Dendritic Cells in Atherosclerosis
Evidence in Mice and Humans
Alma Zernecke

Abstract—Atherosclerotic vascular disease is driven by chronic inflammation involving both innate and adaptive immune responses. Dendritic cells (DCs) are found in healthy arteries and accumulate in atherosclerotic lesions and engage in diverse pathogenic and protective mechanisms during atherogenesis. DCs contribute to early foam cell formation, regulate lipid metabolism, and control pro- and antiatherosclerotic T-cell responses by multifarious mechanisms. We, here, review many roles of DCs and plasmacytoid DCs in experimental models of atherosclerosis and the approaches to target DCs in therapeutic vaccination strategies. We, furthermore, discuss the evidence of the potential function of DCs in human atherosclerosis, and dissect the efforts to harness DC subsets as biomarkers of disease. Finally, we discuss necessary future steps that will help to understand the specific contribution of bona fide DCs in atherosclerosis to move toward novel therapeutic approaches. (Arterioscler Thromb Vasc Biol. 2015;35:00-00. DOI: 10.1161/ATVBAHA.114.303566.)

Key Words: atherosclerosis ■ humans ■ inflammation ■ leukocytes ■ mice ■

Atherosclerotic vascular disease remains the number one cause of death and morbidity in the Western world. It is now well established that atherosclerosis is a chronic inflammatory disease of the vessel wall. Besides macrophages, also other immune cells, namely dendritic cells (DCs) and T cells, can be found within atherosclerotic lesions and contribute to atherogenesis.1,2

In general, DCs have functionally been ascribed to play the main role in initiating antigen-specific adaptive immune responses and maintaining tolerance to self-antigens, whereas macrophages excel in phagocytic processes. The distinction between DCs and macrophages, however, is much debated, in particular because of their partially overlapping phenotypes and functions. In previous studies, mostly promiscuous surface markers were used to discriminate these populations, and DCs were often defined as CD11c+ major histocompatibility complex (MHCII)+ and macrophages as CD11c− F4/80high cells. CD11c, however, can be expressed by activated monocytes/macrophages (MDPs) or common DC precursors (MDPs) either give rise to monocyte-DC precursors (MDPs) either give rise to monocyte progenitors restricted to monocytes and their descendants or commit toward DC precursors. Common DC precursors differ- entiate into plasmacytoid DCs (pDCs) in bone marrow, or can give rise to pre-DCs, which exit the bone marrow, circulate in blood and subsequently develop into classical DCs (cDCs). It should be noted that the exact branching points of cDCs, pDCs and monocytes, and the existence of MDPs or common DC precursors as precursors for cDCs and pDCs, however, have recently been challenged.6 On the basis of these considerations, DCs in mice were proposed to be classified as a separate lineage of mononuclear phagocytes that arises from progenitors that are distinct from precursors of monocytes/macrophages. In the bone marrow common monocyte-DC precursors (MDPs) either give rise to monocyte progenitors restricted to monocytes and their descendants or commit toward DC precursors. Common DC precursors differentiate into plasmacytoid DCs (pDCs) in bone marrow, or can give rise to pre-DCs, which exit the bone marrow, circulate in blood and subsequently develop into classical DCs (cDCs). It should be noted that the exact branching points of cDCs, pDCs and monocytes, and the existence of MDPs or common DC precursors as precursors for cDCs and pDCs, however, have recently been challenged.6 On the basis of these considerations, DCs in mice were proposed to be classified as a separate lineage of mononuclear phagocytes that arises from progenitors that are distinct from precursors of monocytes/macrophages. In the bone marrow common monocyte-DC precursors (MDPs) either give rise to monocyte progenitors restricted to monocytes and their descendants or commit toward DC precursors. Common DC precursors differentiate into plasmacytoid DCs (pDCs) in bone marrow, or can give rise to pre-DCs, which exit the bone marrow, circulate in blood and subsequently develop into classical DCs (cDCs). It should be noted that the exact branching points of cDCs, pDCs and monocytes, and the existence of MDPs or common DC precursors as precursors for cDCs and pDCs, however, have recently been challenged.6

