Dendritic cells in atherosclerosis

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Abstract

Atherosclerosis is a chronic inflammatory disease with activation of both the innate and adaptive arms of the immune system. Dendritic cells (DCs) are potent activators of adaptive immunity and have been identified in the normal arterial wall and within atherosclerotic lesions. Recent evidence points to a functional role for DCs in all stages of atherosclerosis because of their myriad functions including lipid uptake, antigen presentation, efferocytosis, and inflammation resolution. Moreover, DC-based vaccination strategies are currently being developed for the treatment of atherosclerosis. This review will focus on the current evidence as well as the proposed roles for DCs in the pathogenesis of atherosclerosis and discuss future therapeutic strategies.

Keywords

Dendritic cells; Atherosclerosis; Tregs; Efferocytosis

Introduction

Atherosclerosis is a disorder in which the sub-endothelial retention of apolipoprotein B-containing lipoproteins in focal areas of the arterial tree triggers a chronic, nonresolving inflammatory disorder mediated by activation of the innate and adaptive arms of the immune system [1]. The retained lipoproteins, perhaps after oxidative modification, mediate endothelial cell activation and recruitment of monocytes into the subendothelial region or intima. The newly recruited monocytes differentiate into macrophages and DCs, many of which ingest the retained lipoproteins to become cholesteryl ester-laden "foam cells." The macrophages and DCs, along with T cells and other immune cells, mount a maladaptive, nonresolving immune response that promotes progression of the atherosclerotic process [2]. Other features of developing atherosclerotic lesions include the accumulation of myofibroblastic-like vascular smooth muscle cells and expansion of the extracellular matrix proteins, notably proteoglycans, collagen, and elastin. The inflammatory environment, coupled to endoplasmic reticulum (ER) stress, oxidative stress, and likely other processes, can eventually lead to apoptosis of intimal cells as lesions progress. When these apoptotic cells are not efficiently cleared by neighboring phagocytes (efferocytosis), they become

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secondarily necrotic and, together with other processes that trigger primary cell necrosis, promote the formation of the so-called necrotic core [2]. In humans, the presence of a necrotic core in an atherosclerotic lesion, along with thinning of fibrous scar, or "cap," that normally forms over the core, predicts a "vulnerable" plaque phenotype [3] that is susceptible to plaque rupture and luminal thrombosis. This series of events accounts for the majority of plaques that trigger acute myocardial infarction, unstable angina, sudden cardiac death, and stroke.

Dendritic cells (DCs) are uniquely poised to serve at the interface of innate and adaptive immune system and are the most potent cells to activate both naïve and memory T cells. The identification of DCs in human [4] and mouse [5] vascular wall has stimulated tremendous interest in the role of these cells in the pathogenesis of several acute and chronic vascular disorders, including atherosclerosis. How DCs influence the initiation and progression of atherosclerosis is not clear, but their roles in presenting antigen to and activating T cells and secreting cytokines, and perhaps also their ability to become foam cells and to participate in efferocytosis, suggest that DCs have major influence on the pathogenesis of atherosclerosis. This review will focus on the origin and functional role of DCs in the pathogenesis of atherosclerosis and discuss novel approaches of targeting DCs as a therapeutic strategy in atherosclerosis.

Identification and characterization of vascular wall DCs

Lymphoid tissue DCs have been phenotypically classified as classical DCs (cDCs) and plasmacytoid DCs (pDCs) based on cell surface markers and functional criteria. However, the phenotypic identification of DCs in nonlymphoid tissues including within the atherosclerotic plaque has been an area of considerable confusion both in humans and mice because of the overlapping nature of the cell-surface markers used to characterize them, differences in expression markers based on their developmental origin (discussed in the next section), and phenotypic cellular plasticity. For example, CD11c, which is considered a marker of classical DCs in lymphoid tissues, can also be expressed by macrophages in an inflammatory microenvironment such as in the setting of atherosclerosis. A refined approach to identify DCs uses multiple cell-surface markers simultaneously. For example, DCs are $CD11c^{hi}$, $MHC-II^{hi}$, and can be, $CD11b^{\pm}$, $F4/80^{\pm}$, $CD103^{\pm}$, $DEC-205^{\pm}$, $Clec9a^{\pm}$, and $Mertk^{lo/-}$. More recently, transcriptional profiling have added impetus to the use of additional markers such as Flt3, c-Kit, CD272, and CD26, which have been demonstrated to be expressed exclusively by classical DCs and not by several types of tissue macrophages [6]. There are several drawbacks of this approach, including (a) cell-surface marker analysis by flow cytometry, requires dissociation of cells from tissues using enzymatic approaches, which can introduce artifacts. For example, some proteins are more susceptible to cleavage or degradation upon enzymatic digestion [7] and (b) localization of distinct subsets of DCs in the vascular wall or atherosclerotic plaque cannot be determined because of the nature of the assay. However, newer techniques such as "histocytometry" [8], which combines multiplex antibody labeling and powerful analytical microscopy, may overcome these limitations and future studies in this direction are highly warranted.

