Dendritic Cells in Cardiovascular Diseases
Epiphenomenon, Contributor, or Therapeutic Opportunity

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Cardiovascular diseases (CVDs) are one of the leading causes of mortality worldwide.† It has been so for decades, notwithstanding a wide array of – mostly preventive – treatment modalities targeting known risk factors, such as hyperlipidemia, type 2 diabetes mellitus, hypertension, or obesity. Recent technical and conceptual advances have unveiled important contributions of the immune system in the pathophysiology of a variety of CVDs such as atherosclerosis, ischemic stroke, chronic heart failure, and other myocardial conditions like myocardial ischemia and reperfusion, viral myocarditis, and cardiac transplantation.‡ In many of these disorders, so-called danger-associated molecular patterns (DAMPs), released from necrotic tissue and dying cells, can lead to the activation of certain immune cell populations such as monocytes/ macrophages, granulocytes, and T cells, thus aggravating ongoing inflammatory processes at the lesion site. Dendritic cells (DCs) are key modulators of immunity, pivotal in directing innate and adaptive immune responses against microbial, viral, but also modified self-antigens present at the sites of injury. Given the tissue trauma underlying various CVDs, it is not surprising that recent observations have allocated a regulatory role for DCs in CVD-associated immune responses. Interestingly, nondiseased arteries of young individuals were seen to host a network of resident vascular DCs (CD1a S100+ ICAM1+ CD31− CD83− CD86−),§ representing a phenotype related to Langerhans cells in the skin. In agreement, monocyte-derived CD11c+ CD68+ dendritic cells could be detected in the atherosclerosis-prone lesser curvature and aortic sinus in inbred atherosclerosis-susceptible (C57BI/6), but not resistant mouse strains (balb/c).§ Murine vascular resident DCs express an immature phenotype with low expression of costimulatory molecules, and are present in the subendothelial space with occasional probing into the vascular lumen.

DCs have also been observed in human heart and in cardiac valves of healthy C57BL/6 mice.‡ It is assumed that these immature, resident DCs contribute to the maintenance of vascular homeostasis and tolerance by scanning their microenvironment for self- and non–self-antigens. Indeed, Choi and coworkers? were able to show that resident DCs, isolated from the aorta and the valves of wild-type mice, have the capacity to present antigens to CD8+ T cells in vitro and in vivo, indicating that they are fully functional in eliciting a T-cell response. In diseased vessels, the heart and brain of human CVD patients, but also in the circulation, DC subset numbers were reported to be modified, associating DCs with CVD onset and progression.†,‡ This notion is substantiated by a wealth of experimental animal studies addressing the involvement of DCs in CVD. However, it remains mainly unsettled whether the actions of different DC subsets are either detrimental or beneficial for lesion formation. Then again, DC function may depend on lesion stage. This review thus aims to provide an in-depth overview of the role of DC subsets in several cardiovascular conditions in human and experimental animal models to reveal underlying patterns, expose pitfalls and shortcomings in our current understanding of the function of DCs in CVD, and define open/unresolved questions required to enable a thorough appreciation of the validity and potential of DCs as therapeutic target in CVD.

Dendritic Cells: Conductors of Innate and Adaptive Immune Responses

DCs are professional antigen-presenting cells (APCs) that originate from hematopoietic precursors in the bone marrow and are distributed throughout the whole body. DCs have the unique ability to induce T-cell responses by capturing, processing, and presenting antigens to naïve T cells. As such, they are the central mediators of adaptive immune responses.‡ Since the discovery by Steinmann and Cohn,¹ DCs were seen to represent a heterogeneous family of cells, differing in terms of development, migratory cues, compartmentalization, phenotype, and immunologic functions. So far, they are categorized into 4 main subsets: conventional DCs (cDCs), plasmacytoid DCs (pDCs), Langerhans cells, and monocyte-derived inflammatory DCs, characterized by the expression of a panel of specific surface markers.¹⁴ For further details we refer the reader to the online-only Data Supplement Appendix.

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Blood Circulating DC Subsets in Patients With Coronary and Peripheral Arterial Disease

As an indirect measure of DCs’ association with CVD, DC numbers and functionality have been evaluated in the blood of patients with CVD, such as coronary and peripheral artery disease.¹⁵ In 2006, van Vre and coworkers were the first to describe a marked decrease in circulating DCs (circulating cDCs and pDCs) in patients with coronary artery disease (CAD), defined by angiography as ≥50% stenosis in ≥1 coronary arteries.¹⁶ Until now, several studies confirmed a significant decrease in blood DCs (cDCs and pDCs) in CAD patients, irrespective of CAD grade (stable versus unstable angina pectoris, acute myocardial infarction), number of diseased vessels, or subset markers used for DC enumeration.¹⁵,¹⁷–²³ In sharp contrast, Shi and colleagues²⁴ reported increased circulating cDCs and unaltered pDC numbers in patients with stable CAD. By investigating the distribution of circulating DCs in patients with different stages of peripheral arterial disease, including patients with intermittent claudication and critical limb ischemia, Dopheide and coworkers²⁵ showed that blood cDC numbers were increased, whereas pDC numbers were reduced in patients who had peripheral arterial disease in comparison with healthy controls. Of note, both cDCs and pDCs from patients who have critical limb ischemia revealed an immature phenotype, suggesting that severe ischemia and prolonged inflammation in this ailment might lead to an attenuation in the proinflammatory membrane patterns of circulating DC subsets.

In general, most patient studies show declined blood DC numbers in CAD patients. Inconsistent results may be explained by differences in the extent and localization of disease, the timing of blood sampling (before/after a surgical intervention; lesion onset versus progression), the prevalence of risk factors across the patients included in these studies, and the cohort sizes consulted.