Defining DCs
Recently, efforts have been undertaken to unequivocally define DCs and differentiate these from macrophages, taking the ontogeny, phenotype and transcriptional profile into account.6–10 On the basis of these considerations, DCs in mice were proposed to be classified as a separate lineage of mononuclear phagocytes that arises from progenitors that are distinct from precursors of monocytes/macrophages. In the bone marrow common monocyte-DC precursors (MDPs) either give rise to monocyte progenitors restricted to monocytes and their descendants or commit toward DC precursors. Common DC precursors differentiate into plasmacytoid DCs (pDCs) in bone marrow, or can give rise to pre-DCs, which exit the bone marrow, circulate in blood and subsequently develop into classical DCs (cDCs). It should be noted that the exact branching points of cDCs, pDCs and monocytes, and the existence of MDPs or common DC precursors as precursors for cDCs and pDCs, however, have recently been challenged.6

Given these limitations, this review provides an overview about the role of cells ascribed to be DCs in atherosclerosis in mice and humans (Figure), stating the markers used to identify these, and discusses necessary future steps that will help to understand the specific contribution of bona fide DCs in atherosclerosis to move toward novel therapeutic approaches.

1. Koltsova et al noted aortic CD11c+ cells to be pivotal for T-cell activation, whereas lipid uptake was observed by CD11c− CD11b+ cells referred to as macrophages. Because of the paucity of studies clearly discriminating DCs and macrophages (see below) and their functional plasticity, the interpretation of these findings and published literature and a clear attribution to the cell type in atherosclerosis may, therefore, often be difficult.

Key Words: atherosclerosis ■ humans ■ inflammation ■ leukocytes ■ mice ■

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the entire transcriptome revealed a core cDC gene signature absent from monocytes/macrophages and pDCs that include the genes Zbtb46 and Ccr7. CD8α+CD103+ cDCs were furthermore revealed to express Tlr3 and Tlr, and to require the transcription factors BATF3, IRF8, Id2 for their development, thermore revealed to express Tlr3 and Tlr4, and to require the transcription factor E2-2/Tcf4. The transcription factor E2-2/Tcf4 is an essential regulator of pDC developments. Both cDCs and pDCs depend on the growth factor fms-like tyrosine kinase 3 ligand (Flt3L) and Flt3L-dependence can serve as a surrogate marker for common DC precursor origin. In contrast, pDCs depend on the growth factor fms-like tyrosine kinase 3 ligand (Flt3L), and Flt3L-dependence can serve as a surrogate marker for common DC precursor origin. In contrast, pDCs depend on the growth factor fms-like tyrosine kinase 3 ligand (Flt3L), and Flt3L-dependence can serve as a surrogate marker for common DC precursor origin.

Localization of DCs in Atherosclerotic Lesions and the Adventitia

In mice, CD11c+ DCs are frequently located in the aortic intima in areas predisposed to atherosclerosis. Moreover, CD11c+ MHCII+ DCs accumulate in atherosclerotic lesions, including T- and B-cell areas, and harbor cDCs, pDCs, and macrophages. In human tissue, CD123+ pDC numbers were significantly higher in blood and enter tissues under inflammatory conditions, give rise to macrophages and monocyte-derived DCs. It is currently unclear if monocyte-derived DCs and macrophages constitute two distinct lineages, or if they are instead highly plastic cells that acquire different functional modules in response to microenvironmental cues (Tables 1 and 2). In humans, pDCs and 2 types of cDCs (previously referred to as myeloid DCs), namely CD11c+ and CD141+ DCs are discriminated, which are considered homologs of CD11b+ and CD8+CD103+ DCs in mice, respectively. These DC subsets circulate as precursors or in an immature state in blood (Tables 1 and 2) and originate from hematopoietic stem cells in the bone marrow via either granulocyte macrophage progenitors or multilymphoid progenitors. Human homologs of murine DC precursors remain unidentified.