In vitro studies have demonstrated that macrophage foam cells may adopt a DC-like phenotype, including expression of CD11c and MHC-II [9]. However, whether these cells actually exist in atherosclerotic lesions and have the functional capabilities of DCs, notably the ability to present antigen to naïve T cells, is not known. In general, the phenotypic promiscuity of DCs demands that they be defined functionally in terms of their superior ability to present antigens to and activate native T cells. Thus, it is imperative that newly identified subsets of cells hypothesized to be DCs be loaded with model antigens and then

tested for their ability to present antigen to activate naïve antigen-specific transgenic T cells. The ovalbumin-based OVA-OT I/OT II experimental model is often used for this purpose.

Origin of arterial wall DCs

DCs have been identified in the arterial wall of both humans and mice [5, 10]. Interestingly, they are present in higher numbers in the atherosclerosis-prone regions, such as the lesser curvature of the aorta [11]. These regions are exposed to high shear-stress blood flow, and so shear-stress signals might be involved in DC recruitment. However, the specific molecular mechanisms or chemokine receptors involved in recruiting DCs or their precursors to non-atherosclerotic aorta have not yet been identified. In a seminal study, Choi et al. [12] demonstrated that DCs in normal aorta are predominantly located in the intima and can be classified into at least two developmentally distinct subsets, namely, $CD11c^+CD11b^-$ $CD103^+$ and $CD11c^+CD11b^+CD103^-$ DCs. The $CD103^+$ subset in the aortic wall expanded after injection of the DC developmental factor Flt3L and was specifically lost in $Flt3^{-/-}$ mice, demonstrating that this subset was derived from classical Flt3L-dependent pre-DC precursors. By contrast, the $CD11b^+CD103^-$ subset was dependent on the monocyte-related growth factor M-CSF, suggesting derivation from circulating monocyte precursors. Interestingly, the numbers of both subsets expanded rapidly in the intima of atherosclerotic lesions after introduction of hypercholesterolemia into the model. However, the $CD11b^+CD103^-$ subset accounted for most of the intimal DCs, suggesting that monocyte-derived DCs contribute most towards expansion of DC numbers in progressing atherosclerotic lesions. However, the predominant monocyte subset that serves as the precursor of these DCs is not yet clear, with some studies suggesting that $Ly6c^{lo}$ monocytes preferentially differentiate to lesional DCs [13] and others suggesting that they are derived from $Ly6c^{hi}$ monocytes [14]. The observation that $Cx3cr1^{-/-}$ mice have decreased accumulation of lesional DCs suggests that CX3CR1 may play a role in DC recruitment into lesions [15]. However, it is important to note that CX3CR1 is also used by monocytes to adhere to the vascular wall, and so the decrease in intimal DCs in $Cx3cr1^{-/-}$ mice may result primarily from decreased monocyte recruitment, which then secondarily suppresses DC differentiation. Thus, further studies are necessary to understand the specific chemokine receptors that are involved in recruitment of DCs into atherosclerotic lesions. pDCs are another DC subset that is present in atherosclerotic lesions, albeit in smaller numbers than classical DCs [16]. pDCs are derived from the so-called common DC precursor and, unlike classical DCs, express low levels of CD11c and MHC-II and high levels of PDCA-1 and SiglecH. A recent study described another specific subset of DCs, $CCL17^+$ DC, which was present in atherosclerotic lesions but not the healthy vascular wall [17]. These lesional $CCL17^+$ DCs expressed markers of DC maturation, notably high levels of MHC-II, CD40, CD80, and CD86. Currently, it is unclear whether these $CCL17^+$ DCs represent a developmentally distinct subset. Apart from DCs originating from pre-DC precursors and monocytes, there is evidence for local proliferation of $CD11c^+$ DCs in nascent and early lesions, as demonstrated by BrdU labeling [18]. Proliferation was shown to be dependent on GM-CSF [18], a cytokine that is used experimentally to differentiate DCs in vitro from peripheral blood monocytes or bone marrow hematopoietic stem cells. Given the recent demonstration that differentiation of inflammatory DCs from monocytes is not dependent on GM-CSF [19], it will be interesting to determine whether GM-CSF-dependent proliferation is restricted to a specific subset of DCs in atherosclerotic lesions. Finally, the recent identification of a zinc finger transcription factor, Zbtb46, which is specifically expressed in classical DCs and not in pDCs or monocytes [20, 21], may help elucidate the relative contribution of classical DCs derived from pre-DC precursors versus those derived from monocytes in the context of atherosclerosis. For example, breeding the Zbtb46-DTR mouse model [21], which enables depletion of DCs expressing Zbtb46, into an atherosclerosis-prone background may be a useful model in this regard.

