Lymphocytes in Atherosclerosis

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Atherosclerosis is the major cause of cardiovascular disease, and cardiovascular disease remains the primary cause of death in the United States. The primary complication for patients with type 2 diabetes mellitus (T2D) is cardiovascular disease.1–8 Because the incidences of T2D and obesity are drastically increasing in the United States and now worldwide, understanding how atherosclerosis is accelerated in these diseases becomes even more important to discern.

Although many scientific advances in research on atherosclerosis have been made by multiple groups during the past 30 years, particularly about the function of endothelial and smooth muscle biology and macrophage foam cell formation in atherosclerotic plaque development, the roles of immune cells in regulating atherosclerosis onset and progression are still being elucidated. It was widely thought that immune cells played little part in atherogenesis until 1986, when Hansson et al9–12 reported the presence of lymphocytes within atherosclerotic lesions; yet it was many years later until it was widely accepted that immune cells play an important role in atherogenesis.

Since 1986, the viewpoint has changed significantly, but details on lymphocyte subsets and mechanisms are still emerging. Moreover, controversy still exists on the roles that some lymphocyte subsets play in atherosclerosis. These discrepancies in findings are at least partly because of differences in mouse models used, diet composition, and length of time on diet for atherosclerosis measurements. However, despite a few ongoing controversies, there are many studies on immune cells in atherogenesis in mice that show clear findings.

For example, CD4+ T lymphocytes have been shown to accelerate atherosclerosis.19,24–29 Activated CD4 T cells can be distinguished based on their phenotype, and the predominant CD4+ effector T cells studied to date in the context of atherosclerosis are Th1, Th2, and Th17 cells. The Th1 cell, a CD4+ T effector cell that produces interferon-γ, is proatherogenic. For example, CD4+ T lymphocytes have been shown to accelerate atherosclerosis.18,24–29 Activated CD4 T cells can be distinguished based on their phenotype, and the predominant CD4+ effector T cells studied to date in the context of atherosclerosis are Th1, Th2, and Th17 cells. The Th1 cell, a CD4+ T effector cell that produces interferon-γ, is proatherogenic. CD8 cells are much less studied in the context of atherosclerosis, but recent studies suggest that they may be proatherogenic9 although one recent study found that a CD8+CD25+ subset of CD8 cells may be atheroprotective.30 Expect to see more studies emerging on CD8 cells in atherosclerosis in the next few years as interest in this topic is expanding. Of note for further reading, a nice detailed review by Li and Ley31 on T cells in atherosclerosis with a focus on lymphocyte homing to aortic tissue was published this year in ATVB.

Moreover, little is known about the role of innate-like lymphocyte subsets (natural killer cells [NK], γδT cells, and natural killer T cells [NKT]) in atherosclerosis. This article highlights studies on T- and B-lymphocyte subsets and innate-like lymphocyte subsets and their roles in atherosclerosis and related vascular diseases recently published in ATVB.

T Lymphocytes

As noted above, T-lymphocyte subsets have been found to play differing roles in atherosclerosis. Buono et al22 found that numbers of atheroprotective Tregs are reduced in atherosclerotic mice during high cholesterol diet feeding, and that this was a reversible process: on normalization of plasma cholesterol levels in the mice, Treg numbers were restored. Mechanisms for this are likely to be several, but these investigators found that high cholesterol diet feeding influenced Treg migration to aorta. Earlier studies have shown that the cholesterol content of lymphocytes affects their proliferation. Wilhelm et al23 reported in ATVB in 2009 that mice lacking apoA-I and functional high-density lipoprotein had increased atherosclerosis and increased cholesterol-associated CD4 T-lymphocyte proliferation and activation. Studies by Bensinger et al24 and also by our own group25 found that sterol content of lymphocytes increased their proliferation, and this was primarily regulated by the ATP-binding cassette transporter ABCG1. Whether the cholesterol content of Tregs directly affects their proliferation or function is not known, and the role that high-density lipoprotein may play in this process warrants further investigation.

