

Plasmacytoid Dendritic Cells in Atherosclerosis Knocking at T-Cell's Door

Erik A.L. Biessen, PhD; Anette Christ, PhD

The contribution of both innate and adaptive immune processes to the pathophysiology of atherosclerosis is undisputed.¹ Even healthy vessels harbor subendothelial networks of several dendritic cell (DC) subsets, which are considerably expanded in atherosclerosis.² DCs orchestrate innate and adaptive immunity against invading pathogens, as well as altered self-antigens, and have been attributed a central role in atherosclerosis-related immune responses. Nevertheless, considerable controversy exists about the phenotype of discrete DC subsets in, and their impact on, atherosclerosis. Genetic manipulation studies in which the total pool of conventional DCs (cDCs) was ablated or expanded gave rather inconsistent results. These studies not only left unaddressed the question of whether particular cDC subsets play a dominant role in the control of adaptive immune responses relevant to atherosclerosis or of cholesterol homeostasis^{3,4}; they also urged for more refined approaches targeting specific subsets of this heterogeneous cell population.

Article see p 1363

Despite their scant presence in advanced human atherosclerotic lesions, plasmacytoid DCs (pDCs) have drawn considerable attention in this regard. pDCs represent a rare leukocyte population present in blood and peripheral lymphoid organs and sites of inflammation and are thought to augment inflammatory processes by releasing high levels of proatherogenic type I interferons (IFNs).^{5,6} They uniquely express Toll-like receptors (TLRs) 7 and 9, which mediate the induction of type I IFN secretion in response to viral and certain bacterial antigens. In addition, pDCs can act as antigen-presenting cells, albeit less efficiently than cDCs, to activate or inhibit T-cell-mediated adaptive immune responses and immunopathology.⁷ However, recent efforts to address the role of pDCs in atherogenesis by antibody-aided depletion were rather inconsistent and left unsettled whether pDCs are detrimental or beneficial in their pathophysiology.⁸⁻¹⁰

In this issue of *Circulation*, Sage et al¹¹ provide new evidence that pDCs act proatherogenic in murine atherosclerosis by presenting (plaque derived) antigens via major histocompatibility complex (MHC) II and inducing CD4⁺ T-cell immunity. The authors circumvent the potential pitfalls of antibody depletion, deploying 2 elegant genetic loss-of-function models to interrogate the involvement of pDCs in atherosclerosis. For selective pDC deficiency, they took advantage of the fact that expression of the basic helix-loop-helix transcription factor Tcf4/E2-2 is indispensable for proper pDC differentiation. *Ldlr*^{-/-} mice repopulated by *CD11c-Cre* × *Tcf4*^{-/lox} bone marrow displayed 80% to 90% reductions of pDCs (characterized as CD11c⁺ B220⁺ PDCA1⁺) in blood, spleen, lymph node, and aorta, concordant with previous findings of Ghosh et al.¹² Depletion was paralleled by minor increases in CD11c^{hi} MHC II⁺ cDC numbers in spleen and lymph nodes. pDC depletion significantly attenuated plaque development and led to a marked reduction in plaque CD3⁺ T-cell numbers, pointing to a proatherogenic role of pDCs in *Ldlr*^{-/-} mice. This phenotype could be recapitulated in *Ldlr*^{-/-} mice reconstituted by bone marrow from B-cell deficient donor mice, lacking the pIII+IV MHC II transactivator (hence, ablated MHC II deficiency in pDCs and stromal cells; μ MT:*pIII+IV*^{-/-}). The resulting chimeras featured MHC II-restricted antigen presentation defects in pDCs only, with unchanged pDC numbers and TLR 7/9 response. MHC II deficiency in pDCs resulted in elevated levels of splenic IFN- γ -producing CD4⁺ T-helper 1 cells, even in B-cell-sufficient mice. This led the authors to conclude that pDCs act proatherogenic by MHC II antigen presentation. Indeed, aortic pDCs, isolated from *ApoE*^{-/-} and *Ldlr*^{-/-} mice, were shown to ingest and present the prototype antigen Ea in an MHC II-dependent manner.¹⁰ Moreover, Sage et al¹¹ demonstrated that ovalbumin pulsed pDCs can induce OT-II T-cell proliferation, albeit less potently than cDCs. MHC II-deficient pDCs were significantly impaired in their ability to stimulate T-cell proliferation, again confirming the involvement of MHC II. Strikingly, pDCs had a remarkably high capacity for MHC II-dependent presentation of native low-density lipoprotein-derived epitopes to CD4⁺ T-cell hybridoma. Altogether, the authors conclude that MHC II expression by pDCs is required to drive proatherogenic CD4⁺ T-cell responses. These findings align well with previous findings on the considerable antigen-presenting capacity of aortic pDCs from *ApoE*^{-/-} mice,^{9,10} whereas sorted aortic pDCs from hyperlipidemic mice, ex vivo pulsed with ovalbumin and oxidized low-density lipoprotein, were seen to induce strong antigen-specific OT-II CD4⁺ T-cell proliferation in vivo.⁹