Nevertheless, the actual cause for reduced circulating DC numbers remains unaddressed. One possibility for the decrease in circulating DCs might be their enhanced recruitment to the disease site (hence, plaque or the ischemic heart).³⁹ Alterations in circulating DCs have been described in other autoimmune diseases as well, such as systemic lupus erythematosus,²⁶ where markedly lowered blood DC numbers correlated with an accumulation of activated DCs in the inflamed tissue. In analogy, DCs could well be recruited to secondary lymphoid organs to prime naïve T cells.²⁷ Circulating oxidized low-density lipoprotein (oxLDL) or circulating immunocomplexes,²⁸ but also ischemic tissue–derived DAMPs were seen to induce DC activation,²⁹ thus promoting their extravasation to the spleen or lymph nodes. Actually, a recent study has shown in CAD patients a more mature phenotype on a minor subset of circulating CD1c⁺CD14⁻BDCA-1⁺ cDCs, and BDCA-2⁺ pDCs, represented by the upregulation of CD83, CD80, CD86, and CCR7.¹⁷,²⁰ Dopheide and colleagues³⁰ additionally represented that in vitro monocyte-derived DCs from CAD patients revealed an increased expression of costimulatory markers (CD40, CD80, CD86) in comparison with healthy controls. However, it has to be acknowledged that monocyte-derived and classical DCs represent separate subsets, in that the transcriptional signature of monocyte-derived DCs is more reminiscent of that of macrophages than of classical DCs.³¹ Conceivably, these blood DC subsets exert distinct functions, and should be considered apart. Second, the apparent blood DC depletion could however also be explained by increased DC turnover, because of increased circulating cholesterol levels. Indeed, Alderman and colleagues³² have demonstrated in vitro that high concentrations of oxLDL provoke DC apoptosis. Otherwise, declines in DC numbers could be a temporary response to acute ischemia. Third, reduced blood DC numbers might also result from decreased release from bone marrow. Interestingly, the group of Bult has shown diminished plasma levels of FMS-like tyrosine kinase 3 ligand in CAD patients, a growth factor that is responsible for DC differentiation and release from bone marrow.³⁰ Fourth, the altered blood DC abundance, phenotype, and function could be owing to CAD patient’s medication, including aspirin, statins, β-blockers, and angiotensin-converting enzyme inhibitors. Although supported by several in vitro studies,³³–³⁶ the validity of this notion needs further investigation.

Recapitulating, circulating DC decline cannot be exclusively linked to the compartmentalization of this subset, be it to atherosclerotic plaque or lymphoid organs, because other covariates may modify blood DC numbers and functionality as well. This needs to be further addressed in future studies. Moreover, these observational studies leave unaddressed whether DCs are active contributors or just casual bystanders.

DC Involvement in Vascular Inflammatory Processes

Beside their presence in atherosclerosis, DC attendance has been described in other chronic inflammatory vasculopathies, such as giant cell arteritis, Takayasu arteritis, and Kawasaki disease.³⁷–³⁹ It is hypothesized that they contribute to the first critical steps in disease pathogenesis through breakdown of vascular tolerance. In these vasculopathies, resident DCs are located in the adventitia and adventitia-media border, and cDC numbers are seen to increase with disease progression.³⁵ Dense infiltrates of mature cDCs and T cells have been described at later stages as well, reflective of DC-initiated, antigen-specific immune responses. Considering that DC networks are present in healthy arteries and that they function as professional APCs, they might well be involved in disease onset and progression through the presentation of modified (self-)antigens to T cells. The actual triggers to activate vascular DCs are yet unknown, as is their relative contribution to immune priming in comparison with other vascular resident APCs such as macrophages. Additionally, the recent comparative analysis of the transcriptional network of DC versus macrophage lineages has helped to redefine DC-specific molecular signatures in lymphoid and nonlymphoid tissues, allowing a better discrimination between DC subsets and macrophages.³¹ These novel insights will help to appreciate whether DCs, described in vasculature,⁶ are bona fide DCs or tissue resident macrophages. Altogether, a functional role for DCs in the pathogenesis of these diverse inflammatory vasculopathies remains to be established, because studies are of a rather descriptive nature.
Functional Role for cDCs in Atherosclerosis

In human atherosclerotic plaques, fully mature DCs (CD83+ CD86+) accumulate within the rupture-prone atherosclerotic plaque shoulder where they produce T-cell chemotactic (CCL19 and CCL21) and proinflammatory cytokines (interleukin-12 [IL-12], IL-23, and tumor necrosis factor α [TNF-α]). Mapping of plaque-residing DCs revealed a close contact between DCs and activated T and natural killer T cells, suggesting that DCs tune or even orchestrate immune responses relevant to atherosclerosis. Of note, many of the histology studies are thwarted by the moderate/poor specificity of most DC markers, rendering the immunohistochemical detection of DCs a delicate issue. In fact, CD11c, an established DC marker regularly used to detect classical DCs within the atherosclerotic lesions, is also expressed on a wide scope of cells including tissue macrophages, natural killer cells, natural killer T cells, and a subset of T and B cells. Actually, all available cDC surface markers fail to conclusively identify this subset by immunohistochemistry. To circumvent this pitfall, the use of more refined or additional techniques, such as flow cytometric cell isolation, with the use of a panel of monocytic and DC surface markers, including CD11c, CD11b, F4/80, MerTK, CD64, Ly6C, is recommended to better appreciate DC presence and phenotype in different stages of atherosclerosis. Otherwise, DCs could be isolated by flow cytometry cell sorting for subsequent signature mapping (whole-genome arraying).

Mouse models have been very insightful in elucidating DC functions in atherosclerosis. For instance, lipid accumulation in the initial stages of atherosclerosis was recently shown to be directed and regulated by intimal CD11c+ DCs, residing in the atherosclerotic-prone lesser curvature of the aortic arch. As already discussed above, the combined expression of CD68 and CD11c by these vascular resident DCs may be suggestive of a macrophage rather than bona fide DC phenotype, a notion that requires further validation.

