The role of the vascular dendritic cell network in atherosclerosis

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1Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, Georgia; 2Division of Cardiology, Emory University, Atlanta, Georgia, 3Department of Pediatrics, Emory University, Atlanta, Georgia; 4Epithelial Pathobiology Research Unit, Department of Pathology and Laboratory Medicine, Emory University, Atlanta, Georgia; and 5Department of Bioinspired Science, Ewha Womans University, Seoul, Korea

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Albers-Grill N, Denning TL, Rezvan A, Jo H. The role of the vascular dendritic cell network in atherosclerosis. Am J Physiol Cell Physiol 305: C1–C21, 2013. First published April 3, 2013; doi:10.1152/ajpcell.00017.2013.—A complex role has been described for dendritic cells (DCs) in the potentiation and control of vascular inflammation and atherosclerosis. Resident vascular DCs are found in the intima of atherosclerosis-prone vascular regions exposed to disturbed blood flow patterns. Several phenotypically and functionally distinct vascular DC subsets have been described. The functional heterogeneity of these cells and their contributions to vascular homeostasis, inflammation, and atherosclerosis are only recently beginning to emerge. Here, we review the available literature, characterizing the origin and function of known vascular DC subsets and their important role contributing to the balance of immune activation and immune tolerance governing vascular homeostasis under healthy conditions. We then discuss how homeostatic DC functions are disrupted during atherogenesis, leading to atherosclerosis. The effectiveness of DC-based “atherosclerosis vaccine” therapies in the treatment of atherosclerosis is also reviewed. We further provide suggestions for distinguishing DCs from macrophages and discuss important future directions for the field.

atherosclerosis; dendritic cells; shear stress; vascular inflammation; vessel wall physiology

ATHEROSCLEROSIS IS AN INFLAMMATORY disease that typically affects arterial regions that are exposed to disturbed patterns of blood flow (d-flow), and is characterized by the infiltration of immune cells into the artery wall, followed by the development of lipid laden plaques (67, 82, 116, 124, 232). The evidence suggests that local blood flow conditions (d-flow) induce aberrant endothelial cell (EC) function (36, 162, 164) and immune cell recruitment in atheroprone regions of the arterial wall (2, 55, 66, 67), establishing a preatherosclerotic, stable immune cell infiltrate under steady-state conditions (22–24, 45, 108). The development of atherosclerosis occurs when the preatherosclerotic arterial microenvironment is coupled with other proatherosclerotic risk factors such as disruptions in gut homeostasis (237), hyperlipidemia, homocysteinemia, hypertension, diabetes, stroke, periodontal disease, and autoimmunity (194). All of these risk factors commonly produce systemic inflammation, which interacts with d-flow to promote further arterial immune cell infiltration and culminates in the establishment of a sustained, proatherogenic immune response against vascular self- and modified self-antigens (7, 83). The common presence of immune cells in the artery wall during both health and disease presents a major challenge to the field today to decipher the nature of vascular immune responses and determine how systemic and local environmental cues influence immune cell function and overall vascular inflammatory tone to either maintain a healthy, preatherosclerotic steady state, or develop atherosclerosis. A growing literature suggests that specialized innate immune cells called dendritic cells (DCs) play a key role as central orchestrators of the immune response important in the establishment and support of both proatherogenic (67, 114, 167) and regulatory (37, 212) responses within the artery wall.

ATHEROSCLEROSIS AND THE VASCULAR IMMUNE RESPONSE

Long considered to be a lipid-mediated disease, the development of maladaptive vascular inflammation and the failure of inflammation-resolving regulatory processes are now known to be key pathogenic events during atherosclerosis (217). Both the innate and the adaptive “arms” of the immune response have been shown to play important roles in atherosclerosis (7, 82, 83).

Innate Immunity and Atherosclerosis

Innate immune responses mounted by ECs, DCs, macrophages, and neutrophils play a critical role in the initiation of atherosclerosis (132). ECs express mechanosensors, which sense the physical forces associated with blood flow and pressure, and pattern-recognition receptors (PRRs) that respond to lipid and d-flow conditions by inducing expression of chemokines and adhesion molecules to recruit immune cells into the artery wall (36). A diverse array of these chemokines and their receptors have been identified which govern immune cell recruitment during atherogenesis includ-
ing C-C chemokine ligand type 2 (CCL2), macrophage inhibitory factor, C-X-C chemokine ligand type 16 (CXCL16), and C-X3-C chemokine ligand type 1 (CX3CL1) (monocyte/macrophages, DCs), and CXCL1 and CXCL8 (neutrophils) (67, 115). Disruption of PRR signaling or cell adhesion in knockout mouse studies prevents the acquisition of resident and atherosclerotic immune cell populations and blocks atherosclerosis (19, 44, 83, 149). Macrophages also express a broad repertoire of PRRs which mediate various important effector functions including extracellular cholesterol uptake, clearance of cell debris, nitric oxide synthesis, antigen presentation, and the production of atherogenic proinflammatory mediators such as cytokines and eicosanoids (83). The dependence of the atherogenic process on macrophage function is demonstrated by studies in mouse models of atherosclerosis, such as apolipoprotein E-deficient (ApoE−/−) or low-density lipoprotein receptor-deficient (LDLR−/−) mice that, in combination with macrophage-deficient osteopetrotic (op/op) mice, exhibit an almost total ablation of disease (97%) (184). Similarly, the inducible deletion of monocyte/macrophages using the CD11b-DTR mouse model significantly disrupts the development of atherosclerotic plaques in ApoE−/− or LDLR−/− mice (184, 185, 211). Neutrophils have also been shown to enter nascent atherosclerotic lesions in mice where they make a small, but significant, contribution to early, but not later, stages of the disease (55).

Adaptive Immunity and Atherosclerosis

While the innate components of the vascular immune response are clearly responsible for the initiation of atherosclerosis, agents of adaptive immunity are also present throughout the course of atherosclerosis. For instance, T-cells are recruited into the artery wall in parallel with macrophages via similar mechanisms involving chemokines and adhesion molecules (85). Both T- and B-cells develop antigen-specific immune responses against atherosclerosis-associated vascular antigens such as oxidized low-density lipoprotein (oxLDL) and heat shock protein-60 (HSP-60), which has been observed in both mice and humans (7). The lack of T- and B-cells in recombination-activating gene-deficient or severe combined immunodeficiency (scid/scid) mice leads to a marked reduction of atherosclerosis (>70%) in LDLR−/− and ApoE−/− mice (191, 250). The majority of T-cells that accumulate in the artery wall during atherogenesis are TCRβ+CD4+ cells (CD4+ T-cells) that bear an activated Th1 effector phenotype and secrete large amounts of the proinflammatory cytokine interferon-γ (IFNγ) (7, 82, 84, 242, 251). Other types of effector CD4+ T-cells such as Th17 T-cells, which secrete the proinflammatory cytokine IL-17, have also been implicated in promoting atherosclerosis (69, 221, 227). Effector CD4+ T-cells are critical for the development of atherosclerosis, and their adoptive transfer reconstitutes disease in scid/scid ApoE−/− mice (250). In humans, oxLDL- or HSP-60-reactive CD4+ T-cells have been found in both the plaques and the circulating blood of patients where they correlate positively with plaque inflammation and the incidence of clinically active disease (82, 129, 177, 210, 239). B-cells, on the other hand, play a largely protective role in atherosclerosis, especially through the production of antibodies specific for oxLDL (83). In summary, macrophage and T-cell studies clearly show that innate and adaptive immune responses are required for the development of atherosclerosis, with innate immune components playing a critical role in the initiation of disease while adaptive CD4+ T-cell responses drive lesion growth and progression.

Macrophage and T-cell Control of Atherosclerosis

While both macrophages and CD4+ T-cells are required for atherosclerosis development, both cell types represent heterogeneous cell types capable of regulating inflammation as well. Both inflammatory M1 and regulatory M2 macrophages are present in atherosclerotic plaques and can be distinguished by the cytokines they secrete upon PRR ligation (67, 68, 121, 243). M1 macrophages contribute to inflammation within atherosclerotic lesions by secreting proinflammatory cytokines such as IL-12, IL-23, IL-6, IL-1, and TNF-α, and differentiating into foam cells (67, 68, 121). M2 macrophages help regulate inflammation by clearing away cell debris (a process known as efferocytosis) and producing large amounts of anti-inflammatory IL-10 (67, 140). Similar to the dichotomy between M1 and M2 macrophages, proinflammatory CD4+ T-cell responses occur alongside regulatory CD4+ T-cell (Treg) responses. Tregs potently suppress inflammation and have been shown to inhibit atherosclerosis by secreting anti-inflammatory, antiatherogenic cytokines such as IL-10, IL-13, and transforming growth factor-β (TGF-β) (1, 17, 112, 138, 139, 154).