The fact that the μ MT:*pIII+IV*^{-/-} → *Ldlr*^{-/-} model displayed normal-type I IFN responses after pDC activation but mirrored the phenotype seen in the pDC depletion model challenges the notion that pDC-released IFN- α is

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From the Department of Pathology, Cardiovascular Research Institute Maastricht, Maastricht University Medical Center, Maastricht, The Netherlands (E.A.L.B.); Division of Infectious Diseases and Immunology, Department of Medicine, University of Massachusetts Medical School, Worcester, MA (A.C.).

Correspondence to Erik A.L. Biessen, PhD, Department of Pathology, Cardiovascular Research Institute Maastricht, Maastricht University Medical Center, P Debyelaan 25, PO Box 616, 6200 MD Maastricht, The Netherlands. E-mail Erik.biessen@mumc.nl

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a key driver in atherogenesis. This is surprising in view of the studies of Döring et al⁹ reporting increased IFN- α content in murine plaques and serum in high-fat diet-fed *ApoE*^{-/-} mice, which was reduced after PDCA1 antibody depletion of pDCs. In their study, plaque pDC activation was attributed to complexes of DNA and the antimicrobial protein Cramp within the atheroma, which had the capacity to induce IFN- α production by pDCs in vitro.⁹ The observed mode of action mechanism is reminiscent of the pathophysiology of autoimmune diseases characterized by a type I IFN signature and double-stranded DNA-targeted immune responses, such as psoriasis or systemic lupus erythematosus.¹³ In line with Döring et al,⁹ Macritchie et al¹⁰ reported markedly reduced lesion sizes in the aortic root and aorta of *ApoE*^{-/-} mice on PDCA1 antibody-induced pDC depletion. However, in the latter study, IFN- α levels were not affected by hypercholesterolemia or by pDC depletion, reflecting limited, possibly plaque-restricted pDC activation.¹⁰ Supportive of this, we were unable to detect any effects of prolonged pDC depletion or Western-type diet feeding on IFN- α expression in plasma and spleen, a finding that was corroborated in human coronary artery disease.⁸ Using another PDCA1-specific depletion antibody (120G8), our group observed exacerbated atherosclerosis in the carotid artery and aortic roots of *Ldlr*^{-/-} mice after pDC depletion. Plaque expansion was accompanied by increased plaque T-cell numbers and more activated peripheral CD4⁺ T cells. 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.⁸

These discrepant findings are intriguing and could relate to differences not only in mouse models and mouse health status but also in the pDC depletion antibody, although it is targeting the same antigen, or antibody administration regime. Moreover, (target cell-bound) depletion antibodies could well exert different patterns of Fc γ receptor activation than the isotype control, potentially leading to disparate leukocyte activation, whereas acute massive cell death has been seen to elicit immunosuppressive or stimulatory effects, depending on its context. The aforementioned limitations underpin the importance of more refined genetic approaches, such as the *CD11c-Cre \times Tcf4^{-flox}* and the μ MT:*pIII+IV*^{-/-} models, deployed by Sage et al,¹¹ to firmly establish the role of DC subsets, such as pDCs in the pathophysiology of atherosclerosis.

