzyme B–mediated apoptosis of macrophages, smooth muscle cells, and endothelial cells, which, in turn, leads to necrotic core formation and further augments inflammation by TNF secretion. Depleting CD8 T cells by monoclonal antibody in Apoe−/− mice ameliorated atherosclerosis by reducing lipid and macrophage accumulation, apoptosis, necrotic cores, and monocyte chemottractant protein 1, interleukin 1β, interferon γ, and vascular cell adhesion molecule 1. Transfer of CD8 T cells into lymphocyte-deficient Apoe−/− mice increased lipid and macrophage accumulation, apoptotic cells, necrotic cores, and IL-1β in atherosclerotic lesions. Transfer of CD8 T cells to Apoe−/− mice increased atherosclerosis with age in Apoe−/− mice. NKT cell–deficient Apoe−/− mice transplanted with bone marrow from B-cell–deficient μMT mice showed increased atherosclerosis. Also, B cells from Apoe−/− mice adoptively transferred to B-cell–deficient Apoe−/− μMT mice attenuated the disease. In contrast, recent studies show that depleting B cells with anti-CD20 antibody decreases atherosclerosis.79,80 In these experiments, antibody-producing plasma cells remain unaffected by depletion.
coronary artery disease and animal models of atherosclerosis. In LDLr−/− mice, the titer of IgG autoantibodies correlates positively with disease progression. In humans, the role of ox-LDL-specific IgG remains controversial because some epidemiological studies have reported positive and others negative correlations with disease progression. B-2 cells may also amplify inflammation through T-cell activation and Th1 polarization. A subset of B-2 cells called innate response activator B cells arise in both human and mice, produce GM-CSF (granulocyte-macrophage colony-stimulating factor), and aggravate atherosclerosis by shifting the adaptive immune response toward Th1.

**Lymphocyte Homing in Atherosclerosis**

Although CD4 T cells, CD8 T cells, NKT cells, and B cells are all present in atherosclerotic lesions, it is largely unknown how these cells migrate to the lesion sites. Recent studies suggest that some chemokines, their receptors, and L-selectin play a role in regulating lymphocyte migration to the atherosclerotic aorta (Figure 1). Although many of the lymphocytes may primarily home to the adventitia and not the plaque, lymphocyte recruitment to the adventitia versus plaque is not distinguished in most studies. Therefore, our review will discuss lymphocyte homing to the aortic wall. T and B cells are thought to be trafficking between the spleen and aortic wall through the blood and from aortic wall to draining lymph node through lymphatics (Figure 2).

**B-Cell Homing in Atherosclerosis**

**L-Selectin**

B and T cells are present in the normal (nonatherosclerotic) aortas, suggesting that constitutive homing mechanisms must exist to allow lymphocytes traffic into the aortic wall. Lymphocyte recruitment to normal and atherosclerotic aortas was found to be partially L-selectin dependent. Adoptive transfer of WT and L-selectin−/− lymphocytes to WT mice showed that L-selectin−/− T- or B-cell migration into the aortic wall was only 50% of that of WT lymphocytes. To determine the role of L-selectin in lymphocyte homing into mature plaques, tertiary lymphoid organs (TLO) are induced, but the distribution of CCL19 and CCL21 has not been reported.
atherosclerotic aortas, L-selectin−/− lymphocytes were trans-
ferred to Apoe−/− mice. L-selectin−/− B cells displayed a 57%
reduction in migration into atherosclerotic aortas of recipient
mice in comparison with migration of WT B lymphocytes.
L-selectin−/− T cells demonstrated a similar 50% reduction
in homing to atherosclerotic aortas. The partial reduction
of lymphocyte homing in the absence of L-selectin suggests
that migration of T and B lymphocytes into the athero-
sclerotic aorta is regulated by L-selectin and other adhesion
molecules.95

