Plasmacytoid Dendritic Cells in Atherosclerosis:

Knocking at T-Cell’s Door

Running title: Biessen et al.; PDC impact on atherosclerosis

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Journal Subject Code: Atherosclerosis:[134] Pathophysiology

Key words: atherosclerosis, Editorial, immunology, vaccination
The contribution of both innate and adaptive immune processes to the pathophysiology of atherosclerosis is undisputed. Next to cytokines, a range of danger-associated molecular pattern molecules (DAMP) are being released in plaque, amongst others by apoptotic and necrotic cells and due to proteolysis of extracellular matrix material. These proinflammatory agents will activate several leukocyte populations to perpetuate the inflammatory process. Even healthy vessels harbor subendothelial networks of several dendritic cell (DCs) subsets, which are considerably expanded in atherosclerosis. 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 if particular cDC subsets play a dominant role in the control of adaptive immune responses relevant to atherosclerosis or of cholesterol homeostasis, they also urged for more refined approaches targeting specific subsets of this heterogeneous cell population.

Despite their scanty presence in advanced human atherosclerotic lesions plasmacytoid dendritic cells (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 pro-atherogenic type I interferons (IFN). They uniquely express Toll-like receptors (TLR) 7 and TLR9, which mediate the induction of type I IFN secretion in response to viral and certain bacterial antigens. Additionally, pDCs can act as antigen-presenting cells, albeit less efficiently than cDCs, to activate or inhibit T-cell mediated adaptive immune responses and immunopathology. Recent
efforts to address pDC’s role in atherogenesis by antibody-aided depletion were however rather inconsistent and left unsettled whether pDCs are detrimental or beneficial in its pathophysiology.

In this issue of *Circulation*, Sage and colleagues provide new evidence that pDCs act pro-atherogenic 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 two 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 indispensible for proper pDC differentiation. *Ldlr<sup>−/−</sup>* mice repopulated by CD11c-Cre x Tcf4<sup>−/lox</sup> bone marrow displayed 80-90% reductions of pDCs (characterized as CD11c<sup>+</sup> B220<sup>+</sup> PDCA1<sup>+</sup>) in blood, spleen, lymph node and aorta, concordant with previous findings of Reizis and coworkers. Depletion was paralleled by minor increases in CD11c<sup>hi</sup> MHCII<sup>+</sup> cDC numbers in spleen and lymph nodes. PDC depletion significantly attenuated plaque development and led to a marked reduction in plaque CD3<sup>+</sup> T-cell numbers, pointing to a pro-atherogenic role of pDCs in *Ldlr<sup>−/−</sup>* mice. This phenotype could be recapitulated in *Ldlr<sup>−/−</sup>* mice reconstituted by bone marrow from donors lacking pIII+IV MHCII transactivator (hence MHCII expression in pDCs, B cells and stromal cells) and B-cell μMT (‘μMT:pIII+IV<sup>−/−</sup>‘). These chimeras feature MHCII-restricted antigen presentation defects in pDCs only, with unchanged pDC numbers and TLR7/9 response. MHCII deficiency in pDCs resulted in elevated levels of splenic IFNγ producing CD4<sup>+</sup> Th1 cells, even in B-cell sufficient mice. This led the authors to conclude that pDCs act pro-atherogenic by MHCII antigen presentation. Indeed aortic pDCs, isolated from *ApoE<sup>−/−</sup>* as well as *Ldlr<sup>−/−</sup>* mice, were shown to ingest and present the
prototype antigen Eα in an MHCII-dependent manner. Moreover, Sage and colleagues demonstrated that ovalbumin pulsed pDCs can induce OT-II T-cell proliferation, albeit less potent than cDCs. MHCII-deficient pDCs were significantly impaired in their ability to stimulate T-cell proliferation, again confirming the involvement of MHCII. Strikingly, pDCs had a remarkably high capacity for MHCII-dependent presentation of native low-density lipoprotein (nLDL)-derived epitopes to CD4+ T-cell hybridoma. Altogether, the authors conclude that MHCII expression by pDCs is required to drive pro-atherogenic CD4+ T-cell responses. This aligns well with previous findings on the considerable antigen-presenting capacity of aortic pDCs from ApoE−/− mice, while sorted aortic pDCs from hyperlipidemic mice, ex-vivo pulsed with ovalbumin and oxidized LDL, were seen to induce strong antigen-specific OT-II CD4+ T-cell proliferation in vivo.