Functional role of DCs in atherosclerosis

Role of DCs in lipid uptake and lipid metabolism

As mentioned earlier (Fig. 1), a subpopulation of cholesteryl ester-laden foam cells in atherosclerotic lesions appears to be derived from DCs. The mechanism by which DCs internalize lipoproteins to become foam cells remains to be determined, but the process likely involves multiple processes including macropinocytosis, receptor-mediated endocytosis, scavenger receptor-mediated uptake, direct uptake from circulation via dendritic processes that extend into the vascular lumen, and efferocytosis of cholesterol-rich apoptotic cells. Interestingly, depletion of vascular DCs in CD11c-DTR mice following injection of diphtheria toxin decreases the accumulation of lipids in nascent lesions, suggesting that DCs are an important mediator of early lesional lipid accumulation [22]. The functional significance, if any, of lipid accumulation by DCs is not known and will be discussed below.

Interestingly, apart from the role of DCs in lipid uptake in early lesions, several lines of evidence suggest that DCs in other sites might be involved in regulating systemic cholesterol levels. For example, in Western diet-fed $Ldlr^{-/-}$ and $Apoe^{-/-}$ mice in which the lifespan of DCs was enhanced by CD11c-specific transgenic expression of the anti-apoptotic protein Bcl-2, there was a decrease in the level of VLDL and LDL cholesterol [23]. Conversely, acute ablation of DCs upon injection of diphtheria toxin in a CD11c-DTR mice led to an increase in plasma cholesterol levels in $Ldlr^{-/-}$ and $Apoe^{-/-}$ mice [23]. The precise mechanisms by which DCs regulate systemic cholesterol levels is not known, but recent studies suggest that DCs might influence cholesterol absorption from the intestine as well as fecal excretion of sterols [24]. These data illustrate the extent of immune-metabolism cross-talk and highlight the central role of immune cells in modulating metabolic disorders.

Role of lipids in modulation of DC function

The macrophage has been the predominant lesional cell type studied to assess the affect of lipid accumulation on cell function. For decades, investigators had assumed that cholesterol accumulation promoted macrophage "activation." However, Glass and colleagues have recently presented in vitro evidence that cholesterol accumulation by macrophages can actually promote an anti-inflammatory response through activation of the transcription factor LXR by desmosterol [25]. Whether this pathway exists in cholesterol-loaded DCs has not yet been reported. Other macrophage consequences of lipid accumulation that may or may not apply to DCs include the ability to efflux cholesterol and/or possibly emigrate from lesions, which may promote the clearance of extracellular lipid from developing lesions. Finally, oxidation products of sterols and other lipoprotein-derived lipids could potentially trigger inflammation, oxidative and ER stress, and/or cell death in lesional DCs as they are thought to do in lesional macrophages.