The impact of lipid uptake by DCs on their functionality remains a controversial subject. Dyslipidemia was seen to lead to an accumulation of DCs and macrophages within the atherosclerotic plaque. As reported in several studies, hyperlipidemic conditions and direct exposure of (splenic) CD11c+ cDCs to oxidized lipoproteins did not seem to impact their antigen-presentation and T-cell priming ability. In a transgenic mouse model with CD11c+ driven overexpression of the antiapoptotic gene hBcl-2, the extended lifespan and enhanced immunogenicity of circulating cDCs was associated with augmented T-cell priming, elevated levels of T helper 1 (Th1) and Th17 cytokines, and increased production of Th1-driven IgG2c antibodies under hyperlipidemic conditions. This major functional DC expansion did not aggravate lesion formation, because it was compensated for by decreased plasma cholesterol levels. Further support for a link between DC function and lipid metabolism was derived from the augmented plasma cholesterol levels after cDC depletion in hyperlipidemic ApoE−/− crossed to CD11c diphertheria toxin receptor (CD11c-DTR) transgenic mice. However, data acquired in this study have to be interpreted cautiously because CD11c is also expressed by several other lineages, and CD11c-DTR–based depletion will affect other subsets as well. In contrast to the above studies, Kopf and coworkers have shown that dyslipidemia did affect cDCs in vitro and in vivo, herein the CD8α− cDC subset, by impairing their response to Toll-like receptor (TLR) stimulation during Leishmania major infection. OxLDL appeared to be directly responsible for this effect, because it uncoupled TLR-mediated signaling in splenic cDCs, dampening DC activation and Th1 responses. Whether or not these findings also apply for humans remains to be addressed.

Next to classical lymphoid-resident and blood circulating cDC subsets, several studies have examined the impact of oxLDL on monocyte-derived DCs, which is of relevance because a majority of intimal DCs are thought to originate from monocytes. In vitro exposure to oxLDL during differentiation of human monocytes with granulocyte-macrophage colony-stimulating factor resulted in phenotypically mature DCs with upregulated HLA-DR, CD40, and CD86 and induced capacity to T-cell activation. However, incubation with high concentrations of oxLDL attenuated DC function and induced apoptosis. This may in part be associated with the strong oxLDL-induced downregulation of CCR7 and its ligand CCL21 by monocyte-derived DCs, which likely will impact their migratory capacity to and in plaque. It will be of interest to investigate and compare the effect of (modified) lipoproteins on in vitro FMS-like tyrosine kinase 3 ligand–cultured DCs, because they represent the classical in vivo equivalents. In contrast to the above-described data, Blueml and colleagues have shown that oxidized phospholipids impair monocyte-derived DC maturation by blocking TLR3- and TLR4-mediated upregulation of costimulatory molecules and induction of proinflammatory cytokines in human DCs. Taken together, considerable controversy exists on DC subset function(s) in hyperlipidemia-associated atherosclerosis, which is fostered among others by the apparent promiscuous expression patterns of alleged DC markers and the significant plasticity of both DCs and macrophages. But the definition of transcriptional DC versus macrophage signatures will undoubtedly help to reassess the actual presence of bona fide resident DCs within the vasculature. Additionally, it will be of importance to uncover how early and more advanced stages of hyperlipidemia impair DC (precursor) homeostasis, including their development in the bone marrow, DC mobilization into the circulation, peripheral phenotype, and migratory routes. In addition, extensive knowledge about DC actions within a lipid-rich environment such as the atherosclerotic plaque is lacking. How lipid uptake and prolonged intracellular storage interfere with signaling pathways responsible for DC activation is still controversial and will require further study.

A Functional Role for Plasmacytoid DCs in Atherosclerosis

The group of Weyand and coworkers has recently shown the presence of CD123+ pDCs in human carotid atherosclerotic plaques, mainly located in the shoulder region that was also enriched in interferon-α (IFN-α) positive cells, thus associating pDC presence with IFN-α production. Furthermore, pDC numbers were significantly increased in unstable in comparison with stable human lesions. In vitro CpG-induced IFN-α release by pDCs induced a 10-fold upregulation of tumor
necrosis factor-related apoptosis-inducing ligand expression on CD4+ T cells, thus promoting apoptosis of vascular smooth muscle cells and endothelial cells, processes that tremendously contribute to plaque destabilization. However, these data leave unaddressed whether pDCs are functional in the atherosclerotic plaque in vivo. We and others have recently shown that CD123 displays only moderate specificity for human pDCs, being colocalized also with CD68+ macrophages and with ASMA+ vascular smooth muscle cells. Use of the human pDC-specific marker BDCA-4 by our group revealed the scanty and equivalent presence of pDCs in human stable and unstable plaques. Moreover, we demonstrated that expression of IFN-α in human unstable versus stable carotid endarterectomy tissue specimens did not differ, suggesting that in chronic low-grade inflammatory processes, such as atherosclerosis, pDC activation may not be a prominent feature.

Our group has recently shown that selective depletion of pDCs by 120G8 monoclonal antibody administration in Ldlr−/− mice fed a high-fat diet exacerbated lesion size in the carotid artery and the aortic roots, and promoted plaque T-cell accumulation and peripheral CD4+ T cell activation, as well. pDCs isolated from atherosclerotic mice suppressed CD4+ T-cell proliferation in an indoleamine-2,3-dioxygenase-dependent manner, suggestive of an atheroprotective role for pDCs in atherosclerosis. In contrast to our study, Döring and colleagues and MacRitchie and colleagues, as well, reported significantly decreased diet-induced lesion formation in the aortic root and the aorta of pDC-depleted ApoE−/− mice, whereas plaques showed a more stable phenotype. Both groups investigated the impact of pDC depletion by use of the PDCA-1 depletion antibody on early lesion development (4 weeks of high-fat diet feeding). These controversial findings are intriguing. A seeming paradox was in the presence of pDCs in the atherosclerotic plaque. Whereas pDCs could barely be detected in mouse atherosclerotic lesions in Ldlr−/− mice, they were detected in lesions of ApoE−/− mice, mainly in the plaque shoulder, at which pDC abundance was increased with high-fat diet feeding and lesion progression.