T-Cell Homing in Atherosclerosis

**CCR7**

CCR7 is a chemokine receptor expressed on activated den-
dritic cells, naïve T and B cells, central memory T cells,
and some Tregs.96,97 It is known to regulate T-cell homing
to lymph nodes and Peyer’s patches. Both CCR7 and its 2
ligands, CCL19 and CCL21, have been identified in mouse
and human atherosclerotic lesions,98 suggesting a pathological
role of CCR7 in atherosclerosis. However, the role of CCR7
in atherosclerosis is still controversial as results from different
studies are contradictory. Genetic deletion of CCR7 in Apoe−/−
mice increased lesion size by increasing T-cell accumulation
in atherosclerotic lesions.99 Ccr7−/− Apoe−/− mice had increased
T cells in the blood, bone marrow, and spleen, as well as in
atherosclerotic lesions. Competitive repopulation experiments
revealed that T cells from Ccr7−/− Apoe−/− mice migrated poorly
into lymph nodes compared with CCR7 competent T cells.99
However, in Ccr7−/− Ldlr−/− mice, CCR7 deficiency was reported to reduce plaque development
with increased CD3 and CD4 T-cell accumulation in the aor-
tic root.100 These findings suggest a complex role for CCR7
signaling in different experiment mouse models. The increased
number of T cells in the aorta of these CCR7 deficiency mod-
elts might be because of the impaired efflux of T cells from
the inflamed tissue to the lymph node. CCR7 has also been
reported to play a role in regulating macrophage/dendritic cell
egress from the plaque to the draining lymph nodes.101

The limitation of these studies is that global CCR7 knock-
out mice were used. Besides T cells, CCR7 is expressed on
activated dendritic cells and B cells. T-cell–specific CCR7
knockout atherosclerotic mice may be better suited to study
the role of CCR7 in regulating T-cell homing to the athero-
sclerotic aorta. Until conditional CCR7 (and other chemokine
receptor) knockout mice become available, adoptive trans-
fer of chemokine receptor knockout lymphocytes may be
an approach that can more directly address the role of these
receptors in T-cell homing.

**CCL5 and its Receptors CCR1 and CCR5**

CCR1 and CCR5 are receptors for CCL5.102 In atheroscle-
rotic plaques, CCR1 and CCR5 are expressed on various
cell types including monocytes, macrophages, and Th1 cells.
CCL5 can be expressed by monocytes, macrophages, T cells,
and smooth muscle cells. CCL5 acting on CCR1 and CCR5
mediates leukocyte arrest (transition from rolling to adhesion)
and transendothelial diapedesis. Ccr1−/− ApoE−/− mice show
increased plaque size and increased CD3 T cells in the aortic
root.102 Consistent with this result, Ccr1−/− bone marrow trans-
plantation to Ldlr−/− mice increases lesion size and CD3 T-cell
number in the thoracic aorta, as well,103 suggesting that CCR1
may have an overall atheroprotective role.

Genetic deletion studies in ApoE−/− mice suggest that CCR5
has a proatherogenic role in neointimal plaque formation.
Ccr5−/− Apoe−/− mice are protected from diet-induced atherosclerosis and show a more stable plaque phenotype, reduced mononuclear cell infiltration, reduced T-cell infiltration, reduced Th1-type immune responses, and increased IL-10 expression. Suggesting CCR5 is more important than CCR1 in regulating T-cell homing to the aorta. In a murine model of reperfused myocardial infarction, CCR5-null mice exhibited enhanced inflammation. The effect was associated with impaired recruitment of CD4+Foxp3+ Tregs, suggesting that CCR5 may play a role in regulating Treg homing to the aorta.

The CCR5 antagonist TAK-779, which is an inhibitor of both CCR5 and CXCR3, dramatically reduced atherosclerosis in the aortic root and carotid arteries of Ldlr−/− mice. The number of T cells in the plaque was reduced by 95%, concurrently with a 98% reduction in area staining for IFN-γ, suggesting a role of CCR5 and CXCR3 in regulating Th1-cell homing to the aorta. CCL5 antagonist treatment in Ldlr−/− mice similarly showed reduced progression of established atherosclerosis and decreased CD4+ T-cell infiltration in the aorta.