In terms of how lipids may affect functions more specific to DCs, studies have shown that native LDL as well as the oxidatively modified forms of LDL can induce DC maturation, with upregulation of costimulatory molecules and inflammatory cytokines, in a manner that could influence the migratory and T cell-stimulatory capacity of DCs [26–28]. In this context, it is interesting to note that DCs under hyperlipidemic conditions have normal T cell stimulatory capacity [29], while their ability to migrate from peripheral tissues to secondary lymphoid organs is severely impaired [30]. This could have multiple implications in atherosclerosis: (a) impaired migration could impede the inflammation resolution function of DCs, such as cholesterol efflux, clearance of dead cells, and antigen presentation to T cells, which may be critical in inducing T cell tolerance to self or modified self antigens in atherosclerotic plaques; (b) local retention and maturation of DCs could promote the local

secretion of inflammatory cytokines, leading to enhanced monocyte and T cell recruitment; (c) local activation or reactivation of antigen-specific T cells by antigen-presenting DCs could lead to exacerbation of local inflammation; and (d) secretion of collagenases and matrix metalloproteases by mature DCs could promote collagen and extracellular matrix degradation, leading to thinning of the protective fibrous cap and a vulnerable plaque phenotype.

The inability of DCs to migrate under hyperlipidemic conditions appears to be due to deficient upregulation of the migratory chemokine receptor CCR7. The evidence for this stems from the observation that surgical transplantation of diseased aorta into normocholesterolemic animals led to regression of atherosclerotic lesions with emigration of CD11c^{hi} cells expressing high levels of CCR7 [31]. Moreover, neutralization of CCL19 and CCL21, ligands for CCR7, prevented the regression of lesions in the surgical aortic transplant model demonstrating that the emigration was dependent on expression of CCR7 [31]. However, when lesion regression was induced by treating $Apoe^{-/-}$ mice with viral vectors encoding Apoe, CCR7 did not affect lesional myeloid cell numbers [32]. Moreover, $Ccr7^{-/-} Apoe^{-/-}$ tended to have smaller lesions, arguing against a role for CCR7 in myeloid cell egress [32]. However, it is important to note that CCR7 is also important for homing of T cells [33], which could have influenced the phenotype of the CCR7-deficient lesions. Interestingly, recent studies in mice demonstrated that treatment with statins led to lesion regression, which was associated with increased expression of CCR7 in lesional cells [34]. While the primary beneficial effect of statins is related to their ability to lower plasma apoB-containing lipoproteins, it is theoretically possible that additional, incremental benefit may be derived by restoration of the inflammation resolution function of DCs. All of these studies call for additional studies and improved models to understand the mechanisms and consequences of CCR7 regulation and other factors that may affect DC trafficking, such as cellular guidance signals [35], during atherosclerosis progression.

DC maturation, antigen presentation, and atherosclerosis

Prior to antigen exposure, DCs exist in the immature state, characterized by the capacity to capture antigen but not yet expressing high levels of MHC-I/II antigen presenting module or the T cell costimulatory molecules CD80, CD86, and CD40. Upon TLR-mediated activation, DCs undergo the process of maturation, which is associated with downregulation of several modes of antigen capture, notably phagocytosis and efferocytosis, upregulation of MHC-I, MHC-II, CD80, CD86, and CD40, and migration to draining lymph nodes for antigen presentation to nodal T cells. Interestingly, both human and animal studies have demonstrated that mature DCs accumulate in the atherosclerotic plaques with lesion progression [10]. This is consistent with the idea that DC migration is defective under hypercholesterolemic conditions as described above. The signals that contribute to DC maturation in atherosclerotic lesions are not known, but several candidates include inflammatory cytokines, DNA and RNA fragments derived from necrotic cells, and cholesterol crystals. DC maturation is associated with pro-inflammatory cytokine secretion as well as the ability to robustly activate naïve T cells in an antigen-specific manner, both of which are known to accelerate atherosclerosis [36]. Indeed, early studies demonstrated that atherosclerosis is decreased in mice lacking the T cell costimulatory molecules CD80 and CD86 ($CD80^{-/-} CD86^{-/-}$), which are upregulated during DC maturation, and in mice deficient in CD74 (MHC-II associated invariant chain), which regulates antigen loading on MHC-II [37, 38]. These data suggested that DC maturation and antigen presentation to T cells led to a pro-atherogenic phenotype. However, deletion of CD80/CD86 is associated with severe systemic defect in regulatory T cell (Treg) development [39], a subset of T cell that is known to be atheroprotective [40]. Similarly, deficiency of CD74 is associated with a dramatic decrease in the number of peripheral T cells [38] which could have secondary

consequence on atherosclerosis independent of DC function. In this context, a more specific way to decrease the ability of DCs to present antigen to and activate T cells can be accomplished by blocking DC maturation through DC-specific knockdown of MyD88 (*Cd11cCre$^+$Myd88$^{fl/fl}$*) [41]. When these mice were placed on an atherosclerosis background, there was defective activation of both effector T cells (Teff) and Tregs in lesions, but the net effect was increased atherosclerotic lesion development [42]. These data demonstrate that DC maturation and antigen presentation play a major role in the development of atheroprotective Tregs, which has a dominant effect over the pro-atherogenic role of DC-activated Teff cells.