It is clear that innate and adaptive immunity work together in concert to drive atherosclerosis in the artery wall, and the loss of either macrophages or CD4+ T-cells potently stymies disease progression. However, specialized subsets of macrophages and CD4+ T-cells are also responsible for critical regulatory processes as well. A growing literature suggests that DCs are essential mediators in maintaining tolerance in preatherosclerotic, steady-state arteries (37, 212), which fail in the context of hypercholesterolemia and other proatherogenic stimuli and instead promote proatherogenic immunity (67, 114, 167).

DENDRITIC CELLS AND VASCULAR INFLAMMATION

DCs are innate immune cells that, while developmentally related to macrophages, play a unique role as central orchestrators of the immune response. DCs express PRRs such as Toll-like receptors (TLRs), which they use to sense pathogens, lipids, and other biomolecules (183). Along with macrophages, DCs also represent a class of professional antigen-presenting cells, which express high levels of the major histocompatibility class II (MHC-II) molecule and link innate and adaptive immune responses by presenting endogenous and exogenous antigens to T-cells. In line with their role in controlling T- and B-cell responses, DCs play an integral part in directing immune responses against pathogens and cancer cells but are also essential for the maintenance of self-tolerance and the prevention of autoimmunity (10, 11, 114, 208). DCs are a heterogeneous group of cells that share many properties with tissue macrophages including phenotype, tissue localization, and their ability to sample extracellular antigens, sense environmental injuries, and induce adaptive immune responses (11). However, DCs distinguish themselves by their unique stellate (or “dendritic”) morphology and their superior ability to migrate to the tissue-draining lymph nodes and activate both naïve and memory T-cells (46, 188).
Development and Function of DC Subsets

DCs reside in relatively low numbers throughout the peripheral tissues of the body and in greater numbers within secondary lymphoid tissues, such as the lymph nodes and spleen, as well as in specialized lymphoid tissues associated with the gut, the lungs, and the liver.

DCs consist of distinct subsets that differentiate along distinct developmental pathways and possess different abilities to process antigens, respond to environmental stimuli, and engage distinct effector lymphocytes (91). This division of labor makes it important to first understand the developmental origins of DCs to better understand how they orchestrate local immune responses in the context of a disease such as atherosclerosis. Most DCs depend on fms-like tyrosine kinase 3 (Flt3)-Flt3 ligand (Flt3L) signaling for their differentiation and development and are defined as classical or conventional DCs (cDCs) (41, 92, 157, 173) (Fig. 1).

Classical DCs derive from a common dendritic precursor cell (CDP) of the hematopoietic stem cell lineage, which traffic into tissues via the blood as cDC-restricted precursors called pre-DCs, where they then locally differentiate into DCs. Classical DCs are divided into two subsets defined by the distinct developmental programs required for their differentiation in lymphoid (lymph node and spleen) and nonlymphoid tissues (intestine, lung, liver, artery wall, etc.). Lymphoid CD8+ cDCs and nonlymphoid CD103+ cDCs arise from a differentiation program dependent on the transcription factors basic leucine zipper transcription factor, ATF-like 3 (Batf3) (94), interferon regulatory factor 8 (IRF8) (5, 200, 220), and inhibitor of DNA protein 2 (Id2) (81, 88). CD8+/CD103+ cDCs excel in the cross-presentation of cell-associated antigens to CD8+ T-cells and are thus critical to the development of CD8+ T-cell responses (92). Lymphoid tissues also contain CD4+ cDCs which require the transcription factors v-rel reticuloendotheliosis viral oncogene homolog B (Relb) (88), IRF2 (99), IRF4 (215), and recombination signal binding protein for immunoglobulin κJ region (RBPJ) (31) for development and preferentially present antigen to CD4+ T-cells (88).

Nonlymphoid tissue DCs that are functionally analogous to lymphoid CD4+ cDCs represent a heterogeneous subset commonly referred to as CD11b+ DCs. These cells commonly lack expression of the nonlymphoid cDC marker CD103 and instead express many markers common to macrophages such as signal-regulatory protein-α (SIRPα) and F4/80 (92). CD11b+ DCs are found in many nonlymphoid tissues throughout the body, and comparative gene expression studies found that

![Fig. 1. Lineage of established dendritic cell (DC) and macrophage subsets.](http://ajpcell.physiology.org/)
while CD11b+ DCs from different tissues expressed variable gene profiles, gene expression in these cells bears some resemblance to that of splenic CD4+CD11b+ cDCs (27, 150, 233). These cells develop independently of the CD103+ cDC transcription factors Batf3, IRF8, and Id2 and remain intact in the nonlymphoid tissues of mice deficient in those genes (5, 74, 81, 88, 94, 200, 226). Adoptive transfer studies have shown that CD11b+ DCs can derive from pre-DCs in vivo (74). However, the majority of nonlymphoid tissue resident CD11b+ DCs differentiate from monocytes (34, 37, 88, 199). CD11b+ DCs have been shown to differentiate in response to several growth factors, including macrophage colony stimulating factor (M-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), and Flt3L, which further reflects their heterogeneity (74, 101, 148, 198). However, more recent work suggests that all resident nonlymphoid tissue DCs including Flt3-Flt3L-dependent CD103+ cDCs and CD11b+ DCs, but not lymphoid tissue DCs, rely on GM-CSF for homeostatic proliferation within nonlymphoid tissues (77). Despite this heterogeneity in gene expression and development, CD11b+ DCs found in different nonlymphoid tissues share the ability to potentiate stimulate CD4+ T-cells, but not CD8+ T-cells (92).

**Characterizing Vascular Dendritic Cell Subsets**

DCs are present within healthy, but atherogenic, regions of the vasculature, such as bifurcations and curvatures, and accumulate in robust numbers in atherosclerotic plaques (37, 38, 45, 151, 167, 238) (Table 1). The following sections will discuss these vascular DC subsets in depth and summarize the current knowledge of their phenotype and origin.

**CD11b+ DCs.** The most abundant DCs found in mouse aorta are CD11b+ DCs, which constitute 50–60% of DCs in healthy mouse aorta (37). CD11b+ DCs localize primarily in intima where they reside in close proximity to ECs and CD103+ cDCs (37). Like CD11b+ DCs found in other nonlymphoid tissues, vascular CD11b+ DCs lack CD103 and express many common macrophage markers including CD11b, F4/80, CD14, and CX3CR1 (also known as fractalkine receptor) (34, 35, 37). Isolated aortic CD11b+ DCs have been shown to exhibit cardinal DC functions, such as the capacity to activate whole alogeneic CD4+ T-cells in vitro as efficiently as splenic cDCs (37). Typical of mature DCs, they are also nonphagocytic and exhibit stellate “dendritic” morphology in healthy, nonatherosclerotic atherosclerotic curvatures (37). CD11b+ DCs can also emigrate from atherosclerotic plaques under nonatherosclerotic conditions and travel to draining lymph nodes via the afferent lymphatics (130).

The origin of resident vascular CD11b+ DCs is difficult to determine owing to the heterogeneity of nonlymphoid tissue CD11b+ DCs, which have been shown to derive from both monocyte and pre-DC precursors (74). Aortic CD11b+ DCs express the C-type lectin DC-SIGN (CD209a) which is typically expressed by monocyte-derived CD11b+ DCs in other tissues, in addition to the M-CSF receptor (CSF1R, also known as CD115), which is expressed by monocytes and macrophages (34, 37, 199). Studies in M-CSF-deficient op/op mice, which lack monocytes and macrophages, showed a marked reduction

Table 1. Phenotype and ontogeny of currently defined vascular dendritic cell subsets

<table>
<thead>
<tr>
<th>CD103+ cDCs</th>
<th>CCL17+ cDCs</th>
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<th>CD11b+ DCs</th>
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<td>Resident?</td>
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<tr>
<td>Localization</td>
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<td>Intima; adventitia</td>
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DC, dendritic cell; CDP, common dendritic cell precursor; GMCSFR, granulocyte/macrophage colony-stimulating factor receptor; M-CSFR, macrophage colony-stimulating factor receptor (CSF1R).
in resident aortic CD11b+ DCs, consistent with a monocytic origin (37). However, the lack of monocytes and M-CSF in op/op mice did not completely ablate aortic CD11b+ DCs, suggesting that these cells can develop from pre-DCs as well, without the requirement for M-CSF. This has been found to occur in liver, lung, and kidney where adoptive transfer studies showed that CD11b+ DC populations can derive in vivo from pre-DCs in addition to monocytes (74, 92). Experiments performed in Flt3−/− mice found that Flt3 deficiency failed to impact CD11b+ DC numbers in the aorta, suggesting that resident vascular CD11b+ DCs derive primarily from the M-CSF-dependent, monocyte-dependent pathway (37). Further studies using adoptive transfer or parabiotic mouse models are required to identify whether vascular CD11b+ DCs can develop from nonmonocyte precursors in vivo.