**CXCR3 and its Ligand CXCL10**

CXCR3 is expressed on activated Th1 cells, B cells, NKT cells, and endothelial cells. It has been shown that Th1 cells in human atheroma express high levels of CXCR3. The importance of CXCR3 for Th1-cell differentiation was highlighted recently by the discovery that CXCR3 is required for optimal generation of Th1 cells in vivo. Recent studies showed that a CXCR3 antagonist significantly inhibited atherosclerotic lesion formation in the aortic valve leaflet area and the entire aorta in Ldlr−/− mice. Lymph nodes draining the aorta were significantly smaller in treated mice and contained more Tregs and fewer activated T cells. The markers for Treg cells (transforming growth factor-β, Foxp3, and CTLA-4) were significantly increased in treated mice. Genetic deletion of CXCR3 in Apoe−/− mice reduced atherosclerotic lesion development within abdominal aortas. This reduction of lesion formation was correlated with a decrease of T-cell content in the aorta, an upregulation of anti-inflammatory molecules such as IL-10, IL-18–binding protein, and endothelial nitric oxide synthase, and an increased number of Tregs within atherosclerotic lesions. Consistent with the above results, genetic deletion of the CXCR3 ligand, CXCL10, in Apoe−/− mice significantly decreased lesion area in the aorta. T-cell accumulation in the aorta was significantly diminished, whereas Treg number and activity were enhanced as assessed by increased message for the Treg marker Foxp3, as well as increased immunostaining for the Treg-associated cytokines IL-10 and transforming growth factor-β. mRNAs encoding for chemokines and chemokine receptors associated with Tregs, including CCR4, CCR8, CCL17, and CCL22, were also increased in the aortic arch of CXCR3−/− mice.

These results suggest that CXCR3 and its ligand CXCL10 play a role in balancing Th1 and Tregs in atherosclerosis. Moreover, in Cxcr3−/− Apoe−/− mice, cells in the atheroma of the mice mainly express CCR5, suggesting that CCR5 may play a role in Treg homing to the aorta in this model.

**CXCR6 and its Ligand CXCL16**

CXCR6 is expressed on some T cells and NKT cells. It is a chemokine receptor that regulates Th1-cell homing. Apoe−/− mice deficient in CXCR6 show reduced atherosclerotic plaque formation associated with a lower content of CXCR6+ T cells and CD68+ macrophages and decreased IFN-γ expression in the aorta. Short-term homing experiments demonstrated that CXCR6 is involved in the recruitment of CXCR6+ T cells into the atherosclerotic aorta wall. The only known ligand of CXCR6 is CXCL16, which is upregulated during atherosclerosis. It is a transmembrane chemokine that possesses both chemotactic and ox-LDL scavenger activity. In contrast to CXCR6 deletion, CXCL16 deletion in Ldlr−/− mice increased atherosclerosis without an effect on T-cell content in the aorta compared with Ldlr−/− mice. The increase of plaque formation may be explained by loss of the ox-LDL scavenger function of CXCL16, suggesting that it is more influential than the chemokine function in atherosclerosis.

**Macrophage Migration Inhibitory Factor and its Receptors CXCR2 and CXCR4**

Migration inhibitory factor (MIF) is an atypical chemokine involved in the pathogenesis of atherosclerosis. On stimulation by ox-LDL, endothelial cells, smooth muscle cells, and macrophages express MIF. The expression of MIF has been shown to correlate with increased intima-media thickening and lipid deposition in the aorta of mice and in advanced human carotid artery plaques. CXCR2 and CXCR4 are functional receptors of MIF; MIF binding to CXCR2 or CXCR4 triggers calcium influx, induces a rapid activation of integrins, and can subsequently mediate integrin-dependent arrest and chemotaxis of monocytes and T cells.

Evidence of MIF playing a role in the disease progression of atherosclerosis has been shown in recent studies. Neutralizing MIF by neutralizing antibody in Apoe−/− mice impaired the atherogenic recruitment of macrophages and the aortic expression of inflammatory mediators. Mif−/− Ldlr−/− mice had significantly reduced abdominal aorta lipid deposition and intimal thickening from aortic arch throughout the abdominal aorta. Blocking MIF by neutralizing antibody in Apoe−/− mice resulted in plaque regression and reduced monocytic and CD3+ T-cell content in plaques, suggesting that MIF also affects T-cell recruitment to the aorta.

**Inhibitor of Differentiation-3 and CCR6**

Inhibitor of differentiation-3 (ID3) is important for atheroprotection in mice, and a polymorphism in the human ID3 gene has been implicated as a potential risk marker of atherosclerosis in humans. The ID3−/− mouse develops a Sjögren-like syndrome with lachrymal and salivary gland lymphocytic infiltrates, raising the interesting possibility that Id3 may regulate B-cell homing to sites of disease.