Other key questions related to DC-mediated T cell activation in the setting of atherosclerosis pertains to the identity of the antigen(s) in atherosclerosis, the site of antigen acquisition by DCs, as well as the site of DC-Tcell interaction. Among the lesional antigens that have been hypothesized to be important are oxidized LDL, Hsp60, Hsp65, and β2-glycoprotein I [43]. One idea is that lesional DCs acquire these and/or other antigens by macropinocytosis, scavenger receptor mediated uptake, or efferocytosis of antigen containing dead cells and then process and present those antigens on their MHC-I and MHC-II modules. Alternately, circulating DCs, peripheral tissue DCs, or lymphoid-tissue DCs may capture and then present circulating atherosclerosis-relevant antigens, such as oxidized LDL. It is interesting to note that in the aforementioned model of defective DC maturation [42], Treg numbers were decreased not only in the atherosclerotic lesions and in draining mediastinal and iliac lymph nodes, but also in nondraining inguinal lymph nodes. These data raise the possibility that DCs acquire certain antigens outside of atherosclerotic lesions per se.

The primary site of DC-mediated antigen presentation to T cells in atherosclerosis is an active area of investigation. The demonstration of oligoclonal expansion of T cells [44], as well as the finding that T cells are in close proximity to DCs as imaged in fixed aortic tissues [45] and in live images of aortic explants [46], have raised the interesting possibility that the atherosclerotic intima itself may be a site of antigen presentation and T cell activation. However, it is unlikely that this possible scenario represents the site of priming of naïve T cells, because the emigration of naïve T cells into peripheral tissues is limited. Rather, DCs may interact with activated/ memory T cells in lesions, which is consistent with the expression of activation markers by the T cells that interact with intimal DCs [46]. Moreover, in an aortic explant model, only T cells derived from hypercholesterolemic animals demonstrate interaction with aortic DCs [46]. These date reinforce the idea that the T cells that directly interact with DCs in lesions are pre-activated. The interaction of mature DCs with activated/memory T cells could have pathologically and clinically relevant consequences because of elicitation of a local inflammatory response, especially in advanced lesions where these DCs are sequestered because of deficient emigration. Another potential site of DC-mediated antigen presentation to T cells could be the arterial tertiary lymphoid organs (ATLO), which have been described in the adventitia adjoining advanced atherosclerotic lesions of mice [47]. These are sites with high endothelial venules, follicular DCs, and well-defined T and B cell areas. The mechanisms by which these ATLOs are formed are currently unknown. However, lymphotoxin-β receptor-expressing vascular smooth muscle cells have been hypothesized to play a role in their organization [47].

Role of DCs in maintaining Treg cell homeostasis

Tregs are a subset of CD4$^+$ T cells that suppress inflammation in atherosclerosis by inhibiting pro-atherogenic Th1 T cell responses [40] and by suppressing activation of endothelial cells [48] and macrophages via secretion of anti-inflammatory cytokines such as TGF-β and IL-10 [42, 49]. The current literature suggests that the atheroprotective response is mediated by both natural, thymus-derived as well as by adaptive or inducible Tregs,

which are derived by activation of naïve T cells in peripheral lymph nodes [50]. However, the relative contribution of natural vs. adaptive Tregs in suppressing inflammation in atherosclerosis is not known. The studies in this area are limited because of the lack of specific markers to distinguish the two populations [51]. The methylation status of CpG-rich Treg-specific demethylated region of the FoxP3 locus is the most precise marker of adaptive Tregs, because they are predominantly demethylated at this region while this region in natural Tregs is methylated. However, such analyses are not amenable in the context of atherosclerosis because of the small numbers of vascular Tregs that can be isolated from atherosclerotic aorta. In this context, recent demonstration of markers such as Helios [52] and Neuropilin [53] for distinguishing natural vs. adaptive Tregs warrants further investigation in the setting of atherosclerosis.