Tregs may be also protective in other vascular diseases. In a recent study in ATVB, Tregs were shown to protect against abdominal aortic aneurysm. In a well-established inducible mouse model of aneurysm using angiotensin II, selective depletion of Tregs using CD25 antibodies enhanced susceptibility of mice to aneurysm and promoted aortic rupture.46 Furthermore, these authors found that IL-10 played an important role in the protection against angiotensin II–induced aneurysm in this model. To address whether there is a link between T2D or obesity and lymphocyte function, recent work in ATVB
shows that T-cell frequencies are changed in the adipose tissue of obese subjects. McLaughlin et al.\textsuperscript{47} found that both visceral and subcutaneous fat depots in overweight and obese human subjects contained elevated numbers of both CD4 and CD8 T cells. Furthermore, these investigators found that Th2 frequencies in both fat pad depots correlated with reduced incidence of insulin resistance. Thus, adipose tissue of obese subjects likely contains cytokines and antigenic stimuli for modulation of T-cell numbers and activation. One possible mediator of increased T-cell recruitment to adipose tissue is the chemokine receptor CXCR3. In obese mice, CXCR3 expression was higher on stromal vascular cells of adipose tissue.\textsuperscript{48} CXCR3-deficient mice possessed fewer T cells in adipose depots and showed reductions in proinflammatory cytokines. A second novel mediator of T-cell infiltration and accumulation in adipose is CD11a, a b2 integrin. Jiang et al.\textsuperscript{49} showed that CD11a is upregulated on CD8 cells from obese mice, and that CD8 T cells from CD11a-deficient mice failed to migrate to adipose tissue in vivo. This study is of interest because it supports a novel role for this integrin in lymphocyte homing to adipose tissue, which will be important for immunity associated with T2D and obesity.

Despite accepted knowledge that lymphocytes significantly affect lesion development, we know little about the detailed mechanisms involved or the cross talk that occurs between myeloid cells and lymphocytes in the artery wall. Several studies in \textit{ATVB} this year reported how novel cytokines and other novel T-cell modulators affected atherogenesis. For example, IL-19 is an anti-inflammatory cytokine produced by Th2 lymphocytes. Atherosclerotic-susceptible low-density lipoprotein receptor (LDLR)\textsuperscript{−/−} mice treated with recombinant IL-19 developed less aortic atherosclerosis, and this was accomplished by polarization of CD4\textsuperscript{+} lymphocytes to a Th2 phenotype, with decreased interferon-\gamma and IL1\beta expression and increased expression of GATA3 and Foxp3 transcription factors.\textsuperscript{50} This study suggested that IL-19 was a potent inhibitor of atherosclerosis through its effect on T-cell polarization. Another novel molecule, T-cell immunoglobulin and mucin domain-3 (Tim-3), acts as a negative regulator of immune responses. In a recent report in \textit{ATVB}, treatment of mice with anti-Tim3 antibody depleted Tim-3 and increased lesion development.\textsuperscript{51} This was accompanied by increased numbers of activated CD4\textsuperscript{+} effector T cells and reduced numbers of Tregs. Related to Th2 polarization is a recent study in humans that indicated that increased numbers of Th2 lymphocytes in blood was associated with reduced risk of myocardial infarction.\textsuperscript{52} suggesting that Th2 bias may indeed be atheroprotective.

Macrophages and dendritic cells (DCs) are known to present antigen to T cells and also to secrete cytokines that participate in T-cell phenotypic switching in the vascular microenvironment. Three studies published recently in \textit{ATVB} cite new mechanisms by which myeloid cells influence T-cell activation in atherosclerosis. One interesting article, in particular, relayed findings where oxidized LDL, but not native LDL, stimulated DC activation.\textsuperscript{53} These oxidized LDL-stimulated DC were able to induce T-cell proliferation and activation, and these DC polarized the CD4 cells to a Th1 or Th17 bias. Mechanistically, this was shown to be caused, in part, by action of heat shock proteins. Interestingly, treatment of oxidized LDL–stimulated DC with the anticoagulant Annexin A5 caused the DC to produce both IL-10 and transforming growth factor-\beta, which promoted differentiation of naive T cells to Treg cells in vitro. A second article explored how toll-like receptor (TLR) 9 signaling in myeloid cells protected mice against atherosclerosis.\textsuperscript{54} Although the exact mechanisms for the increased atherosclerosis in TLR9\textsuperscript{−/−} apoE\textsuperscript{−/−} mice are unclear, the data support the notion that TLR9 signaling may be atheroprotective: in the absence of TLR9 signaling, there is an accumulation of DC in atherosclerotic lesions that cause recruitment and activation of CD4\textsuperscript{+} T cells. Related to the above work on Tim-3, these investigators also found an increase in Tim-3 in the atherosclerotic lesions of TLR9-deficient mice, supporting the proatherogenic role for Tim-3 noted above.