The link between ID3 and atherosclerosis has been confirmed recently in mice and humans. In Id3−/− Apoe−/− mice, atherosclerosis is increased compared with Apoe−/− mice.
Humans carrying an allele of ID3 that contains a single nucleotide polymorphism that alters ID3 protein function show increased carotid intima-media thickness, an imaging measure of preclinical atherosclerosis. In Id3<sup>−/−</sup> Apoe<sup>−/−</sup> aortas, B-cell content was found to be decreased compared with Apoe<sup>−/−</sup> mice. B cells transferred from Id3<sup>−/−</sup> Apoe<sup>−/−</sup> mice into B-cell–deficient mice reconstituted the spleen, lymph node, and blood similar to B cells from Id3 competent Apoe<sup>−/−</sup> mice; however, aortic reconstitution and B-cell–mediated inhibition of diet-induced atherosclerosis was significantly impaired. The chemokine receptor CCR6 was identified as a target of ID3, because ID3 regulates the expression of CCR6. In Id3<sup>−/−</sup> Apoe<sup>−/−</sup> mice, CCR6 expression in B cells was decreased compared with Apoe<sup>−/−</sup> mice, and CCR6 is required for B-cell recruitment into the aortic wall.

In conclusion, the chemokine receptors CXCR3, CXCR6, and CCR3 and the adhesion molecule L-selectin seem to be involved in T-cell homing and CCR6 and L-selectin in B-cell homing to the aortic wall. Almost nothing is known about homing of lymphocyte subsets like Th1 and Treg, which are known to be relevant for atherosclerosis. Thus, this area is ripe for further investigation.

Conclusions

Lymphocytes play a vital role in the pathogenesis of atherosclerosis, but our understanding of lymphocyte recruitment in atherosclerosis is limited. In this review, we summarize what is known about chemokines (CCL5, CCL19, CCL21, CXCL10, and CXCL16), chemokine receptors (CCR5, CCR6, CCR7, CXCR3, CXCR6, and CXCR2/CXCR4), and other factors (CD62L, MIF, and ID3) with respect to lymphocyte homing to the atherosclerotic aorta. Some of these chemokines show specificity or preference for lymphocyte subsets: CCL5, CXCL10, and CXCL16 and their receptors CCR5, CXCR3, and CXCR6 for Th1, CXCR6 and CXCR4 for Tregs. It is not known how these receptors and ligands specifically promote homing of Th1 cells and Tregs to the vascular wall. Most published experiments were done in global chemokine receptor knockout mice. Because chemokine receptors are expressed on cells other than T and B lymphocytes, these experiments often remain inconclusive. Some studies such as the CCR7<sup>−/−</sup> Apoe<sup>−/−</sup>, CXCR6<sup>−/−/−</sup> Apoe<sup>−/−</sup>, CD62L<sup>−/−</sup> and Id3<sup>−/−</sup> Apoe<sup>−/−</sup> included adoptive transfer experiments using the receptor knockout lymphocytes and are thus more convincing. Lymphocyte-specific chemokine receptor knockout mice will enable better experiments to investigate the role of homing receptors in lymphocyte trafficking in atherosclerosis.

Disclosures

None.

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Understanding this aspect of adaptive immunity in atherosclerosis may support the development of novel therapeutic strategies for atherosclerosis.

Other factors involved in lymphocyte homing to the atherosclerotic aorta. Because chemokine receptors are known pharmacological targets, understanding this aspect of adaptive immunity in atherosclerosis may support the development of novel therapeutic strategies for atherosclerosis.

Significance

Extensive studies have shown that chemokines and their receptors are promising therapeutic targets for atherosclerosis and have opened up new avenues for treating inflammatory diseases. Murine models have provided proof of the role of chemokines such as CCR2/CCL2, CX3CR1/CX3CL1, and CCR5/CCL5 in the different stages of disease. However, the chemokines that regulate adaptive immunity and lymphocyte trafficking in the pathogenesis of atherosclerosis are still unclear. This review summarizes our understanding of chemokines, chemokine receptors, and other factors involved in lymphocyte homing to the atherosclerotic aorta. Because chemokine receptors are known pharmacological targets, understanding this aspect of adaptive immunity in atherosclerosis may support the development of novel therapeutic strategies for atherosclerosis.
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