The fact that the μMT:plIII+1IV−/− 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 IFNα is a key driver in atherogenesis. This is surprising in view of the studies of Döring and colleagues 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. This mechanism is reminiscent of the pathophysiology of autoimmune diseases characterized by a type I IFN-signature and dsDNA-targeted immune responses, such as psoriasis or systemic lupus erythematosus (SLE). In line with Döring and colleagues, MacRitchie and colleagues reported markedly reduced lesion sizes in the aortic root and aorta of ApoE−/− mice upon PDCA1 antibody induced pDC depletion. However, in the latter study IFNα
levels were neither affected by hypercholesterolemia nor by pDC depletion, reflecting limited, at best plaque-restricted pDC activation\textsuperscript{10}. Supportive of this, we were unable to detect any effects of prolonged pDC depletion or western type diet feeding on IFN\alpha expression in plasma and spleen, a finding that was corroborated in human coronary artery disease\textsuperscript{8}. Using another PDCA1 specific depletion antibody (120G8), our group observed exacerbated atherosclerosis in the carotid artery and aortic roots of \textit{Ldlr}\textsuperscript{-/-} mice after pDC depletion. Plaque expansion was accompanied by increased plaque T-cell numbers and more activated peripheral CD4\textsuperscript{+} T-cells.

PDCs isolated from atherosclerotic mice suppressed CD4\textsuperscript{+} T-cell proliferation in an indoleamine-2,3-dioxygenase–dependent manner, suggestive of an atheroprotective role for pDCs in atherosclerosis\textsuperscript{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 - even though targeting the same antigen - or antibody administration regime. Moreover, (target-cell bound) depletion antibodies could well exert different patterns of Fc\gamma receptor activation than the isotype control, potentially leading to disparate leukocyte activation, while 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 x Tcf4\textsuperscript{-/-}\textsuperscript{flo}x and the “μMT:pIII+Iv\textsuperscript{+/–}” models, deployed by Sage and coworkers, to firmly establish the role of DC subsets such as pDCs in the pathophysiology of atherosclerosis.

It is unclear why pDC-MHCII deficiency has such profound impact on CD4 T-cell priming, given unaltered abundance of cDCs. Although pDCs were reported to mediate protective adaptive immune responses\textsuperscript{14}, their antigen presenting capacity is generally viewed as inferior to that of cDCs. Non-activated pDCs express only low levels of MHCII and co-
stimulatory molecules and are therefore less potent in T-cell priming. Upon TLR activation
pDCs can acquire cDC-like features, with lowered Bst2 and increased CD11c and MHCII
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 do express scavenger receptors such as CD36 (and
CD205 in humans), shaped to internalize (modified) lipoproteins and contained neo-epitopes,
while oxidized LDL in turn was seen to upregulate CD36 and MHCII expression\(^\text{15}\). Conceivably,
lipoprotein uptake by pDCs effects a phenotypic switch (up-regulating MHCII and co-
stimulatory molecules) towards a cDC-like antigen presenting cell, able to elicit strong adaptive
immune responses\(^\text{16}\). This could serve as a parallel mechanism of pDCs to acquire \textit{bona fide}
antigen presenting capacity in atherosclerosis next to TLR activation, as previously proposed\(^\text{14}\).
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 upon pDC depletion. It is unclear if this reflects differences in pDC vs cDC co-
localization with T-cells in plaque or plaque draining lymph nodes\(^\text{17}\), or in their trafficking routes
to peripheral (inflamed) lymphoid organs. Nevertheless, the data presented in this issued paper
hints towards more efficient uptake and presentation of plaque neo-epitopes by pDCs than cDCs,
a conception with major implications for future plaque-targeting immunization and vaccination
strategies\(^\text{18}\).

In conclusion, the current study by Sage and colleagues and previously published reports
on pDC depletion illustrate their pleiotropism in the complex pathophysiology of atherosclerosis.
Local and peripheral factors are critical in regulating 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 MHCII dependent CD4 T cell priming and, possibly, type I IFN-driven immune activation, especially in neutrophil enriched foci. On the other hand we can at this point not exclude that pDC will act immunosuppressive during stages of low-grade inflammation\(^1\). Use of a reproducible and precise animal model is vital in this context, as highlighted by the study of Sage and coworkers. Their findings, while 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 towards plaque neo-epitopes, as shown for solid organs as well as haematopoietic stem cell transplantation\(^2\). Alternatively, pDCs primed ex-vivo with plaque epitopes, such as Ep1.B (a novel apolipoprotein E-derived self-peptide) could be employed to promote the generation of regulatory T cells \textit{in vivo} and confer protection against atherosclerosis\(^3\). The future will tell whether pDC exert similar pro-atherogenic activity in human atherosclerosis and whether they will perform superior to other cDC subsets as scaffold in plaque vaccination or immunization strategies.

\textbf{Conflict of Interest Disclosures:} None.

\textbf{References:}


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Circulation, published online September 15, 2014;
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

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