In sum, new work published in \textit{ATVB} showing a protective role for Tregs in other vascular diseases, including aneurysm, support the notion that Tregs serve a protective role in vascular disease. New studies in mice and humans with T2D or obesity suggest that adipose depots are hotspots for immune regulation, with increased myeloid cell content and supporting active CD4 and CD8 lymphocyte recruitment to these fat depots. Understanding the functions of lymphocytes in adipose tissue may aid in preventing complications associated with obesity and T2D. Finally, new mechanisms for how lymphocytes may affect atherosclerosis are emerging, including myeloid cell-lymphocyte cross talk in the artery wall, thus, shedding light on possible new therapies for cardiovascular disease.

**Innate Lymphocytes**

The other lymphocytes, or unusual suspects, as classified by Reardon et al.\textsuperscript{55} in 2005, include \gammadelta lymphocytes, NKT cells, and NK cells. These cells make up a small percentage of lymphocytes, but they seem to pack a powerful punch, and as such, should not be underestimated in disease causation or protection. \gammadelta lymphocytes possess the \gammadelta T-cell receptor and are enriched in skin and in tissues during inflammation. Many \gammadelta cells do not require antigen processing and presentation in the context of major histocompatibility complex molecules to respond to antigen and many recognize non-peptide antigens, including phosphorylated nucleotides. In the context of atherosclerosis, \gammadelta cells producing IL-17 were shown to be significantly elevated by \gammacap3-fold in aortas of apoE\textsuperscript{−/−} mice fed a Western diet for 15 weeks.\textsuperscript{53} However, we studied early atherosclerosis development in T-cell receptor \beta\textsuperscript{−/−} apoE\textsuperscript{−/−} double knockout mice and found no differences in atherosclerosis in mice lacking \gammadelta cells, suggesting that they play a minor role, at least in early atherosclerosis.\textsuperscript{56} NKT cells possess an invariant T-cell receptor that recognizes self and foreign lipid antigens presented by the class I–like molecule CD1d. The unique ability of NKT cells to recognize lipid antigens presented by CD1d suggests that they may play an important role in atherosclerosis, which is considered to be a lipid-driven disease. Most studies have reported that NKT cells are proatherogenic,\textsuperscript{57–64} including a recent study by Li et al.\textsuperscript{65} A recent review on NKT cells in atherosclerosis by
B lymphocytes play a critical role in atherosclerosis. The roles of B lymphocytes in atherogenesis have been studied less than the roles of T lymphocytes to date, but the past few years have been clearly the time of the B cell. B1 and B2 cells are 2 main families of B cells. A review article on targeting B cells in atherosclerosis was recently published in _ATVB_.

In support of this notion is a recent study by Selathurai et al. reports that depletion of NK cells in mice using anti–Asialo-GM1 antibodies attenuated atherosclerosis in ApoE−/− mice. In support of this notion is a recent human study in _ATVB_ that shows that a subset of NK cells have also been shown to be proatherogenic, so the role of B cells in atherosclerosis remains somewhat controversial. However, multiple studies support the concept that B1 cells are atheroprotective because of their production of natural IgM antibodies. A recent study in _ATVB_ showed that mice lacking Id3 had fewer B1-a cells and reduced serum levels of the natural antibody E06 that recognizes the phosphocholine headgroup of oxidized phospholipids. B1-a cell proliferation was found to be caused by Id3-mediated regulation of IL-5 production by natural helper cells, another innate lymphoid cell. In humans, CD19+ B-cell subsets have now been associated with risk of stroke. CD19+CD86+ B cells in blood were associated with higher incidence of developing stroke, and CD19+CD40+ B cells were associated with lower incidence of stroke risk in a cardiovascular cohort of the Malmo Diet and Cancer Study. Finally, Karper et al. have found a link between TLR4 signaling and B-cell numbers and activation in atherosclerosis. Taken together, these studies suggest that B-cell subsets play a critical role in atherosclerosis and mechanisms that influence B-cell function, including transcription factors and receptors, including TLRs, are likely important mediators of atherosclerosis.

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