The current paradigm suggests that adaptive Treg cell development is favored by the presentation of antigen by immature DCs in a noninflammatory environment [54]. However, we recently demonstrated that MyD88 signaling in DCs, which is critical for DC maturation, is also important for generating a protective Treg cell response in the context of atherosclerosis [42]. This finding is consistent with recent data in the literature showing that the development of both Teff as well as Tregs from naïve T cells requires costimulation via CD80/CD86 [54], which is upregulated upon DC maturation. We also showed that the Treg-secreted TGF-β represses MCP-1 production by macrophages, thereby inhibiting the recruitment of inflammatory Ly6c^{hi} monocytes into atherosclerotic intima [42]. These data indicate that in early atherosclerosis, DC-mediated activation of T cells is dominated by an atheroprotective Treg response. However, the evidence that DCs in advanced lesions have a migratory defect (above) raises the interesting question as to whether lesional DCs in this setting can promote the formation of Tregs. This is especially important, because in early atherosclerosis, DC-mediated activation of T cells likely occurs after migration to draining lymph nodes, where a non-inflammatory environment, with low levels of IFN-γ and IL-12, might favor the development of atheroprotective Tregs. In contrast, in advanced atherosclerosis, wherein DC emigration is defective [30], antigen presentation presumably occurs under inflammatory conditions, which might result in strong costimulatory signaling and the development of pro-atherogenic Teff cells. The experimental testing of these hypotheses will require the development of models in which DC maturation could be blocked in a temporal manner. For example, one could utilize an inducible Cre to delete MyD88 in CD11c^{hi} cells only in advanced atherosclerosis. In addition, temporal inducible expression of CCR7 in advanced lesional DCs might rescue the migration defect and thus further test the hypotheses mentioned above.

There is also evidence for specific subsets of lesional DCs that can preferentially activate atheroprotective Treg cells or specifically inhibit their development. For example, the CD103^{+} DCs in lesions have been demonstrated to be tolerogenic DCs and to promote the development of Tregs [12]. These DCs are dependent on Flt3-Flt3L interaction for their development, and their loss in *Flt3*^{−/−} *Ldlr*^{−/−} mice resulted in decreased aortic Tregs, decreased IL10 production, increased production of pro-inflammatory cytokines TNF-α and IFN-γ, and exacerbation of atherosclerosis [12]. It is interesting to note that CD103^{+} DCs in the gut have also been demonstrated to be potent inducers of Treg cell differentiation, which is partly dependent upon their ability to secrete TGF-β and retinoic acid [55], cytokines which play a key role in peripheral Treg differentiation. Moreover, lung CD103^{+} DCs, via secretion of CCL22, has been demonstrated to be important in the recruitment of circulating Tregs [56]. This raises the interesting possibility that vascular CD103^{+} DCs might utilize a TGF-β/retinoic acid pathway and CCL22 to induce differentiation of peripheral Tregs and recruit circulating thymus-derived natural Tregs, respectively, into early atherosclerotic lesions.

In contrast to the CD103^{+} subset of DCs, CCL17^{+} DCs, which are also present in atherosclerotic lesions, appear to restrain Treg development [17]. In particular, CCL17 acting on CCR4 on T cells prevents the differentiation of naïve T cells to Tregs and promotes apoptosis of Tregs [17], and *CCL17*^{−/−} *Apoe*^{−/−} mice show an expansion of lesional Tregs and decrease in lesional macrophages and atherosclerotic lesion size. As described earlier, it is not yet clear whether the CCL17^{+} DCs are a developmentally distinct subset of DC or mature DCs that have been induced to express CCL17.

In summary, DCs appear to play a central role in maintaining Treg homeostasis in developing atherosclerotic lesions. The balance between Treg and Teff activation is a critical factor in determining whether the inflammatory component of atherosclerosis becomes exacerbated, leading to plaque progression, or whether lesional inflammation resolves. DC/ Treg-mediated inflammation resolution could be a major factor in plaque stabilization or, in the setting of correction of hyperlipidemia, and plaque regression.