In contrast, macrophage colony-stimulating factor 1 receptor+ monocytes that circulate in blood and enter tissues under inflammatory conditions, give rise to macrophages and monocyte-derived DCs. It is currently unclear if monocyte-derived DCs and macrophages constitute two distinct lineages, or if they are instead highly plastic cells that acquire different functional modules in response to microenvironmental cues (Tables 1 and 2). In humans, pDCs and 2 types of cDCs (previously referred to as myeloid DCs), namely CD11c+ and CD141+ DCs are discriminated, which are considered homologs of CD11b+ and CD8+CD103+ DCs in mice, respectively. These DC subsets circulate as precursors or in an immature state in blood (Tables 1 and 2) and originate from hematopoietic stem cells in the bone marrow via either granulocyte macrophage progenitors or multilymphoid progenitors. Human homologs of murine DC precursors remain unidentified.

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monocyte-derived DCs. These TLOs may participate in primary immune responses in advanced atherosclerosis. Of note, TLO-like adventitial aggregates are also reported in patients with atherosclerotic abdominal aneurysms, which may indicate a relevance of TLO formation also in humans. The mechanisms of their formation and function, as well as their location in humans are unknown and deserve attention in future studies.

Given recent advances in defining bone fide DCs, an in-depth characterization of the ontogeny of vascular and adventitial DCs in healthy and atherosclerotic arteries is now warranted. For example, fate mapping studies will bring clarity to the origin of vascular DCs versus macrophages, such as the one exploiting DNGR-1 expression to trace cDC precursors. Subsequently, their transcriptional profiling could help identifying genes specifically regulated during disease development. This knowledge is requisite for further characterizing the phenotype and function of distinct DC subsets in atherosclerosis.

**Table 1. DC Subsets in Mice and Their Role in Atherosclerosis**

<table>
<thead>
<tr>
<th>Mouse DC Subsets</th>
<th>Monocyte-Derived DCs/Macrophages</th>
<th>General Markers: CD11c⁺ MHCII⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subset markers</td>
<td>cDC2</td>
<td>cDC1</td>
</tr>
<tr>
<td>Other markers</td>
<td>CD11b⁺</td>
<td>CD8⁺</td>
</tr>
<tr>
<td></td>
<td>CD172a/Sirpx⁺</td>
<td>XCR1⁺</td>
</tr>
<tr>
<td></td>
<td>CX3CR1⁺</td>
<td>CADM⁺</td>
</tr>
<tr>
<td></td>
<td>CD64⁺</td>
<td>CLEC9A⁺</td>
</tr>
<tr>
<td></td>
<td>MerTK⁻</td>
<td>TLR3⁺</td>
</tr>
<tr>
<td></td>
<td>MHCII−</td>
<td>TLR5⁻</td>
</tr>
<tr>
<td></td>
<td>CD206⁻</td>
<td></td>
</tr>
<tr>
<td>Precursors</td>
<td>MDP→monocyte progenitor→different monocyte subsets</td>
<td>MDP→CDP→pre-DC</td>
</tr>
<tr>
<td>Transcription factors</td>
<td>...</td>
<td>Zbtb46</td>
</tr>
<tr>
<td>Receptor dependence</td>
<td>M-CSFR</td>
<td>RelB, PU.1, RBP-J, IRF4</td>
</tr>
<tr>
<td>Findings in atherosclerosis</td>
<td>Present in healthy and atherosclerotic aortae¹¹,¹²,¹⁴</td>
<td>Present in healthy aortae¹⁴</td>
</tr>
<tr>
<td></td>
<td>As discussed elsewhere¹⁵</td>
<td>Expand Tregs¹</td>
</tr>
</tbody>
</table>

CAD indicates coronary artery disease; cDC, classical DC; CDP, common DC precursor; DC, dendritic cell; Flt3, factor fms-like tyrosine kinase 3; M-CSFR, macrophage colony-stimulating factor receptor; MDP, monocyte-DC precursor; MHC, major histocompatibility complex; and pDC, plasmacytoid DC.