Conversely, MacRitchie and colleagues described the constitutive presence of mostly immature pDCs in noninflamed aortic tissue of normolipidemic mice, at numbers similar to those seen in atherosclerotic ApoE−/− mice. Nevertheless, the antigen presentation capacity of aortic pDCs from ApoE−/− mice was enhanced. In line with this, Döring and colleagues showed that sorted aortic pDCs from hyperlipidemic mice, ex vivo primed, were capable of triggering T-cell stimulation in vivo. Finally, baseline IFN-α levels were below detection levels or not affected by pDCs in our study and the study of MacRitchie and coworkers, whereas Döring and colleagues demonstrated elevated IFN-α levels in plaque (mRNA) and serum in high-fat diet–fed ApoE−/− mice, being reduced after pDC depletion. It must be noted that these groups used different methodologies, such as the use of the depletion antibody, administration regimen, and mouse models. For example, the ApoE−/− model displays more aggressive atherosclerosis than the Ldlr−/− model, which may favor pDCs switching to an immunogenic mode. The use of pDC depletion antibodies (120G8, mPDCA-1 or 927) targeting BST2 has been shown to eliminate pDCs quite efficiently. BST-2 is restricted to pDCs in the steady state. Bst-2 expression was, at transcriptional level, reported to be induced on other cell subsets on type I IFN stimulation, albeit still markedly lower than that of pDCs. Nevertheless, in the context of atherosclerosis, we, Döring and colleagues, and MacRitchie and colleagues, as well, demonstrated the specific depletion of pDCs by Bst-2–specific monoclonal antibody, thus excluding undesired side effects due to nonspecific depletion.

Regarding the complex pathophysiology of atherosclerosis, pDCs could well exert dual functions in early and advanced stages of disease, dependent on their microenvironmental context. During episodes of fulminant plaque inflammation pDCs acquire proatherogenic functions by rapid secretion of type I IFNs and proinflammatory cytokines, whereas, during low grade chronic inflammatory stages, pDCs may act tolerogenic by inhibiting proliferation of CD4+ T cells. Indeed, pDCs are involved in the pathogenesis of a range of autoimmune diseases characterized by a type I IFN signature, whereas alternatively activated pDCs are considered to contribute to tolerance induction. Further studies are warranted to elucidate the actual pathways that are activated in pDCs during different stages of atherosclerosis by using more advanced animal models, such as conditional E2-2 knockout. Nevertheless, these findings clearly identify this cell type as an interesting new target for future therapeutic intervention studies in the treatment of atherosclerosis.

CVD Risk Factors: Contribution of DCs?

Type 2 diabetes mellitus (T2D) and hypertension are major risk factors for the development of atherosclerosis and its cardiovascular complications. Chronic inflammation is thought to accelerate the progression of these pathological conditions. DCs are likely to contribute here by triggering cell-mediated immune responses. The next 2 sections outline the current knowledge on potential DC functions in T2D and hypertension.

A Role for DCs in T2D Patients With Atherosclerotic Complications

Insulin resistance and hyperglycemia in T2D are associated with a systemic proinflammatory state (increase in proinflammatory cytokines such as IL-6, activation of immune cells) that facilitates the development of atherosclerosis. In vitro studies have shown that advanced glycosylation end products and hyperinsulinemia enhance DC maturation and induce an antigen-specific T-cell activation, thus supporting a contributory role of DC (subsets) on the immune reactions in diabetic atherosclerosis. Yao and colleagues recently reported a significant decline in circulating cDCs in T2D patients with unstable angina pectoris versus healthy controls and T2D patients, whereas pDC numbers remained mainly altered. cDCs showed a more mature and activated phenotype, evidenced by the upregulation of CD86 and the enhanced capability to stimulate T-cell proliferation in vitro. Reduced circulating DC numbers in T2D patients with atherosclerotic complications was attributed to an increased trafficking into the inflamed vulnerable plaque or to neighboring lymph nodes, because patients had significantly increased levels of fractalkine, a chemokine that is mainly involved in the recruitment...
of Ly6C<sup>low</sup> monocytes to sites of inflammation, but has thus far not been linked to classical DC chemotaxis. In contrast to these findings, Orfao and coworkers showed both quantitatively and functionally impaired proinflammatory cytokine response by circulating DCs from T2D patients with atherosclerotic complications. Conceivably, the increased plasma TNF-α levels observed in patients with diabetic atherosclerosis may underlie this impairment, because it can tone blood DC differentiation. This notion is encouraged by studies describing an inverse correlation between blood DC numbers and plasma TNF-α concentrations in T2D. Of note, medication used for glycemic control and for the treatment of diabetes-related comorbidities (angiotensin-converting enzyme antagonists, angiotensin receptor blockers, or statins) could be partly causal in the altered blood DC abundance. Altogether, although DC function is clearly perturbed in T2D, the present state of knowledge does not allow segregating atherosclerosis from T2D intrinsic DC effects.