**CD103+ classical DCs.** CD103+ cDCs represent a resident vascular DC subset with a possible immunoregulatory role in atherosclerosis. These cells are found in the intima of steady-state aorta, especially within aortic sinus, and reside in close proximity to ECs and CD11b+ DCs (37). Phenotypically, vascular CD103+ cDCs resemble CD103+ cDCs found in other nonlymphoid tissues such as small intestine, liver, and lung, and lack expression of macrophage markers like CD11b and CX3CR1 (37, 147). Similar to CD103+ cDCs in other tissues, the proportion of CD103+ cDCs among total vascular DCs ranges between 20% and 30% in steady-state aorta (37, 92). As described above, CD103+ cDCs differentiate from common dendritic precursor cells and enter tissues as pre-DCs. They depend on Flt3-Flt3L signaling for differentiation, proliferation, and survival (126, 157, 173), and as such, are absent from aorta in Flt3−/− mice (37). Comparative microarray studies suggest that as a family, CD103+ cDCs have similar gene expression profiles to splenic CD8+ cDCs, which are adept at the cross-presentation of cell-associated antigens to CD8+ T-cells, suggesting that these two DC subsets may be functionally related (150, 206). Intimal CD103+ cDCs exhibit classical DC functions showing a typical “dendritic” morphology in vivo and the ability to potently activate allogeneic T-cells in mixed leukocyte reaction assays (37). They also lack the common macrophage ability to phagocytize latex beads in vitro.

**CCL17+ DCs.** In contrast to the previously described vascular DC subsets, which are all present in the intima or adventitia of healthy arteries, recent work identified a distinct subset of aortic DCs characterized by the expression of the DC-derived chemokine CCL17 (4, 238). These cells bear a CD11c+ MHC-II+ CD11b+ phenotype but do not express CSF1R (CD115) or F4/80, differentiating them from other CD11b+ DCs, which express both of these common macrophage markers (Table 1). Interestingly, studies in CCL17-GFP reporter mice found that CCL17+ DCs are absent from the arterial wall of healthy arteries but accumulate in the intima and adventitia of atherosclerotic arteries (238). However, another study characterizing aortic DC populations found a discrete CD11b−F4/80− DC subset in steady-state aorta that expanded in response to exogenous Flt3L treatment but was only slightly reduced in Flt3−/− mice (37). As the vast majority of resident vascular CD11b+ DCs express both F4/80 and CSF1R, it is possible that these CD11b−F4/80− DCs represent CCL17+ DCs under noninflamed steady-state conditions when they do not produce CCL17. CCL17+ DCs express high levels of C-C chemokine receptor type 7 (CCR7), which is commonly expressed in DCs and is largely responsible for their ability to migrate (238). However, the origin of aortic CCL17+ DCs is still unknown.

Like other mature DC subsets, CCL17+ DCs display increased expression of costimulatory markers CD40, CD80, and CD86, suggesting that they are capable of activating CD4+ T-cells within the plaque. Indeed, multiphoton microscopy studies showed that CCL17+ DCs colocalize with CD4+ T-cells in atherosclerotic lesions and form distinct immune synapses with them (238). Direct examination of the CD4+ T-cell priming capacity of CCL17+ DCs showed that ovalbumin (Ova)-loaded CCL17+ DCs exhibited a reduced capacity to activate Ova-specific CD4+ OT-II T-cells compared with splenic cDCs (238). However, this effect was shown to depend entirely on CCL17 expression as DCs from CCL17−/−GFP/GFP mice were able to activate OT-II T-cells as effectively as splenic cDCs.

**Plasmacytoid DCs.** Nonconventional plasmacytoid DCs (pDCs) reside primarily in the arterial adventitia (167), although they are also found clustered with DCs in the shoulder regions of atherosclerotic lesions in humans (246) and mice (108). Unlike other conventional DCs, mouse pDCs express low levels of both CD11c and MHC-II and express the markers plasmacytoid DC antigen-1 (PDCA-1) and sialic acid-binding immunoglobulin-like lectin H (Siglec-H) (20). In humans, pDCs are identified by expression of IL-3 receptor-α chain (CD123) (78) and blood DC antigen 2 and 4 (BDCA-2 and BDCA-4) (56). pDCs derive directly from common dendritic precursor cells in the bone marrow in a Flt3L-dependent manner and then travel to lymphoid and nonlymphoid tissues via the blood, rather than entering peripheral tissues as a precursor cell (126, 158, 231). Development of pDCs has been shown to depend on the transcription factors E2–2 (Tcf4) (40, 72) and IRF8 (200, 226), among others (122). Comparative microarray studies show that pDCs exhibit similar gene expression profile regardless of their tissue of origin, suggesting that pDCs comprise a single DC subset of common developmental origin (150). In contrast to the previously described DC subsets, pDCs are relatively poor activators of T-cells (167). Instead, pDCs provide a potent defense against viral and some microbial infections via their unique ability to produce large amounts of potent proinflammatory cytokines termed type I interferons, especially IFNα (104, 110, 111).

**Challenges Discriminating Between DCs and Macrophages.** Distinguishing macrophage and DC subsets in the vascular wall has proven difficult as several vascular DC subsets express classic monocyte/macrophage markers such as CD11b, F4/80, CX3CR1, SIRPα, and Ly-6C (37, 92, 97, 114, 187, 238). Vascular DCs have been commonly defined as CD11c−MHC-II− cells in the atherosclerosis literature, regardless of their expression of macrophage markers (68, 114). However, this definition is not ideal as CD11c−MHC-II− macrophage populations have been reported previously (88). Additionally, monocytes and macrophages have been shown to upregulate the classical DC marker CD11c under hypercholesterolemic conditions (76, 245), and MHC-II expression during inflammation (114, 262), making it likely that CD11c−MHC-II− macrophages also exist in atherosclerotic lesions. The overall promiscuity of phenotypic markers makes phenotyping alone insufficient for the identification of novel
vascular DC subsets. Thus, phenotypic characterization should be validated by functional and morphological analyses (Table 2).

To summarize, it is clear that at least two and possibly three distinct conventional DC subsets reside in the intima of healthy, atheroprone arterial regions, which all possess the ability to efficiently activate naïve T-cells (Table 1). These include the following, in order of abundance: 1) CD11b+ DCs, which are similar to CD11b+ DC populations in other tissues in their expression of macrophage markers F4/80, CSF1R, and CX3CR1; 2) CD103+ cDCs, which are developmentally related to splenic CD8+ cDCs; and 3) a third, poorly defined CD11b+F4/80− DC subset which bears a phenotypic resemblance to CCL17-producing DCs. Further work is needed to define the phenotype and function of resident CD11b+F4/80− DCs and to determine whether these are the same as CCL17-producing DCs found in atherosclerotic plaques. Also, further work must be done to elucidate the developmental pathways responsible for CD11b+ DC differentiation as this information may provide good animal models for the subset-specific depletion of these heterogeneous nonlymphoid tissue DCs.