**Trafficing of DCs**

In mice, the 3 major DC precursors in blood include Flt3⁺ pre-DCs and pDCs, and macrophage colony-stimulating factor⁺ monocytes. The loss of both CD103⁺ and CD11b⁺ cDCs in the nonatherosclerotic aorta in Flt3/Flt3l-deficient mice indicates that monocyte-derived DCs and bone fide cDCs are already present prior disease development. Whereas blood monocytes systemically increase, it is unclear whether circulating DCs or their precursors are altered in hyperlipidemia. Importantly, however, DCs rapidly expand during atherosclerotic lesion growth, and a reduction in CD103⁺ cDCs and monocyte-derived DCs is evidenced in atherosclerotic arteries of mice lacking Flt3 and macrophage colony-stimulating factor, respectively. A decrease in CD11c⁺ DC numbers in Cx3cr1⁻/⁻ mice may relate to diminished DC or monocyte recruitment. These data suggest that DCs or their precursors are recruited during disease development. Elucidating the DC subsets/precursors in blood and other organs during atherosclerosis may contribute to resolve the question of the origin of vascular DCs and could facilitate the identification of factors that regulate circulating and plaque DC numbers. Blocking the recruitment of specific DC subsets/precursors may be tested as therapeutic approaches to limit lesion size and local inflammation. Notably, CD11c⁺ MHCII⁺ DCs can also proliferate in early lesions. The contribution of DC (precursor) recruitment versus local proliferation remains to be defined.

**Circulating DCs: Biomarkers of Disease?**

In humans, several contradictory studies have addressed whether circulating DC numbers correlate with disease severity and thus may serve as biomarkers of disease. Shi et al.²² have identified numbers of total Lineage⁻ HLA-DR⁺ DCs, CD11c⁺ cDCs but not CD123⁺ pDCs to be increased in patients with coronary artery disease (CAD) compared with controls. In contrast, Yilmaz et al.²⁰ and Wen et al.²¹ reported decreased circulating BDCA-1⁺ cDC but not BDCA-2⁺ or CD123⁺ pDC precursors in patients with unstable CAD. Van Vrè et al.²⁵ and Van Brussels et al.²⁶ observed reduced BDCA-1⁺
cDCs and BDCA-2+ pDC numbers in patients with CAD, which were identified as independent predictors of the presence of or subsequent therapeutic procedures in stable CAD. Moreover, total DC, BDCA-1+ or CD11c+ cDC and BDCA-2+ or CD123+ pDC numbers were shown to be decreased in patients with CAD irrespective of disease severity. The mechanisms responsible for the decline in blood DCs in atherosclerosis may include an enhanced recruitment to lymphoid organs or sites of inflammation, as suggested by their accumulation in vulnerable lesions, an increased DC turnover, or a decreased production or release from bone marrow. Interestingly, decreased numbers of blood Lin−HLA-DR−CD11c+ cDCs and CD123+ pDCs in CAD correlated with decreased plasma FLt3L, suggesting that reduced DC counts in CAD may, in part, be because of an impaired DC differentiation from progenitors.

Although these studies have failed to unequivocally identify certain DC subsets as biomarkers of disease, the majority of studies observed a decline in DC numbers in CAD. With a finer delineation of circulating DC subsets/precursors, including CD141+ cDCs, their abundance may prove to be useful as potential biomarkers of disease.

**Emigration of DCs**

The ability to migrate to secondary (or tertiary) lymphatic tissue is considered functional characteristic of DCs. Although it is unclear where T-cell sensitization occurs, DCs may emigrate from the vessel wall after antigen uptake and home to lymphatic tissue. Indeed, CD11c+ cells seem to be able to leave atherosclerotic plaques in a chemokine-dependent manner.