**A Role for DCs in Hypertension**

T cells that likely involve their priming by APCs, such as DCs, with the capacity to present neoantigens, generated by necrotic and apoptotic cells. However, less is known regarding the role of DCs in hypertension. DC accumulation in alveolar lesions of human and experimental pulmonary arterial hypertension has been described. Recently, Vinh and colleagues showed that the CD28-blocking agent abatacept prevents angiotensin II–induced hypertension in mice, supporting a contributory role for DCs as APCs in hypertension. Additionally, they observed increased activated DC numbers in spleen and lymph nodes of hypertensive mice. However, these data leave unaddressed whether DCs are the primary cell type responsible for antigen presentation. The more abundant vascular macrophages in the vessel wall might as well function as APCs. Interestingly, the renin-angiotensin-aldosterone system can by itself initiate/modulate innate and adaptive immune responses and inflict target-organ damage as shown by the group of Mueller and coworkers in a compound transgenic rat model harboring human renin/angiotensin genes. Activation of the angiotensin-1A receptor—among others expressed on DCs—promoted DC migration to the kidneys, and their activation, potentially inducing renal damage. Moreover, I<sub>d</sub> mice, lacking Langerhans cells and CD8<sup>+</sup> DCs, infused with angiotensin II remained normotensive and failed to develop albuminuria and renal damage, firmly establishing a role for Langerhans cells and CD8<sup>+</sup> DCs in angiotensin II–induced hypertension. These data support the idea that angiotensin II itself can influence T-cell fate both directly or indirectly.

**DC Contribution to Ischemic Stroke: Friend, Foe, or Bystander?**

Immune mechanisms were only recently recognized to contribute to the pathophysiology of ischemic stroke and involve both the innate and adaptive immune system. A potential role for DCs as potent mediators of inflammation in stroke has not been investigated extensively. Yilmaz and colleagues have shown that circulating cDCs, and pDC numbers, as well, were transiently reduced in patients with acute stroke. They postulated that circulating DCs are recruited into the infarcted brain to elicit antigen-specific immune responses through T-cell activation. Indeed, HLA-DR–expressing cDCs colocalized with T cells in dense infiltrates around cerebral vessels in the stroke area. The pro-oxidant conditions of ischemia-reperfusion may give rise to the formation of neoepitopes, which can be well presented by APCs, such as HLA-DR–expressing resident microglia or recruited DCs. It cannot be excluded that other factors, such as increased cell apoptosis, may contribute to the declined circulating DC numbers, as it has been shown for lymphocytes after stroke. Interestingly, the group of Magnus and coworkers revealed in a rodent stroke model (temporary middle cerebral artery occlusion) the early accumulation of DCs, peaking on day 3 after reperfusion. DCs showed strong and sustained upregulation of major histocompatibility complex class II, but an absent concomitant upregulation of costimulatory molecules, possibly leading to disrupted T-cell activation. Although such a phenotype is conceivable, this warrants further study on whether DCs are active contributors to local immune responses after stroke, either in an immunogenic or immunosuppressive way.

**DC Involvement in the Diseased Heart**

**Circulating DCs in Patients With Heart Failure**

The association between circulating DCs and heart failure has only been subject of a few scattered studies, and the study outcomes are rather divergent. Athanassopoulos and colleagues revealed an increase in total blood DCs in patients with chronic heart failure (nonischemic dilated cardiomyopathy) in comparison with controls owing to an increase in the mature fraction of the cDC subset (CD83<sup>+</sup> CCR7<sup>+</sup>), suggesting a possible Th1 response in end-stage heart failure. In seeming contrast, Sugi and colleagues showed that total circulating DC numbers transiently declined, but residual DCs appeared more activated during the acute decompensated phase of heart failure. Similar to the decline in circulating DC counts in CAD patients, the authors associate diminished DC numbers in heart failure with DC recruitment from systemic circulation into the damaged tissue in response to released DAMPs. Clearly, other confounders with the potential to modify circulating DC counts (eg, development in the bone marrow and release into the circulation, cell apoptosis, trafficking routes) have to be taken into account. To conclude, a direct involvement of DCs in the pathophysiology of heart failure, although plausible, remains elusive. Moreover, further studies are warranted to precisely define the cause of systemic DC modifications, taking different disease stages (acute versus chronic) into account (Table).

**Myocardial Ischemia and Reperfusion**

A role for DCs in the pathogenesis of cardiac ischemia/reperfusion injury is not well-established. In 1993, the group of Ferrans demonstrated the rapid accumulation of interstitial DC in the border zones 7 days after myocardial infarction (left coronary artery ligation) in the rat heart. DCs tended to be assembled in small clusters with CD4<sup>+</sup> T cells, which disappeared 21 days after coronary ligation. It is assumed that
### Circulating DC Populations in Human CVD

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<th>AMI</th>
<th>CAD excluded</th>
<th>Early CAD</th>
<th>Moderate CAD</th>
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<td>(AIS and AHS)</td>
<td>correlates with stroke severity</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>AHS: 31</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Type 2 diabetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR: 15</td>
<td>UAP: 18</td>
<td>↓</td>
<td>(BDCA-1+ mDC)</td>
<td>↓</td>
<td>(BDCA-2+ pDC)</td>
<td>Blank48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR: 44 (no atherosclerotic complications)</td>
<td>EXP: 11 (atherosclerotic complications)</td>
<td>↓</td>
<td>(CD33+)</td>
<td>↓</td>
<td>(CD33-)</td>
<td>Corrales65</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR: 21</td>
<td>T2D: 21</td>
<td>=</td>
<td>subgroup &quot;poor glycemic control&quot; within T2D</td>
<td>less functional (all T2D)</td>
<td></td>
<td></td>
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</tbody>
</table>

(Continued)
these DCs are involved in postischemic short-term cyto-
protective responses through TLR2 and TLR4 stimulation
by tissue-released DAMP.77 Maekawa and coworkers,78 on
the contrary, demonstrated in a murine myocardial infarc-
tion model (left coronary artery ligation) that deletion of
interleukin-1 receptor-associated kinase-4 , a downstream
effector of the TLR/MyD88 axis, had beneficial effects on
survival and function after myocardial infarction by blunting
DC mobilization into the heart and attenuating local
inflammatory processes. Of note, cardiomyocytes and resi-
dential macrophages also express TLR2 and TLR4 and are
implicated in low-grade inflammation during chronic myocard-
itis (days 15–90 postinfection) remains unclear. In particu-
lar, pDCs, the major type I IFN producers in response to viral
infection, have not received much attention so far, but may
be likely mediators in antiviral defense in the heart, although
endogenous type I IFN release by infected cardiomyocytes
could limit viral replication in the heart, as well (Figure).