**Establishment of vascular dendritic cells in flow-disturbed arterial regions**

DC infiltration in the vasculature is a localized phenomenon dependent on EC responses to blood flow conditions. Atheroprone regions of the vasculature differ from athero-resistant regions in the physiological blood flow patterns and hemodynamic forces they are exposed to, with atherosclerosis preferentially occurring in branches and curvatures where blood flow is disturbed and exerts nonuniform and irregular shear forces on the vascular endothelium (36). Disturbed flow results in several key changes in endothelial cell biology and function that alter the normal inflammatory status of the endothelium, allowing these regions to preferentially acquire resident DC and macrophage populations (36, 38, 108, 176). There is extensive evidence showing that exposure to d-flow induces EC inflammation, leading to the upregulation of adhesion molecules such as intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) (57, 100, 161, 162, 214, 235). These molecules mediate leukocyte adhesion to inflamed endothelium and are essential for leukocyte entry into the arterial intima under steady-state and disease conditions (44, 108). In vivo studies of d-flow show numerous changes in EC gene expression in response to experimentally induced d-flow in the carotid artery (163, 164). In addition to upregulation of ICAM-1 and VCAM-1, d-flow also induces expression of various chemotactic molecules necessary for early leukocyte homing to the arterial wall. These include CCL2 (MCP-1), which is responsible for recruitment of monocytes and some DCs to the arterial wall (203, 225), CCL9, a chemokine involved in CD4+ T-cell trafficking (86, 153) and in attracting CD11b+CCR1+ DCs to the gut (248), CXCL16, which has been implicated in recruiting T-cells, DCs, and macrophages into atherosclerotic lesions (65), and CXCL12, which is expressed in human and mouse atherosclerotic lesions but whose role in atherosclerosis remains unclear (115). Other important chemokines have been found that bring DCs into the artery wall, such as CCL5 (RANTES) and CX3CL1 (fractalkine) (33, 128, 247).

Electron microscopy studies show that intimal DCs are organized into subendothelial cell networks along with T-cells and resident macrophages in flow-disturbed regions of human large arteries such as the aorta, carotid and coronary arteries (Fig. 2) (23, 25, 241, 242). DCs have also been observed in the steady-state aortas of mice that reside adjacent to ECs and extend dendritic processes into the lumen, presumably to sample antigen in the blood (38). DCs, with their long dendritic morphology, represent a key, organizing element of the vascular cell network, integrating small groups of vascular immune cells and screening vascular tissue and the blood for potentially harmful antigens (241, 242). Several studies have shown that

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<th>Table 2. Salient features necessary for the proper identification and characterization of vascular dendritic cell subsets</th>
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<td>CD26</td>
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<td>PDCA-1</td>
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2. Expression of Inducible Markers of DC Maturation
   - CD40  
   - CD80  
   - CD86  
   - CD83  

3. DC-Specific Morphological Characteristics
   - Exhibit classic, stellate, "dendritic" morphology  
   - Potent activation of naïve, effector, and memory T cells;  
   - Promotion of antiatherogenic Th2 responses vs. proatherogenic Th1/Th17 responses;  
   - Ability to migrate from peripheral tissue to lymph nodes.

DC, dendritic cell; MO, monocyte; MΦ, macrophage.
beyond pathogen screening, immature DCs serve an important and integral role in the tolerization of immune responses against autoantigens by regulating local tissue inflammation (51, 180). In recent years, a more substantial understanding of how resident DCs and macrophages maintain vascular homeostasis has emerged.

**ROLE OF DENDRITIC CELLS IN VASCULAR HOMEOSTASIS**

Resident leukocyte populations play a critical role in the homeostasis of numerous tissues in the body including the gut, liver, and lungs (92, 222). It has been previously postulated that vascular DCs play an important role in the maintenance of arterial homeostasis as well (22, 26, 242). As central mediators of proinflammatory effector T-cell and anti-inflammatory Treg responses, it is likely that DCs serve as regulators of vascular inflammation and are important both in the maintenance of vascular homeostasis and in the initiation and progression of atherosclerosis. Overall, the vascular DC network seems to play three key roles in resolving inflammation in the arterial wall, as follows: 1) the uptake and storage of intimal and circulating lipids; 2) the clearance of lipids, apoptotic cells, and debris from the artery wall via a migratory mechanism; and 3) the maintenance of vascular Treg responses (Fig. 3). These roles parallel the already well-established functions of DCs, including antigen uptake, processing, and presentation to T-cells (lipid uptake and Treg activation); and the migration of nonlymphoid DCs to lymphoid organs to prime T-cell responses (lipid/inflammatory mediator clearance and Treg priming).

**Role of Vascular DCs in Lipid Metabolism**

Apolipoprotein B (ApoB)-containing lipids such as LDL are a potent inflammatory stimulus in the artery wall, and the retention of these inflammatory lipids in the arterial intima is widely recognized as one of the earliest stages in the initiation of atherosclerosis (219). Early intimal lipoprotein retention occurs as a natural consequence of EC responses to d-flow (36) and is aggravated by hypercholesterolemia (219). Several lines of evidence point to an important role for DCs in regulating circulating cholesterol and LDL levels in normo- and hypercholesterolemic mice. First, in studies in which DC survival and lifespan were enhanced by transgenic expression of the antiapoptotic gene hBcl-2 under the CD11c promoter, hBcl-2-DCs were found to decrease plasma lipids in both LDLR−/− and ApoE−/− mice, even under hypercholesterolemic conditions (70). Conversely, DC ablation using the CD11c-DTR mouse model increased blood lipids (70). The decreased plasma lipids seen in hBcl-2-DC mice also corresponded with a slight reduction in atherosclerotic plaque volume and lipid content in both mouse models despite enhanced hyperlipidemia-induced Th1 T-cell and anti-oxLDL B-cell responses (70). These data imply that DC-dependent metabolism of vascular lipids helps to control inflammation locally in the artery wall, although vascular inflammatory cytokine production was only tested in spleen and not in aorta.

A series of en face immunostaining studies suggest that DC-dependent control of plasma cholesterol is mediated by vascular DCs that reside within the aortic intima of normolipidemic LDLR−/− mice (176, 254). These resident DCs proliferated in situ in a GM-CSF-dependent manner within days after exposure to a hyperlipidemia-inducing cholesterol-rich diet, actively took up lipid, developed characteristic foam cell morphology, and gained expression of the foam cell marker CD68. DC depletion in CD11c-DTR mice disrupted this proliferative DC-foam cell response, while blockade of monocyte recruitment by pertussis toxin treatment was ineffective, suggesting a cDC-mediated process (176, 254). Indeed, modified LDL exposure induces GM-CSF expression in endothelial cells (186), and GM-CSF treatment expectedly downregulates serum cholesterol (102). This cholesterol-regulating role of DCs likely influences the establishment of vascular inflammation during atherogenesis as hypercholesterolemia is a major risk factor for atherosclerosis. How this DC-mediated function influences the development of atherosclerosis is still unclear as GM-CSF deficiency decreased atherosclerosis in inducibly
hyperlipidemic LDLR<sup>−/−</sup> mice (205), but aggravated the disease in spontaneously hyperlipidemic ApoE<sup>−/−</sup> mice (52), while effectively curtailing CD11c<sup>+</sup> cells that are phagocytized by M2 macrophages or possibly by immature DCs as part of the inflammation-resolving process of efferocytosis (2). Under hyperlipidemic conditions, lipid uptake and efferocytosis likely lead to DC-fom cell formation, and mature DCs and foam cells emigrate from the vessel wall in a C-C chemokine receptor type 7 (CCR7)-dependent manner, clearing inflammatory cells, lipids, and apoptotic cell debris from the intimal space and preventing necrosis and persistent inflammation (3). Vascular Treg numbers are maintained by mature CD103<sup>+</sup> cDCs and pDCs, which induce Tregs from naïve CD4<sup>+</sup> T-cells by secreting retinoic acid (RA) and indoleamine 2,3-dioxygenase (IDO). Proinflammatory T-cell function is regulated by Treg-produced IL-10 and transforming growth factor-β (TGF-β), maintaining an intimal microenvironment conducive to the polarization of regulatory M2 macrophages, which in turn secrete more anti-inflammatory cytokines (4). Blue lines indicate homeostatic functions that occur under steady-state conditions. Magenta lines indicate functions that are thought to occur under steady-state conditions, but play a proinflammatory role in atherogenesis. MHC-II, major histocompatibility complex class II; CCL, C-C chemokine ligand; HSP-60, heat shock protein-60; Treg, regulatory T-cell; effector T-cell (Teff).