When the aortic arch of Apoe−/− mice with established atherosclerotic lesions was transplanted into wild-type recipients, blockade of the CCR7 ligands CCL19 and CCL21 inhibited plaque regression and preserved CD11c+ cell content, suggesting that CCR7 mediates the egress of DCs during lesion regression. In different models of lesion regression, however, deficiency of CCR7 did not affect myeloid cell content, and lead to increased lesion formation in Apoe−/− but reduced plaque development in Ldlr−/− mice, findings that may also relate to altered T-cell trafficking. Whether DC emigration plays a role during atherogenesis remains to be defined, but may be impaired by a reduced migratory ability of DCs under conditions of hyperlipidemia.

**DCs Take Up Lipids and Control Cholesterol Homeostasis**

In the arterial intima, lipid accumulations can be found within vascular CD11c+ DCs in Ldlr<sup>−/−</sup> mice after only few days of hypercholesterolemia, where they adopt a foam cell–like appearance that may constitute the earliest stages of plaque formation. When the aortic arch of Apoe<sup>−/−</sup> mice with established atherosclerotic lesions was transplanted into wild-type recipients, blockade of the CCR7 ligands CCL19 and CCL21 inhibited plaque regression and preserved CD11c+ cell content, suggesting that CCR7 mediates the egress of DCs during lesion regression. In different models of lesion regression, however, deficiency of CCR7 did not affect myeloid cell content, and lead to increased lesion formation in Apoe<sup>−/−</sup> but reduced plaque development in Ldlr<sup>−/−</sup> mice, findings that may also relate to altered T-cell trafficking. Whether DC emigration plays a role during atherogenesis remains to be defined, but may be impaired by a reduced migratory ability of DCs under conditions of hyperlipidemia.

### Table 2. DC Subsets in Humans and Their Role in Atherosclerosis

<table>
<thead>
<tr>
<th>Human DC Subsets&lt;sup&gt;a,b,21&lt;/sup&gt;</th>
<th>Monocyte-Derived DCs/ Macrophages</th>
<th>(Myeloid) cDCs</th>
<th>pDCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subset markers</td>
<td>CD1c/BDCA-1&lt;sup&gt;+&lt;/sup&gt;</td>
<td>CD141/BDCA-3&lt;sup&gt;+&lt;/sup&gt;</td>
<td>...</td>
</tr>
<tr>
<td>Other markers</td>
<td>CD11b&lt;sup&gt;+&lt;/sup&gt;</td>
<td>CD123/IL3R&lt;sup&gt;+&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CD11c&lt;sup&gt;+&lt;/sup&gt;</td>
<td>CD303/BDCA-2&lt;sup&gt;+&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CD64&lt;sup&gt;+&lt;/sup&gt;</td>
<td>CD304/BDCA-4&lt;sup&gt;+&lt;/sup&gt;</td>
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<td></td>
<td>CD14&lt;sup&gt;+&lt;/sup&gt;</td>
<td>XCR1&lt;sup&gt;+&lt;/sup&gt;</td>
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<td></td>
<td>CD16&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>CADM&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>CD206&lt;sup&gt;+&lt;/sup&gt;</td>
<td>CLEC9A&lt;sup&gt;+&lt;/sup&gt;</td>
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<tr>
<td>Precursors</td>
<td>Blood CD1c&lt;sup&gt;+&lt;/sup&gt; cDCs</td>
<td>Blood CD141&lt;sup&gt;+&lt;/sup&gt; cDCs</td>
<td>CD303&lt;sup&gt;+&lt;/sup&gt; blood pDCs</td>
</tr>
<tr>
<td>Immature phenotype in blood</td>
<td>Zbtb46</td>
<td>IRF8, BATF3, IRF4, ID2</td>
<td>E2-2/TCF4</td>
</tr>
<tr>
<td>Transcription factors</td>
<td>As discussed elsewhere</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Receptor dependence</td>
<td>Flt3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Findings in atherosclerosis</td>
<td>Increased in blood in CAD&lt;sup&gt;22&lt;/sup&gt;</td>
<td>Decreased in blood in CAD&lt;sup&gt;23–28&lt;/sup&gt;</td>
<td>Increased in unstable lesions&lt;sup&gt;20,29&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Decreased in blood in CAD&lt;sup&gt;23–28&lt;/sup&gt;</td>
<td>Increased in unstable lesions&lt;sup&gt;20,29&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Present in atherosclerotic lesions&lt;sup&gt;16,20,30&lt;/sup&gt;</td>
<td>Increased in unstable lesions&lt;sup&gt;16,30&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inflammatory amplifiers/ upregulation of TLR4 on cDCs/cytotoxic CD4&lt;sup&gt;+&lt;/sup&gt; T cells responses&lt;sup&gt;20,31&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CAD indicates coronary artery disease; cDC, classical DC; DC, dendritic cell; Flt3, factor fms-like tyrosine kinase 3; MHC, major histocompatibility complex; and pDC, plasmacytoid DC.