Viral Myocarditis

Even less is known about a contribution of DCs to the patho-
genesis of viral myocarditis. Viral infections can inflict
significant damage on cardiomyocytes by (1) immune
response–mediated, (2) autoimmune-mediated, or (3) direct
virus–induced myocardial injury, which can cause myocard-
itis and dilated cardiomyopathy. Many animal studies have
been performed in this field by using models of myocarditis
cauised by Coxsackie virus B3.79 In response to viral infec-
tions, immune cells, such as cardiomyocytes, endothelial cells,
fibroblasts, and DCs, were reported to infiltrate up to 5 days
postinfection, and were supposed to limit viral replication by
the release of proinflammatory cytokines (IL-1α, IL-1β, IL-6,
IL-18, TNF-α, TNF-β, and IFN-γ).80,81 In the subacute phase
(within 2 weeks after viral infection) the release of progeny
virus into the interstitium stimulates the infiltration of proin-
flammatory natural killer cells and macrophages, followed by
a considerable influx of antigen-specific CD4+ Th and cyto-
toxic CD8+ T cells, which recognize viral antigens presented
via major histocompatibility complex molecules on the sur-
face of infected cardiomyocytes.81,82 Interestingly, in a mouse
model of Coxsackie virus myocarditis, it has been shown that
CD8+ DCs, next to cardiomyocytes, are implicated in CD8+
T-cell priming, thereby curtailing viral infection in the acute
phase (within 8 days postinfection).83 Whether or not DCs are
implicated in low-grade inflammation during chronic myocard-
itis (days 15–90 postinfection) remains unclear. In particu-
lar, pDCs, the major type I IFN producers in response to viral
infection, have not received much attention so far, but may
be likely mediators in antiviral defense in the heart, although
endogenous type I IFN release by infected cardiomyocytes
could limit viral replication in the heart, as well (Figure).

DC-targeted Therapeutic
Opportunities in CVD

Targeting DCs in Cardiac Transplantation:
Contributors to Graft Rejection or Useful Tools in
Tolerance Induction

The role of DCs as modulators of allo- and autoreactive T-cell
responses after transplantation has been extensively studied.84
DCs hold promise as therapeutic tools to ameliorate or prevent
graft rejection or graft-versus-host disease and to treat autoim-
mune diseases.85,86 DCs are implicated in the recognition of
alloantigens by the host’s immune system. As Larsen and col-
leagues87 demonstrated, mature donor-derived DCs are hort-
ing to T-cell areas in the draining lymph nodes in the first days
after transplantation, where they trigger naïve T cells by pre-
senting graft-derived epitopes. These DCs potentially contri-
ute to acute rejection of cardiac allografts. Similarly, Kofler
and colleagues88 reported pronounced infiltration of recipient
DCs into the cardiac allograft, picking up and processing the
donor antigens, and activating the recipient’s adaptive immune
system in the first postoperative year after human heart trans-
plantation. In summary, DCs appear to be the main culprits
in organ rejection, but have otherwise been shown to mediate
transplant tolerance by preventing T-cell–mediated immunity.
The group of Bromberg and coworkers89 has demonstrated
an essential role for pDCs as phagocytotic APCs in tolerance
induction in vascularized cardiac grafts, in that adaptive transfer
of tolerized pDCs induced regulatory T-cell development
and prolonged graft survival in mice. Likewise, a single preoperative infusion of donor-mobilized immature pDCs in combination with anti-CD154 monoclonal antibody was able to effectively suppress allograft rejection and prolonged graft survival in mice.90 These findings are concordant with a recent study in humans that examined total peripheral blood DC numbers in patients with clinical heart transplantation, revealing significantly diminished DC frequency 1 week after heart transplantation, which probably reflects immunologic quiescence through adequate immunosuppression.91

In summary, adoptive transfer of preprimed (tolerogenic) DCs seems to hold great promise for preventing graft rejection after cardiac transplantation. For all that, a better understanding of how graft-infiltrating DCs function may also help to appreciate their contributory roles in chronic heart failure and postmyocardial infarct healing.

**DCs as Potential Therapeutic Tools in the Treatment of Atherosclerosis**

As alluded to, DC-based vaccination and immunization strategies, based on the application of ex vivo antigen-loaded or genetically engineered autologous DCs to tune T-cell responses have meanwhile evolved into a viable therapeutic option for cancer.92 This success has inspired several groups to explore the potential of DC-based vaccination for atherosclerosis. Studies in animal models so far are at least encouraging and support the notion that DC-based vaccination and immunization hold promise for therapeutic immunomodulation of atherosclerosis.93 Kuiper and coworkers have shown that oxLDL-pulsed mature DCs transferred into Ldlr⁻/⁻ mice reduce atherosclerotic lesion size. DC vaccination led to quenched Th1 responses and elevation of oxLDL-specific IgG titers indicating that oxLDL-pulsed DCs may confer protection against atherosclerosis, by favoring humoral immune responses to oxLDL.94 A recent study proposed immunotherapy with DCs, pulsed with apolipoprotein B 100 in the presence of IL-10 to render them immnosuppressive, as an effective strategy to attenuate atherosclerosis. DC immunotherapy resulted in dampened T-cell immunity and diminished atherosclerotic lesion formation in mice.95 Taken together, the outcome of these studies indicates that DC vaccination emerges as a new, potentially powerful approach in the treatment of atherosclerosis, although translation of these largely animal experimental findings to human disease needs further investigation. Atherosclerosis-associated (neo) epitopes such as apolipoprotein B100 peptides (eg, P2, P45, P210), next to malondialdehyde fibronectin, double-stranded DNA, or heat shock protein 60, which were shown to be effective in immunization studies by Nilsson and coworkers,96,97 may hold particular promise as targets for vaccination. Of note, therapy timing and the immunologic status of the patient are additional critical issues that need to be considered carefully, given that atherosclerosis mainly affects elderly individuals. Indeed, age-dependent alterations in expression and function of innate immune receptors and signal transduction pathways may translate in defective DC activation, thus diminishing the DC-based vaccination efficacy.98 Besides, DC vaccination studies based on adaptive transfer of monocyte- or bone marrow–derived DCs to mouse models of atherosclerosis, should be interpreted with caution, because DCs in vitro matured with growth factors other than FMS-like tyrosine kinase 3 ligand do not represent bona fide DCs in every aspect.91