It is unclear why MHC II deficiency of pDC has such a profound impact on CD4 T-cell priming, given the unaltered abundance of cDCs. Although pDCs were reported to mediate protective adaptive immune responses,¹⁴ their antigen-presenting capacity is generally viewed as inferior to that of cDCs. Nonactivated pDCs express only low levels of MHC II and costimulatory molecules and are, therefore, less potent in T-cell priming. On TLR activation pDCs can acquire cDC-like features, with lowered Bst2 and increased CD11c and MHC II expression. However, antigen uptake by pDC-expressed Bst2, combined with TLR activation, was found to induce strong antigen-presenting immune responses that were equivalent to those in cDCs.

Relevant to atherosclerosis, pDCs express scavenger receptors, such as CD36 and CD205, shaped to internalize (modified) lipoproteins and contained neoepitopes, whereas oxidized low-density lipoprotein, in turn, was seen to upregulate CD36 and MHC II expression.¹⁵ Conceivably, lipoprotein uptake by pDCs effects a phenotypic switch (upregulating MHCII and costimulatory molecules) toward a cDC-like antigen-presenting cell, able to elicit strong adaptive immune responses.¹⁶ This could serve as a parallel mechanism of pDCs to acquire bona fide antigen-presenting capacity in atherosclerosis next to TLR activation, as proposed previously.¹⁴ Whether this is opportune in atherosclerosis remains to be addressed, but the unaltered pDC BST2/CD11c expression in atherosclerotic *Ldlr*^{-/-} mice seems to speak against this notion.

An obvious question to be asked is why cDCs do not compensate for the loss in T-cell priming ability on pDC depletion. It is unclear whether this reflects differences in pDC versus cDC colocalization with T cells in plaque or plaque-draining lymph nodes¹⁷ or in their trafficking routes to peripheral (inflamed) lymphoid organs. Nevertheless, the data presented in the study of Sage et al¹¹ hints toward more efficient uptake and presentation of plaque neoepitopes by pDCs than cDCs, a conception with major implications for future plaque-targeting immunization and vaccination strategies.¹⁸

In conclusion, the current study by Sage et al¹¹ and previously published reports on pDC depletion illustrate their pleiotropism in the complex pathophysiology of atherosclerosis. Local and peripheral factors are critical in regulating the pDC phenotype to favor immune activation or tolerance. At early stages of atherosclerosis and during episodes of fulminant plaque, inflammation proatherogenic pDC functions seem to predominate and involve MHC II-dependent CD4 T-cell priming and, possibly, type I IFN-driven immune activation, especially in neutrophil-enriched foci. On the other hand, we cannot at this point exclude that pDC will act immunosuppressive during stages of low-grade inflammation.¹⁹ The use of a reproducible and precise animal model is vital in this context, as highlighted by the study of Sage et al.¹¹ Their findings, although clearly adding to our understanding of pDC functionality in atherogenesis, also identify pDC-based immunotherapy as an attractive new target for treating atherosclerosis. pDCs could be instructed to mediate tolerance toward plaque neoepitopes, as shown for solid organs and hematopoietic stem cell transplantation.¹⁸ Alternatively, pDCs primed *ex vivo* with plaque epitopes, such as Ep1.B (a novel apolipoprotein E-derived self-peptide), could be used to promote the generation of regulatory T cells in vivo and confer protection against atherosclerosis.²⁰ The future will tell whether pDCs exert similar proatherogenic activity in human atherosclerosis and whether they will perform superiorly to other cDC subsets in plaque vaccination or immunization strategies.

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Disclosures

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