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*a, b* References are not provided in the main text but are included in the table. The table details the expression markers and functions of DC subsets in the context of atherosclerosis.
effects remain to be addressed. Gautier et al.\(^4\) speculated that DCs may contribute to an enhanced lipoprotein uptake and clearance from the circulation. Whether this primarily occurs within lesions, or also in other organs, for example, in spleen, has not been addressed. Of note, DT-treated CD11b-DTR Apoe\(^{-/-}\) mice with a depletion of monocytes/macrophages, neutrophils, and CD11b\(^{+}\) DCs did not show any changes in cholesterol levels,\(^4\) which may be because of confounding effects of other cell populations or indicate that mostly CD11b\(^{+}\) DCs control cholesterol metabolism. The loss of CD103\(^{+}\) cDCs in atherosclerotic Flt3\(^{-/-}\)-Ldlr\(^{-/-}\) mice, however, did similarly not affect lipid levels.\(^4\)

Lipid uptake may be mediated by scavenger receptors of modified LDL (eg, CD36) and be controlled by ABCA1/ABCG1-mediated cholesterol efflux pathways, reverse cholesterol transport, activity of peroxisome proliferator-activated receptor-\(\gamma\), or liver X receptors, as recently reviewed in macrophages\(^5\); in addition, fluid-phase endocytosis of native or modified LDL by DCs may contribute to lipid uptake.\(^1\)

In future studies, these hypotheses will have to be experimentally tested. Especially in humans, a correlation between lipid levels and circulating or plaque DC numbers remains to be investigated. In addition, it will be interesting to characterize DC numbers and phenotype both in lesions and the circulation with regard to the expression of mediators that could control cholesterol metabolism in mice and humans. Enhancing the lipid-lowering potential of DCs could be an interesting approach to limit hypercholesterolemia and atherosclerosis.

**DCs as Antigen Presenting Cells That Control T-Cell Activation and Phenotype**

As discussed, DCs may emigrate from the vessel wall to home to lymphatic tissue, or interact with T cells in the adventitia. It may also be conceivable that circulating antigens can directly be ingested by DCs in the spleen or lymph node and that DCs at these sites contribute to T-cell priming. The presence of oligodendroglial T cells and CD4\(^{+}\) T cells reactive to disease-related antigens in human lesions indicates that priming of T cells or re-encounter of antigen may (also) occur locally at sites of inflammation in human atherosclerosis.\(^1,2\)

**Classical DCs**

In principle, vascular DCs bear the capacity to interact with T cells. In model systems, CD11c\(^{+}\) MHCI\(^{+}\) DCs take up injected antigen from the bloodstream, and after sorting are capable of inducing antigen-specific MHCI or MHCI\(^{+}\)-restricted T-cell proliferation in vitro.\(^4,5\)\(^6\) Furthermore, aortic DCs promote tumor necrosis factor-\(\alpha\) and interferon (IFN)-\(\gamma\) production in exogenously added antigen-specific T cells from Apoe\(^{-/-}\) mice ex vivo,\(^5\) suggesting that DCs can cause local T-cell activation and proinflammatory cytokine production.