**DC Migration and Efferocytosis Resolve Vascular Inflammation**

The resolution of vascular inflammation, and probably lipid metabolism as well, is closely tied to the related processes of DC migration and efferocytosis, by which lipids, apoptotic cells, and other proinflammatory debris are phagocytosed and removed from the artery wall (130, 187, 217). Migratory inflammatory cells bearing a phenotypic resemblance to CD11b<sup>+</sup> DCs have been shown to constitutively leave the artery wall and migrate to the draining lymph nodes under steady-state conditions as well as during atherosclerosis regression (130, 224). One study found that during experimentally induced plaque regression in a surgical transplant model, lipid-laden foam cells upregulated the DC-typic chemokine receptor CCR7 and migrated through adventitial lymph vessels to local lymph nodes, leading to lesion regression and the egress of intimal lipids (224). Inhibition of CCR7 signaling using antibodies against CCR7 ligands CCL19 and CCL21 inhibited DC-foam cell egress and prevented lesion regression (224), suggesting that CCR7-mediated DC egress plays a major role in resolving vascular inflammation through the removal of inflammatory lipid components. Comparative genomics studies have found that CCR7 expression is restricted to DC subsets within the myeloid lineage (150), supporting data which suggest that the majority of lesional foam cells are in fact DCs (176). Migratory vascular DCs have also been credited with the removal of cell debris and apoptotic cell bodies (a process known as efferocytosis), which is critical for the resolution of inflammation (118, 187, 204, 217). In the context of atherosclerosis, efferocytosis prevents postapoptotic secondary necrosis, which supplies the artery wall microenvironment with further proinflammatory mediators, leading to necrotic core formation (201, 216). In the literature, efferocytosis has primarily been attributed to M2 macrophages (217). However, the developmental and phenotypic overlap between CD11b<sup>+</sup> DCs and macrophages may entail overlapping functional roles as well. Other work suggests that immature DCs, which still retain their phagocytic capacity (10), enter the artery wall where they participate alongside M2 macrophages in the effe-
rocytosis of intimal apoptotic cells (229, 230) and induce tolerogenic immune responses as suggested by in vitro studies (234). Apoptotic cells have been found in the phagosomes of DCs in the afferent lymphatics (59), suggesting that DC-mediated effrocytosis does occur in the vasculature. Another possibility is that phagocytic monocytes may transform into DCs to transport cell debris to lymphoid organs (189), which is particularly relevant in the context of intimal lipid retention, where oxLDL has been shown to induce phagocytic monocyte differentiation into mature DCs that secrete IL-12 and effectively stimulate T-cells (178).

The contribution of CCR7-dependent constitutive DC migration to tissue homeostasis has been demonstrated in other tissues such as the intestines (96, 136) and the skin (170), where the maintenance of immune tolerance relies on the constitutive transport of commensal bacteria (in intestine) and apoptotic cells (in skin and intestine) to draining lymph nodes by DCs (62, 79, 170). CCR7-deficient mice exhibited markedly reduced intestinal DC migration and impaired oral tolerance (244), emphasizing that DC migration and presentation of auto-antigens to draining lymph node T-cells in the steady state is an essential aspect of peripheral immune tolerance.

Not much is known of how vascular DCs maintain tolerance against proatherogenic auto-antigens such as HSP-60 and oxLDL in the artery wall. The breakdown of effrocytosis and foam cell emigration is a key step in the pathogenesis of atherosclerosis that precedes the chronic persistence of vascular inflammation (217). However, atheroerosclerosis studies in CCR7-deficient mice have yielded conflicting results. One study found that atheroerosclerosis was attenuated in CCR7−/−LDLR−/− mice (131), while a more recent study found that CCR7 deficiency increased atheroerosclerosis severity in ApoE−/− mice by 80% (236). However, both studies also found that CCR7 deficiency disrupted T-cell homing during atherogenensis, which may further complicate disease outcome in a DC-independent manner. Tissue-specific knockout of CCR7 in CD11c-expressing cells would be a particularly useful model to explore the role of CCR7-mediated DC trafficking in the maintenance of vascular tolerance.

Role of DCs in Maintaining Vascular Treg Responses

Lastly, DCs have been shown to support vascular Treg responses within the artery wall. As discussed earlier, Tregs are vitally important to the control of inflammation based on their ability to secrete anti-inflammatory cytokines and inhibit local, proatherogenic Th1 T-cell responses (1, 17, 112, 138, 139, 154). Decreased Treg function occurs under conditions of chronic hypercholesterolemia, and the gradual loss of vascular Tregs corresponds well with the development of atherosclerosis (137). A strong role has been shown for resident intimal CD103+ cDCs in maintaining vascular Treg homeostasis. As discussed above, the lack of Flt3 results in the loss of these Flt3/Flt3L-dependent cDCs in aorta and other tissues (37, 126, 157, 173). In athertonpro Flt3−/−LDLR−/− mice, loss of CD103+ cDCs led to decreased aortic Tregs, reduced IL-10 production, and increased proinflammatory cytokine production (IFNγ, TNF-α), which aggravated atheroerosclerosis development (37).

Interestingly, studies of DC function in lung and skin show that CD103+ cDCs are effective at cross-presenting antigens to CD8+ T-cells, but not to CD4+ T-cells like Tregs, whereas CD11b+ DC subsets in both these tissues are effective at activating CD4+ T-cells (15, 29, 48). The evidence suggests that CD103+ cDCs support Tregs within tissues via the factors and cytokines they secrete. For instance, CD103+ cDCs in the lung uniquely secrete high levels of CCL22, a chemokine important in the recruitment of Tregs and Th2 T-cells (14), and thus may play a role in attracting Tregs into the vessel wall. CD103+ cDCs are also known to reside within the intestinal mucosa, where they excert at inducing the conversion of naïve Foxp3+ T-cells into Foxp3+ Tregs in the presence of TGF-β (43, 49, 50, 213). Several lines of evidence suggest that CD103+ cDC-derived retinoic acid is responsible for Treg induction in the intestines (43, 50, 105, 213, 222), although more work is needed to determine whether CD103+ cDC-derived retinoic acid plays an important role in promoting vascular Treg responses (192). This casts a potential role for CD103+ cDCs in attracting circulating Tregs to the vessel wall and/or inducing the conversion of local naïve T-cells into Tregs. In contrast, CD11b+ DCs have been implicated in the local expansion of both effector T-cells and Tregs as shown in the dermis (146), suggesting that CD11b+ DCs may contribute to vascular Treg homeostasis as well.

Similar to the way CD103+ cDCs support Treg homeostasis through indirect mechanisms of CCL22 and retinoic acid secretion, an atheroprotective pathway has been identified in pDCs which relies on the synthesis and release of indoleamine 2,3-dioxygenase (IDO) in response to interferon signaling (107, 182). IDO release from pDCs has been shown to facilitate the differentiation of Tregs from naive CD4+ T-cells and can control effector T-cell responses by influencing their tryptophan metabolism (107, 182). In light of the proatherogenic effects of pDC-produced IFNα, this implies a more complex, homeostatic role for pDCs in regulating atherosclerosis. Indeed, multiple studies using pDC depletion techniques have produced conflicting results. In one study, investigators found that pDC depletion aggravated atherosclerosis development and led to increased T-cell proliferation in atherosclerotic lesions (47), suggesting a regulatory role for pDCs in atherosclerosis. Further work suggests that atheroprotective pDC function is IDO dependent, as therapeutic enhancement of IDO expression by oral administration of eicosapentaenoic acid inhibited conventional DC maturation and resulted in diminished lesional effector T-cell numbers and substantial lesion regression (160). However, a second study found that pDC depletion significantly decreased early lesion development and that specific stimulation of pDCs with type A CpG oligonucleotides led to increased disease (53). Together, these data demonstrate a complex, context-dependent role for pDCs in mediating vascular inflammation, requiring further research to determine how pDCs influence vascular inflammatory tone in response to biologically relevant microenvironmental cues.

The importance of DC-dependent vascular Tregs in maintaining the inflammatory tone of the artery wall is demonstrated by a recent study in which DC maturation, which is critical for T cell activation, was inhibited by the deletion of the common PRR signaling protein MyD88 in CD11c+ cells. Transplant of CD11c-MyD88−/− bone marrow into LDLR−/− mice fed a high-fat diet inhibited both inflammatory effector CD4+ T-cell and atheroprotective Treg responses in aorta but led to an unexpected increase in atherosclerosis due to the increased recruitment of Ly-6Chi monocyte-derived inflammatory cells (212). This surprising finding highlights the impor-
tance of vascular Treg-derived cytokines such as IL-10 in controlling both innate and adaptive immune responses in the artery wall. In addition to targeting proatherogenic CD4+ T-cells, IL-10 also targets M1 macrophages and DCs, suppressing the production of proinflammatory cytokines (IL-1, IL-6, IL-12, and TNF-α) (155, 217) and stimulating the conversion of M1 macrophages to the M2 subtype (143). IL-10 also enhances other homeostatic functions of DCs and macrophages such as increasing efferocytosis both in vivo and in vitro (125), which in turn promotes further IL-10 production (98). A result of this positively reinforced feedback loop is that IL-10 is potent at resolving inflammation and controlling atherosclerosis. For instance, when IL-10 transgenic bone marrow cells were transferred into fat-fed LDLR−/− mice, there was a 47% decrease in lesion size and an 80% decrease in necrotic core area (179).