**Concluding Remarks**

This review summarizes the current state of knowledge on the role of different DC subsets in the pathogenesis of CVDs, pinpoints shortcomings/gaps, and delineates the future perspectives for DCs as a potential therapeutic target in CVD. By now, several DC subsets have been reported to accumulate not only in atherosclerotic or hypertensive vessels, but also in failing, cardiomyopathic, or ischemic heart tissue and in ischemic brain, suggestive of a role in the underlying pathophysiology. DC numbers and functionality in the blood of
patients with CVD have been embraced as a measure of DC association with disease onset and progression, although the cause and implications of these disease-associated changes in DC homeostasis still are subject to discussion. Moreover, the actual value of circulating DCs as biomarkers in CVD needs to be established, but it is fairly improbable that DC subset numbers will offer the precision, specificity, and discriminative power to be useful as a disease biomarker. Of all cardiovascular disorders, atherosclerosis is by far the most extensively studied for impact of DC subsets in its disease ontogenesis, albeit that the majority of these studies involve murine animal models. Much less is known about the contribution of DCs to cardiovascular pathologies such as stroke, heart failure, and myocardial diseases. Conceivably, also here injury-associated DAMP release will lead to the recruitment and subsequent activation of DC with T-cell priming ability at the inflamed locus, skewing the adaptive immune system toward Th1/17-like immune responses or toward a state of tolerance, dependent on disease stage and environmental context.

Recent findings in mice plead for a beneficial role for primed DCs as therapeutic agents in the treatment of atherosclerosis. Nevertheless, the efficacy of DC immunotherapy for preventing plaque progression and destabilization in humans remains to be seen. In this regard, it will be vital to target the relevant disease-associated antigens for DC pulsing. Donor-mobilized DCs could be otherwise cultured in vitro without further adjuvants, because the use of nonpulsed immature DC has been proven to be an attractive approach to induce tolerance. Alternatively, strategies could be used to instruct endogenous DCs in situ, e.g., by receptor-specific manipulation, albeit that the options for targeting DC subset–specific surface receptors are rather limited.

Summarizing, despite the limitations in our current understanding in DC functions in various CVDs, the recently shown efficacy of DC-based tolerance and immunization strategies in ameliorating murine atherosclerosis and diminishing allograft rejection, in combination with the current dynamics in this rapidly progressing research field inspire confidence that (some of) these approaches will evolve into viable modalities for the treatment, and maybe even prevention, of human cardiovascular disorders.

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Disclosures

None.

References


Dendritic Cells in Cardiovascular Diseases

94. Oviedo-Orta EA, Thomson AW. Dendritic cells in cardiovascular diseases

Key Words: antigens cardiovascular diseases dendritic cells immune system immune system phenomena immunosuppression therapies
Dendritic Cells in Cardiovascular Diseases: Epiphenomenon, Contributor, or Therapeutic Opportunity
Anette Christ, Lieve Temmerman, Bart Legein, Mat J.A.P. Daemen and Erik A.L. Biessen

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Dendritic cells (DC): conductors of innate and adaptive immune responses

1. Dendritic cell development

Both innate and adaptive immunity are built on various leukocyte subsets, which are derived from hematopoietic stem cells (HSC) in the bone marrow. HSCs further differentiate into common lymphoid precursors (CLP) and common myeloid precursors (CMP). The latter gives rise to monocytes/macrophages, granulocytes, DC, and megakaryocytes, which are all belonging to the innate immune system. CLP give rise to T and B cells, but can also differentiate into DC, emphasizing a key role for DC as orchestrators of innate and adaptive immunity. Monocytes and DC share a common progenitor called the monocyte dendritic cell precursor (MDP; lin⁻ ckit<sup>high</sup> CD115<sup>+</sup> CX<sub>3</sub>CR<sub>1</sub><sup>+</sup> Flt3L<sup>+</sup>)<sup>3, 4</sup>, whereas a common DC progenitor (CDP; lin⁻ ckit<sup>low</sup> CD115<sup>+</sup> Flt3L<sup>+</sup>)<sup>5</sup> is restricted to generate conventional DC (cDC) and plasmacytoid DC (pDC).<sup>6</sup> MDP and CDP are confined to the bone marrow.<sup>2</sup> Macrophage colony-stimulating factor (M-CSF) and FMS-related tyrosine kinase 3 (Flt3L) are essential growth factors for the differentiation of the myeloid cell lineage, where M-CSF is critical for the development of monocytes/macrophages and monocyte-derived DC, and Flt3L for DC lineage development.<sup>6</sup>

2. DC subsets

So far, DC are categorized into four main subsets: cDC, pDC, Langerhans cells and monocyte-derived inflammatory DC, based on the expression of a panel of specific surface markers, the development, anatomical location, and functionality.