Several lines of evidence indicate that DC–T cell interactions causally contribute to atherogenesis. For instance, Ldlr\(^{-/-}\) mice lacking the invariant chain of MHCI are protected from atherogenesis, caused by reduced T-cell activation in atheroma.\(^57\) Evidence for DC-intrinsic effector functions in atherosclerosis was, furthermore, obtained by disrupting TGF-\(\beta\) type II receptor-signaling in CD11c\(^{+}\) cells in Apoe\(^{-/-}\) mice, which lead to increased atherosclerotic lesion formation and an expansion of activated effector/memory T cells, indicating that TGF-\(\beta\)-signaling in DCs dampens proinflammatory T-cell responses in atherosclerosis.\(^4\)\(^8\) Several studies have further linked DCs with Treg responses in atherosclerosis. Although a subset of CCL17-expressing CD11b\(^{+}\)-CD11c\(^{+}\)MHCI\(^{+}\) DCs constrains Treg-maintenance and, thereby, drives atherosclerosis,\(^4\) Flt3-dependent CD103\(^{+}\) CD11c\(^{+}\)MHCI\(^{+}\) cDCs promote Treg responses, based on findings showing that Flt3-deficiency reduced aortic CD103\(^{+}\) cDC content, diminished systemic and local Treg numbers and increased atherosclerosis.\(^4\) Moreover, the absence of the key TLR adaptor MyD88 in CD11c\(^{+}\) DCs led to a loss in Tregs, which trumped decreased proatherogenic effector T-cell activation, and entailed an increased atherosclerotic lesion formation, indicating that Treg-mediated suppression of atherosclerosis requires MyD88 signaling in DCs.\(^39\) Thus, specialized DC subsets exert pro- and anti-inflammatory functions in T-cell activation. Interestingly, reduced peripheral Treg numbers in patients with CAD\(^50\) may be in line with an expansion of CCL17\(^{-}\) DCs in atherosclerosis and increased CCL17 serum levels in CAD,\(^51\) or point toward a loss of tolerogenic DC subsets/functions in advanced atherosclerosis.

CCL17-expressing DCs also contribute to the accumulation of T cells in atherosclerotic lesions.\(^3\) Moreover, adoptively transferred Ccr7\(^{+}\) T cells showed a reduced migration into the inflamed aorta, given the expression of CCR7 ligands CCL19/CCL21 by plaque DCs. DCs may also recruit or retain T cells in the inflamed vessel wall.\(^2,40\)

In future studies, it will be important to further investigate the role of specific DC subsets and the effector molecules involved in modulating T-cell responses in atherosclerosis. Identifying genes expressed in certain DC subsets, for example, transcription factors differentially regulating DC subset development, may facilitate their specific targeting. For instance, harnessing the cDC-specific transcription factor Zbtb46\(^3\) may help to define the contribution of cDCs (versus monocytes/macrophages or pDCs) to adaptive immunity and atherosclerosis.

To link experimental observations to clinical manifestations of atherosclerosis, functions and associated DC effector molecules should be explored in human DCs from atherosclerotic plaques and blood, and correlated to disease severity. Targeting DC subsets or their mediators that drive atherosclerosis or enhance atheroprotective Treg responses could be of potential interest for translational therapeutic strategies.

**Plasmacytoid DCs**

Several studies have addressed the role of pDCs in atherosclerosis. pDC-depletion using an antibody against BST2 (120G8) aggravated atherosclerotic lesion development in carotid arteries after collar placement and in aortic roots in diet-fed Ldlr\(^{-/-}\) mice, attributed to a loss of IDO-dependent restraint in T-cell proliferation.\(^18\) Contrary, pDC-depletion with a different antibody against BST2 (anti–PDCA-1) decreased diet-induced lesion formation in 2 independent studies in the aortic root and aorta in Apoe\(^{-/-}\) mice.\(^16,17\) Self-DNA (eg, released
from dying cells or in neutrophil extracellular traps) and an increased expression of the antimicrobial peptide Cramp/LL37 in atherosclerotic lesions was shown to stimulate breakdown of tolerance to self-DNA and promote IFN-α production by pDCs, aggravating early atherosclerosis and antidouble-stranded DNA antibody formation in Apoe<sup>−/−</sup> mice. In addition to releasing type I interferons, pDCs however, also exert typical, cDC functions. pDCs can present antigen via MHCI and stimulate T-cell activation, which is increased on oxidized LDL exposure, and an increased antigen presentation was observed in aortic PDCA-1<sup>+</sup> pDCs in atherosclerotic Apoe<sup>−/−</sup> mice, suggesting that pDC-driven immunity is enhanced in atherosclerosis.