Together, these findings establish vascular Treg responses as a necessary element in the resolution of vascular inflammation by inhibiting inflammatory cytokine production among CD4+ effector T-cells and M1 macrophages, while supporting other homeostatic functions of DCs and macrophages such as efferocytosis.

LOSS OF HOMEOSTATIC DC FUNCTION DURING ATHEROGENESIS

The combination of d-flow-induced EC inflammation and lipid retention presents a significant hurdle to the resolution of inflammatory responses in the artery wall (219). D-flow disrupts vascular homeostasis in several other ways important to the development of atherosclerosis, increasing endothelial permeability to macromolecules such as lipoproteins, increasing EC and smooth muscle cell turnover and proliferation, promoting EC expression of procoagulatory molecules such as von Willebrand factor and tissue factor, which leads to enhanced platelet aggregation and fibrin deposition and promotes tissue inflammation (28, 36, 73, 119). In particular, the d-flow-induced retention of ApoB-containing lipoproteins, such as LDL, presents a potent trigger of the chronic inflammatory response, especially when these lipids are modified by oxidation (217, 219). A new understanding of the molecular mechanisms by which vascular DC homeostasis is disrupted during atherogenesis is emerging in the literature (Fig. 4).

Several key events have been identified that are required for the artery wall to switch from a steady-state microenvironment to a proatherogenic one. First, ApoB-containing lipids are

Fig. 4. Disruption of vascular DC homeostasis during atherogenesis. During atherogenesis, increased subendothelial retention of inflammatory lipid mediators (oxLDL), pathogen-associated molecular patterns (PAMPs), and damage-associated molecular patterns (DAMPs) drives increased vascular DC expansion and maturation. DC maturation blocks the priming of Tregs and silencing of effector CD4+ T-cells by immature/semimature DCs. CCL17-producing DCs also inhibit vascular Tregs. pDC-derived IFNs and T-cell-derived IFNγ induce inflammatory M1 macrophage polarization and sensitize DCs and effector CD4+ T-cells. Oxidized LDL directly downregulates CCR7 expression and blocks DC emigration. This leads to foam cell retention, increased apoptosis, and necrosis, which releases sequestered inflammatory lipids and DAMPs, furthering inflammation in the artery wall. Blue lines indicate antiatherogenic functions. Red lines denote proatherogenic processes.
Pattern Recognition Receptors, DCs, and Atherosclerosis

Tissue inflammation begins with signaling through PRRs, which sense pathogen- and injury-related “danger” signals by recognizing structurally conserved molecules derived from viral and microbial pathogens or by oxidation of endogenous nuclear and cytosolic proteins following tissue injury (106, 218). Pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) are responsible for initiating infectious pathogen and noninfectious inflammatory responses, respectively. PAMPs include classical activators of DCs such as bacterial lipopolysaccharides (LPS), peptidoglycans, unmethylated CpG motifs, and viral nucleic acid variants, while DAMPs include necrotic cells, heat shock proteins, and extracellular matrix breakdown products (16, 167). Several classes of PRRs have been characterized, including NOD-like receptors and TLRs (117). In particular, TLR signaling serves as a crucial checkpoint for DC maturation and the production of immunostimulatory cytokines (11, 145, 223).

Role of TLR signaling in DC maturation and atherosclerosis. A large body of evidence points to TLR signaling pathways as critical mediators of inflammation during atherogenesis, and disruption of TLR signaling potently inhibits atherosclerosis (63). More specifically, in vivo or in vitro stimulation of DCs with TLR ligands (e.g., LPS, CpGs, etc.) induces DC maturation and activation, which includes the robust upregulation of peptide-MHC-II complexes on the cell surface and de novo expression of costimulatory molecules CD40, CD80, and CD86, which are required for the productive activation of naïve CD4+ T-cells (167, 181). In the absence of TLR stimulation under steady-state conditions, immature or semimature IL-10-producing DCs capture apoptotic bodies arising from cell turnover and silence inflammatory T-cells to self-antigens upon antigen presentation (134, 209). These IL-10-producing DCs have also been shown to prime anti-inflammatory Tregs as well (134).

TLR stimulation in DCs also induces the production of immunostimulatory cytokines. DC-derived CCL19 and CCL21 have been implicated in the recruitment of CCR7-expressing DCs and naïve T-cells (167). Additionally, DCs secrete IL-12, which induces the differentiation of proatherogenic IFNγ-producing Th1 T-cells and upregulates T-cell expression of CCR5, which leads to T-cell accumulation within atherosclerotic plaques (247). Thus, by stimulating DC maturation and immunostimulatory cytokine production, TLR ligands effectively convert the tolerogenic microenvironment of the steady-state artery to the proinflammatory milieu of the atherogenic artery.

The potent capacity of TLR signaling to disrupt vascular homeostasis is well demonstrated in knockout mouse models of atherosclerosis. Knockout of MyD88, a signaling molecule downstream of most TLRs, was shown to reduce lesion volume by up to 65% in fat-fed ApoE−/− mice (19). Specifically, signaling through TLR2 and TLR4, which recognize bacterial lipoproteins and LPS, respectively, is of particular importance to atherosclerosis prevention (63). TLR2 and TLR4 are likely responsible for many of the proatherogenic effects of ApoB-containing lipoproteins and heat shock proteins, as these molecules are also capable of binding to TLRs. Oxidized LDL and several other oxidized phospholipid species have been shown to ligate TLR2 and induce angiogenesis and apoptosis in endoplasmic reticulum-stressed vessel wall cells (202, 240). Minimally modified LDL effectively binds to TLR4 (83), and HSP-60 and HSP-70 also signal through TLR2 and TLR4 to stimulate DC maturation and cytokine production (16, 63, 207).

Knockout of either TLR2 or TLR4 inhibits atherosclerosis development in both ApoE−/− and LDLR−/− mice, reduces circulating levels of IL-12 and CCL2, and curbs monocyte recruitment into the artery wall (149, 156). Meanwhile, exposure to the synthetic TLR2 ligand Pam3 greatly enhances lesion volume in LDLR−/− mice in a dose-dependent, TLR2-dependent manner (63).

Role of infection in atherosclerosis. Viral and bacterial infections have also been shown to contribute to the development of atherosclerosis. Numerous pathogenic organisms have been linked to atherosclerosis in both clinical and animal studies [reviewed in ref. 195]. Studies in experimental mouse models have found that infection with several different pathogens, such as Porphyromonas gingivalis, Chlamydia pneumoniae, and Helicobacter pylori, accelerates atherosclerosis, leading to increased lipid and leukocyte accumulation in lesions and increased proinflammatory cytokine production (9, 21, 30, 32, 58, 60, 61, 75, 89, 90, 95, 113, 123, 127, 135, 152, 195, 196). While many infectious pathogens are capable of manipulating immune responses to prolong survival (197), it is likely that much of the proatherogenic effect of infection is mediated through TLR pathways. For instance, C. pneumoniae-accelerated atherosclerosis was shown to depend on TLR2 signaling (159). Whether or not other proatherogenic pathogens also promote disease via TLR signaling remains to be determined. However, in humans, clinical studies have shown that prior infection with atherosclerosis-associated pathogens correlates positively with atherosclerotic coronary artery disease independent of traditional risk factors (253). Furthermore, the link between infectious disease and atherosclerosis is supported by evidence from large population-based studies, which have found molecules specific to at least 20 different pathogenic microorganisms present in the plaques of large subsets of the patient population (195). Together these data indicate that PAMPs derived from atherosclerosis-associated pathogens do localize to atherosclerotic plaques in humans where they can potentially trigger TLR signaling and induce inflammation.