Conventional dendritic cell (cDC) precursors (pre-DCs) leave the bone marrow and migrate via the blood stream to secondary lymphoid organs (lymph nodes, spleen and thymus), and peripheral tissues such as skin, lung, the intestinal tract, kidney and liver, where they convert
into lymphoid resident and migratory cDCs, respectively.\textsuperscript{7} For mouse, cDC are sub-classified into CD11b\textsuperscript{+} and CD11\textsuperscript{-} cDC. The former include CD4\textsuperscript{+} and CD4\textsuperscript{-}CD8\textsuperscript{-} lymphoid-tissue resident cDC, non-lymphoid peripheral tissue CD11b\textsuperscript{+} DC, as well as CD103\textsuperscript{+} CD11b\textsuperscript{+} DC in the gut, which all are principally involved in CD4\textsuperscript{+} T cell priming through major histocompatibility complex (MHC) class II molecules.\textsuperscript{8} The CD11b\textsuperscript{-} cDC subset encompasses lymphoid-resident CD8\textalpha\textsuperscript{+} cDC, as well as the CD103\textsuperscript{+} DC of peripheral tissues, dedicated to efficient cross-presentation of viral antigens via MHC I molecules to CD8\textsuperscript{+} T cells.\textsuperscript{8} The human antipodes of murine CD8\textalpha\textsuperscript{+} cDC are the XCR1\textsuperscript{+}, blood dendritic cell antigen (BDCA)-3\textsuperscript{+}, CLEC-9A\textsuperscript{+} circulating cDC, whereas BDCA-1\textsuperscript{+} cDC resemble the murine CD11b\textsuperscript{+} cDC.\textsuperscript{9} Newly generated cDC have an immature phenotype, characterized by the low surface expression of major histocompatibility complex (MHC) molecules I and II, and T cell co-stimulatory molecules (CD40, CD80, CD86).\textsuperscript{10} While monitoring the environment for the presence of bacterial and viral pathogens, cDC maintain an immature state. Without an overt presence of stimuli, steady-state immature cDC induce and foster peripheral tolerance.\textsuperscript{11} Upon inflammation, cDC capture pathogenic antigens, but also self-antigens from damaged or apoptotic cells through pattern recognition receptors (PRR), such as Toll-like receptors (TLR), C-type lectin receptors (CLR) and CD1 receptors, which in turn induces DC maturation.\textsuperscript{12} This is accompanied by characteristic functional changes of DC, such as loss of endocytic capacity, up-regulation of MHC molecules, T cell co-stimulatory molecules and the production of cytokines such as tumor necrosis factor α (TNF-α), interleukin 12 (IL-12), IL-23 and IL-10.\textsuperscript{12} Concomitant with their maturation process, migratory and lymphoid-resident cDC traffic to the T cell zones in the secondary lymphoid organs to prime and stimulate naïve, memory and effector T cells, and natural killer T (NKT) cells by antigen presentation.\textsuperscript{13} pDC represent an apart DC subset, first described as plasmacytoid T cells or plasmacytoid monocytes in human.\textsuperscript{14-16} They represent a rare leukocyte population, poor in antigen presentation, but specialized in rapid secretion of large amounts of type I interferons (IFN) upon viral and bacterial infection.\textsuperscript{17} Human pDC are typified by the cell surface expression of
BDCA-2, BDCA-4 and CD123 (IL-3Rα), whereas the murine counterpart expresses bone marrow stromal antigen 2 (BST-2), Siglec-H, B220 and Ly6C.\(^\text{18}\) Under healthy steady-state conditions, pDC are present in low numbers, mainly in T cell areas of lymph nodes and spleen, mucosal-associated tissues, thymus and liver, while accumulating in lymphoid and non-lymphoid tissues under pathological conditions.\(^\text{18}\) Viruses and pathogens are sensed through TLR7 and TLR9.\(^\text{19, 20}\) Besides the production of type I IFNs, pDC also secrete IL-12, IL-6, TNF-\(\alpha\), as well as pro-inflammatory chemokines such as CXCL9, CXCL10, CCL3, CCL4 and CCL5. Thereby, pDC can attract CD4\(^+\) and CD8\(^+\) T cells to sites of infection, inducing long-term T cell survival and memory\(^\text{21}\), T helper 1 (Th1) polarization, cytolytic T cell responses\(^\text{22}\), DC maturation\(^\text{23}\) and differentiation of B lymphocytes into immunoglobulin-secreting plasma cells.\(^\text{24}\) Otherwise, pDC have been shown to induce a state of tolerance, thus dampening immune reactions at sites of infections.\(^\text{18}\)

During pathogenic inflammation an additional monocyte-derived DC subset emerges, which is termed the ‘inflammatory DC’. During inflammation, circulating monocytes migrate to the inflamed tissue where they can differentiate into inflammatory macrophages or DC.\(^\text{25}\) Inflammatory (monocyte-derived) DC secrete a specific set of pro-inflammatory cytokines (TNF-\(\alpha\) and nitric oxid) and exert phagocytic activity. Human and mouse inflammatory DC both express CD11b, CD206, CD172a, and FcεRI.\(^\text{7, 8}\)

Langerhans cells (LC) form a separate radioresistant tissue DC subset that comprises 2-3% of epidermal cells, and constitute the first barrier against skin-invading pathogens. It has been shown that LC are maintained under steady-state conditions by local, long-lived, self-renewing precursors that seed the skin already before birth. During inflammation LC are replaced by circulating Gr-\(\text{1}^{\text{high}}\) monocyctic precursors that enter the skin via blood. LC are phenotypically characterized by the expression of CD11c, MHC class II, CD205, and the langerin receptor CD207, which is linked to the intracellular Birbeck granules.\(^\text{26}\)
Supplemental References