Importantly, DC-based vaccination strategies have yielded encouraging results in animal models. For instance, oxidized LDL–pulsed bone marrow–derived DCs have been shown to induce oxidized LDL–specific T cells with a reduced Th1 profile, and to decrease atherosclerotic carotid artery plaque size in Ldlr<sup>−/−</sup> mice. Moreover, tolerogenic bone marrow–derived DCs pulsed with the LDL protein ApoB100 in combination with IL-10 reduced effector T-cell proliferation, inhibited IFN-γ production, and increased the generation of Tregs, which was accompanied by a reduction in atherosclerotic lesion size in Ldlr<sup>−/−</sup> mice transgenic for human ApoB100. These data indicate that DC-based therapeutic approaches harbor potential for the prevention of atherosclerosis. Although in these studies DCs were injected before the commencement of diet to induce atherosclerosis, further experiments are needed to test DC vaccination in mice with established disease. This is of particular importance as the feasibility of vaccination approaches at later stages of atherosclerosis may be prerequisite for an application in patients.

Conclusions
Distinct DC subsets can be found in arterial vessels in healthy mice and humans, and an accumulation of DCs is observed in atherosclerosis. Moreover, an increased abundance of DCs seems to correlate with signs of plaque vulnerability in humans.

Although there is tentative evidence that DCs control lipid uptake, cholesterol metabolism and modulate adaptive immune responses in atherosclerosis, a clear attribution to bona fide DCs and the exact molecular mechanisms engaged are mostly still unclear. Knowledge about the ontology of vascular DC subsets and of genes specifically expressed by these cells will be requisite to experimentally test and provide undisputed evidence of their role in atherosclerosis and of mediators that control recruitment, emigration, lipid uptake, and T-cell responses in mice. The relative scarcity of human tissue, with the exception of blood, has hampered translational efforts of mechanistic findings uncovered in mice. However, blood and human plaque tissue should be used to isolate specific DC subsets for these endeavors. The identification of human homologs of mouse DC subsets and further work in this area will aid to promote translational approaches.

A clear understanding of the functional contribution of DC subsets in atherosclerosis will serve as an essential basis for targeting proatherogenic or tolerogenic cell populations or their mediators as novel therapeutic approaches for treating cardiovascular disease.

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Disclosures
None.

References
3. Satpathy AT, KC W, Albring JC, Edelson BT, Kretzer NM, Bhattacharya D, Murphy TL, Murphy KM. Zbtb46 expression distinguishes classical
presentation by plasmacytoid dendritic cells drives proatherogenic
Circ Res

dritic cells protect against atherosclerosis
Immunity
Atherosclerotic vascular disease and its sequelae, such as coronary artery disease, myocardial infarction, and stroke, remain the leading cause of death and morbidity worldwide. In order for the development of novel therapeutic approaches, a detailed understanding of the key players and their functions in atherosclerosis are prerequisite. Although atherosclerosis is known to be driven by chronic inflammation, little is known about the role of dendritic cells that are found in healthy arteries and accumulate in atherosclerotic lesions in both mice and humans. This review highlights the latest advances in defining dendritic cells and their functions in atherosclerosis. In particular, evidence in mice and humans is combined with a focus on necessary future steps and translational endeavors that should now be tackled to move toward novel therapeutic strategies.
Dendritic Cells in Atherosclerosis: Evidence in Mice and Humans
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