DCs express subset-specific TLR expression. Nonlymphoid tissue DCs express distinct subset-specific repertoires of TLRs, suggesting that specific vascular DC subsets may contribute to TLR-mediated inflammation during atherogenesis. For instance, TLR2 and TLR4 are expressed by CD11b+ DCs, but...
not by CD103+ cDCs, which express TLR3 (150), implicating CD11b+ DCs in the initiation and propagation of vascular inflammation, in response to hypercholesterolemia. Unlike TLRs 2 and 4, which play a strong proatherogenic role, studies of TLR3 have provided mixed results, with one study in ApoE−/− mice showing an atheroprotective role for TLR3 and another study showing that the transplant of TLR3+ bone marrow reduced atherosclerotic plaque burden in fat-fed LDLR−/− recipients (42, 133).

pDCs have also been implicated in atherosclerosis via their expression of TLR7, TLR8, and TLR9, which recognize nucleic acid motifs derived from viral pathogens and mediate IFNα production in response to viral infection (167). IFNα has been shown to enhance atherosclerosis in mice by amplifying the inflammatory capacity of several different cell types present during atherosclerosis (53, 110, 120). In addition to inducing the maturation of conventional, T-cell activating DCs (103) and skewing CD4+ T-cells towards the proinflammatory Th1 effector phenotype (110), IFNα was shown to stimulate IFNγ production and upregulate expression of the death receptor ligand, TNF-related apoptosis inducing ligand (TRAIL) in CD4+ T-cells, resulting in T-cell-mediated killing of vascular smooth muscle cells (166). Furthermore, exposure of conventional DCs to IFNα induced the upregulation of TLR4, sensitizing them to bacterial LPS and modified self-lipid antigens (minimally modified LDL), and increased production of the proinflammatory cytokines TNF-α, IL-12, and IL-23 (166).

In the case of intracellular, antiviral TLRs, it is unclear whether endogenously derived ligands exist within the artery wall as is the case for TLR2 and TLR4. However, viral DNA or RNA from several viruses that infect 80–90% of the human population (i.e., Cytomegalovirus, Hepatitis C, Epstein Barr virus, Herpes simplex viruses, as well as parvoviruses and enteroviruses) have been identified in human plaques (195), suggesting that viral pathogen-derived ligands are readily available to vascular CD103+ cDCs and pDCs.

Role of oxLDL in DC Function and Migration During Atherosclerosis

According to the lipid retention hypothesis of atherosclerosis, oxLDL plays a pivotal role through the induction of foam cell formation, alteration of nitric oxide signaling, initiation of EC activation, and expression of adhesion molecules that accelerate leukocyte homing to the site of atherosclerosis (144, 219). Oxidized LDL appears to play a major role in disrupting homoeostatic DC function as well, which may explain why hypercholesterolemia is so effective at inducing atherosclerosis. Exposure of DCs to oxLDL induces maturation (MHC-II expression, CD40/CD80 expression) and increases their ability to activate naïve T-cells (3). Interestingly, oxLDL can induce monocyte differentiation into mature CD11b+ DCs that secrete IL-12 and effectively stimulate T-cells (178). At high concentrations, highly oxidized LDL is able to induce DC apoptosis as well (3). Together these data indicate that oxLDL is capable of directing DC differentiation, maturation, and priming of oxLDL-specific CD4+ T-cells, which may explain why it dominates the proatherogenic vascular immune response.

CD11b+ DC migration is also impacted by oxLDL exposure. Several studies have shown that the emigration of vascular CD11b+ DCs is disrupted in hypercholesterolemic ApoE−/− mice, leading to rapid intimal lipid accumulation and foam cell formation (130, 176). Aortic transplant studies in mice have shown that under hypercholesterolemic conditions, deficient DC emigration out of the vessel wall led to rapid DC/foam cell accumulation, and atherosclerosis (130, 187, 224). Aortic inflammation is resolved following aortic transplant from diseased ApoE−/− donors into normcholesterolemic C57/BL6 recipients and induces lesion regression characterized by the emigration of large numbers of DCs/foam cells to draining lymph nodes (130, 187, 224). Hypercholesterolemia has global effects on DC function, severely inhibiting DC migration, while stimulating DC maturation and local inflammation in peripheral tissue sites such as the skin (8). Exposure to oxLDL is likely responsible for this disruption in DC trafficking as mature DCs have been shown to downregulate CCR7 upon oxLDL challenge, which directly disrupts their ability to emigrate from the vessel wall (165, 224). Similarly, antibody-mediated inhibition of CCR7 ligands CCL19 and CCL21 block DC-foam cell egress in an aortic transplant model of lesion regression and also prevent lesion regression (224), stressing the importance of oxLDL-mediated suppression of CCR7 signaling in preventing the resolution of vascular inflammation during atherogenesis. However, it is not known what concentrations of intimal oxLDL are required to suppress CCR7 expression in DCs in vivo. Additionally the specific receptors and signaling pathways by which oxLDL controls CCR7 expression have yet to be determined.

DC Disruption of Treg Homeostasis During Atherosclerosis

Vascular Tregs form a critical line of defense against persistent inflammation in the artery wall. As discussed earlier, DCs play an integral role in priming protective vascular Tregs (146, 212), in recruiting them into tissues (14), and in inducing Treg differentiation from naïve CD4+ T-cells (43, 49, 50, 213). New evidence suggests that specialized CCL17-producing vascular DCs (CCL17+ DCs) are responsible for the disruption of Treg homeostasis in aorta during atherogenesis in ApoE−/− mice. As discussed above, studies in CCL17-EGFP reporter mice showed that CCL17 production is unique to a subset of vascular DCs that differ phenotypically from both CD103+ cDCs and CD11b+ DCs based on their expression of CD11b and notable lack of other common CD11b+ DC markers such as F4/80 and CSF1R. More interestingly, active CCL17 production only occurred in the atherogenic ApoE−/− aorta during diet-induced hypercholesterolemia and was absent from the vessel wall in chow-fed, nondiseased animals (238). The CCL17 produced by these DCs was shown to disrupt Treg proliferation both in vivo and in vitro, which ultimately resulted in the loss of functional aortic Tregs under conditions of chronic hypercholesterolemia and the development of atherosclerosis (238), which has also been demonstrated elsewhere (137). Ablation of this anti-Treg function by disruptive insertion of Egrf into both alleles of the Ccl17 gene led to reduced atherosclerosis and decreased accumulation of macrophages and CD4+ T-cells within lesions in CCL17-deficient mice. Additionally, Treg presence and expansion was increased within lesions of CCL17-deficient mice (238). Further exploitation of the mechanism of CCL17 action on Tregs suggests that CCL17 binds to CCR4 expressed on the surface of naïve T-cells and blocks their capacity to differentiate into Tregs.
and LDLR. This surprising finding was later confirmed in both ApoE-deficient rabbit models of atherosclerosis (6, 168, 174).

The effects of native LDL or oxLDL immunization were first limiting atherosclerosis development in mice. The protective strategies directed against proatherogenic inflammatory mediators such as oxLDL, HSP-60, and HSP-65 has proven effective in mouse models. A better understanding of vascular DC development and function in vivo may help us to better understand the mechanisms by which vaccine-based and DC-based immunotherapies work and provide novel diagnostic and therapeutic tools to answer important questions facing the field today.

FUTURE DIRECTIONS AND THERAPEUTIC IMPLICATIONS

On the basis of their broad role in controlling vascular inflammatory responses in both steady-state and atherosclerotic arteries, vascular DCs make an attractive target for antiatherogenic therapies. Indeed, immunotherapeutic vaccination strategies directed against proatherogenic inflammatory mediators like ApoB-containing lipoproteins and heat shock proteins have been fairly successful at limiting atherosclerosis in mouse models. A better understanding of vascular DC development and function in vivo may help us to better understand the mechanisms by which vaccine-based and DC-based immunotherapies work and provide novel diagnostic and therapeutic tools to answer important questions facing the field today.

Immunization in Atherosclerosis

Immunization against atherosclerosis-related autoantigens such as oxLDL, HSP-60, and HSP-65 has proven effective in limiting atherosclerosis development in mice. The protective effects of native LDL or oxLDL immunization were first demonstrated in rabbit models of atherosclerosis (6, 168, 174). This surprising finding was later confirmed in both ApoE−/− and LDLR−/− models of murine atherosclerosis (39, 64, 71, 249). The effectiveness of anti-oxLDL vaccines has been shown to depend on the ability of DCs to initiate protective Th2 CD4+ T-cell and Treg responses, as well as neutralizing antibody responses (mediated by T-cell-dependent B-cell activation) against immunogenic phosphatidylcholine (PC) and ApoB100 residues present in LDL (80, 93, 169). Indeed, immunization strategies involving the adoptive transfer of oxLDL-loaded DCs along with an adjuvant have proven to be an extremely effective therapy, achieving upwards of an 80% reduction in lesion volume in mice (80). Furthermore, immunization of mice with a PC-containing Streptococcus pneumoniae vaccine induced a neutralizing PC-specific antibody response and reduced atherosclerosis in ApoE−/− mice (18), suggesting that immunization regimens against auto-antigenic components tend to produce tolerizing responses against those self-antigens, bolstering homeostatic processes such as Treg function. Similar atheroprotective effects have been seen by immunizing against other atherosclerosis-associated antigens such as HSP-60 and HSP-65 (87, 141, 228). It is also apparent that the context of adaptive immune responses against these antigens can be readily influenced by exposing DCs to anti-inflammatory cytokines like IL-10. One study found that immunization with IL-10-treated DCs loaded with immunogenic oxLDL component ApoB100 created “tolerogenic” DCs that inhibited atherosclerosis (93). Thus, therapeutic immunization regimens provide a useful tool to influence DC control of adaptive immunity towards an atheroprotective response that does not induce an immunocompromised state (as would TLR inhibition). Such techniques are also potentially translatable to human patients as vaccination is a commonly used practice.

Future Directions

Many techniques are available to more reliably phenotype vascular DC and macrophage populations and validate these populations morphologically and functionally. Recent advances in isolating vascular immune cells now allow the extensive phenotyping of cells using flow cytometry techniques (2, 37, 66, 238). For phenotyping in mice, a minimum core panel of anti-CD103, -CD11b, -CD11c, and -MHC-II antibodies should be used, as this effectively discriminates CD103+ cDCs from monocytes, macrophages, and other vascular DC subsets (Tables 1 and 2). Further markers may be added, such as PDCA-1 or Siglec-H as positive markers for pDCs, or CD209a (DCSIGN) as a fairly reliable marker to distinguish CD11b+ DCs from macrophages. Other than intracellular cytokine staining for CCL17, no other markers have been reported for reliably identifying CCL17+ DCs, although these cells are noticeably CD11b+F4/80-, differentiating them from CD11b+ DCs. Other commonly used markers that may be useful are F4/80, CD115, and CX3CR1 (monocytes, macrophages, and CD11b+ DCs); 33D1 and DEC205 (CD4+ lymphoid DCs); and CCR7, CD24, and CD26 (many DC subsets).

Once vascular DC populations are isolated by fluorescence-activated cell sorting or other sorting technologies, it is also necessary to confirm “dendritic” morphology by microscopy and allogeneic T-cell priming capacity using the mixed leukocyte reaction test (37). Alternatively, antigen-specific T-cell priming can be tested in vitro using T-cell receptor-transgenic T-cells against vascular DCs loaded with their specific cognate antigen (238). Lastly, the migration from nonlymphoid to lymphoid tissues is a key aspect that differentiates macrophages from DCs. Previous studies have used complicated surgical transplant or cannulation techniques to measure DC migration (142, 190). However, a recent study identified a migratory DC-specific gene expression profile that may make it possible to measure DC migration by phenotypic marker analysis either in vivo or in vitro (150).

While the accurate phenotypic characterization of vascular DCs is a relatively attainable goal using current techniques, assessing the function of individual DC subsets in vivo has been difficult due to the technical challenge of depleting DCs in a subset-specific manner. DC depletion can be accomplished using the inducible CD11c-DTR mouse model or the constitutive CD11c-DTA model (13, 109, 175). However, these
models cannot be used to reliably deplete specific subsets of DCs and have also been shown to deplete CD11c-expressing non-DC populations of monocytes and macrophages (12, 171), making them unsuitable for such studies. There is some evidence to suggest that the inducible deletion of monocytes and macrophages in the CD11b-DTR mouse does not affect vascular CD11b+ DCs numbers (37) and may thus be useful for studying DC function (i.e., efferocytosis) in the absence of macrophages. A new mouse model was developed called the CAG-Cre/CD11c-iDTR model, in which diphtheria toxin receptor (DTR) expression is induced in CD11c-expressing cells following the Cre-mediated excision of a loxP-flanked stop cassette (172). In this way, DTR expression can be targeted to specific subsets within CD11c-expressing DCs by inserting the Cre transgene under another subset-specific gene promoter, such as CD103 or CD11b. However, no subset-specific CAG-Cre/CD11c-iDTR constructs have been reported in the literature to date.

The targeted deletion of DC subset-specific transcription factors may offer an additional strategy for the subset-specific deletion of vascular DCs. Several major DC transcription factors have already been identified, such as Batf3, IRF8, and Id2 in the case of CD8+ cDCs (5, 81, 94, 200, 220), or Relb, IRF2, IRF4, and RBPJ for lymphoid CD4+ cDCs (31, 88, 99, 215), and knockout mice are available for most of these genes. However, very few atherosclerosis studies have been performed using these knockout models. One study looking at the effect of IRF8 deficiency on atherosclerosis development found that loss of hematopoietic IRF8 expression aggravated atherosclerosis in ApoE−/− mice reconstituted with IRF8+/+ bone marrow (54). Unfortunately, the investigators found that IRF8 deficiency increased neutrophil infiltration and apoptosis and also influenced peritoneal macrophage IL-10 production and efferocytosis of apoptotic neutrophils, suggesting that some of these DC transcription factor knockout models may influence the development and function of other immune cell types as well. Thus, while DC transcription factor knockout mice may prove useful models for the deletion of specific DC subsets, it is necessary to carefully characterize the immune phenotype of these mice.

Lastly, because vascular DC subsets are difficult to isolate in sufficient numbers for adoptive transfer studies, the in vitro derivation of phenotypic and functional equivalents to known vascular DC populations from bone marrow progenitors would be an important advance in the field. These in vitro-derived vascular DC analogs will be useful tools to screen for the key microenvironmental factors, transcription factors, and effector molecules that regulate the vascular DC network and may even have potential as therapeutic agents, as suggested by the effectiveness of DC-based immunotherapy in limiting atherosclerosis development in mice (80, 93).

PERSPECTIVES

Resident vascular DCs play a crucial role in the resolution of vascular inflammatory responses and are likely required for the prevention of atherosclerosis in atheroprone, d-flow-exposed regions of the vasculature. During atherogenesis, homeostatic DC functions break down in response to inflammatory TLR signaling triggered by metabolic stress proteins (oxLDL, heat shock proteins) and pathogen-derived molecules (PAMPs), resulting in a persistent vascular inflammatory response and atherosclerosis (Fig. 5). It is interesting to note that many

![Image](http://ajpcell.physiology.org/ by 10.220.33.4 on June 22, 2017)
functions previously ascribed to vascular wall macrophages may actually be carried out by vascular DCs, including foam cell formation (176) and likely efferocytosis as well. Several studies demonstrate that lipid-rich, CD68+ foam cells upregulate CCR7 and emigrate from the atherosclerotic plaque during lesion regression (130, 224), a process that is unique to DCs. As such, it may be necessary to reexamine the roles of DCs and macrophages in atherosclerosis, as has occurred in other fields such as gut immunobiology (193).

Important questions still remain concerning the developmental pathways and transcription factors required for the differentiation of CCL17+ DCs and CD11b+ DCs in vivo. It is also unknown how microenvironmental factors in the artery wall control the production of DC-derived pro- (CCL17) and anti-inflammatory (IDO and RA) mediators that negatively and positively regulate vascular Treg responses, respectively. Furthermore, much of what is known about the development and origin and function of vascular DCs in the steady-state arterial intima has come from comparison with more detailed studies performed in phenotypically similar DC subsets found in other nonlymphoid tissues. More detailed in vivo studies are needed to assess the role of vascular DCs in mediating vascular homeostasis, and more importantly how to restore those functions in the face of proatherogenic conditions in the artery wall.

**REFERENCES**


Role of Vascular DC Network in Atherosclerosis

Cybulsky MI, Jongstra-Bilen J.


Charo IF, Taubman MB.

Burnett MS, Gaydos CA, Madico GE, Glad SM, Paigen B, Quinn 30.


ROLE OF VASCULAR DC NETWORK IN ATHEROSCLEROSIS