Role of pDCs in atherosclerosis

pDCs have been described in the shoulder regions of both human and mouse atherosclerotic plaques and constitute only a small proportion of the total intimal DC population [57]. However, pDCs are potent secretors of the type 1 interferons IFN-α and IFN-β, both of which are known to be pro-atherogenic [58]. Moreover, the levels of IFN-α in human plaque have been shown to correlate positively with plaque vulnerability. In addition, INF-α has been shown to induce vascular smooth muscle cell apoptosis [57], which might promote detrimental thinning of the fibrous cap, and to sensitize DCs to TLR ligands, which induce pro-inflammatory cytokine production [59]. However, whether pDCs play a pro-atherogenic role is not clear, because depletion of pDCs using an antibody against bone marrow stromal cell antigen 2 led to aggravation of atherosclerosis in a Western diet fed *Ldlr*^{−/−} mouse model [60], whereas in an *Apoe*^{−/−} mouse model there was atheroprotection [16]. Thus, further studies are required to understand the role of pDCs in atherosclerosis.

DC efferocytosis—potential roles in antigen acquisition, inflammation resolution, and necrotic core formation

Efferocytosis is the process by which phagocytes recognize and clear apoptotic cells in a non-inflammatory manner. Immature classical DCs, which are present in early atherosclerotic lesions, and CD103^{+} DCs are potent efferocytes, which raises interesting questions about the importance of this process as a mechanism for antigen acquisition by DCs and subsequent T cell activation. DCs constantly sample the microenvironment for antigens using a multitude of processes including macropinocytosis, receptor-mediated endocytosis, phagocytosis, and efferocytosis. Following uptake, antigenic proteins or lipids are processed and presented on MHC-I or MHC-II to elicit either activation of antigen-specific T cells or to induce T cell tolerance, depending on the specific context. DC efferocytosis-mediated antigen presentation has been hypothesized to be critical in eliciting an immune response in several viral and autoimmune diseases [61]. However, this hypothesis remains to be proven in any context, including atherosclerosis as the molecular machinery that DCs use to carry out efferocytosis and antigen presentation in vivo has not yet been reported (below). In the context of atherosclerosis, DC efferocytosis may have a particularly important influence on plaque progression. In general, efferocytosis results in suppression of inflammation and suppression of DC maturation. Moreover, DC mediated efferocytosis of lesional apoptotic cells could prevent secondary cellular necrosis, which is a major pro-inflammatory signal that accelerates atherosclerotic lesion development as well as necrotic core formation [62]. Additionally, efferocytosis of lipid-laden apoptotic foam cells by DCs, followed by their migration out of earlier lesions, could play a critical role in removing oxidized lipids, cholesterol, and other pro-inflammatory damage-associated

molecular patterns. In this context, several studies have demonstrated that efferocytosis by macrophages plays a critical role in inflammation resolution and prevention of necrotic core formation in advanced atherosclerotic plaques [63–66]. However, there are no published studies till date that address the role of efferocytosis by intimal DCs in antigen acquisition, inflammation resolution, or necrotic core formation. As mentioned above, a major hurdle in the conduct of such studies is the lack of knowledge of the efferocytosis receptors utilized by DCs in vivo. For example, previous in vitro studies have identified CD36, αvβ5, and Axl/ Tyro to mediate DC efferocytosis [67, 68]. However, the relevance of these receptors in vivo in DC efferocytosis is not known. Future studies are necessary to identify the molecular mechanisms of DC efferocytosis in vivo so that molecular-genetic causation experiments can be conducted, i.e., by specifically disrupting DC efferocytosis and then assaying T cell activation and inflammation resolution in atherosclerosis.

As alluded to earlier, the accumulation of mature DCs in advanced atherosclerotic plaque could have several consequences, including amplification of local inflammatory processes, extracellular matrix degradation, and promotion of necrotic core development [62, 69]. With regard to the latter point, it is interesting to note that DCs lose their efferocytosis ability upon maturation [67]. Thus, the accumulation of mature DCs in advanced atherosclerotic lesions [10] could result in pockets of defective efferocytosis leading to focal necrosis. Consistent with this idea, we observed the presence of mature DCs not only in the shoulder region of the advanced murine atherosclerotic plaque but also adjoining the necrotic cores in these lesions (unpublished observations). Additional studies using models of defective DC maturation, such as *CD11cCre $^{+}$MyD88 $^{fl/fl}$* mice [42], or models in which emigration of mature DCs from the advanced plaque might be restored, e.g., through restoration of CCR7, will be required to understand the specific contribution of mature DCs in advanced atherosclerosis.

DC-based vaccine strategies

The understanding that atherosclerosis is a chronic inflammatory disorder that has an autoimmune component shaped by both cellular as well as humoral immunity has raised the possibility of using vaccination approaches in the treatment of atherosclerosis [43]. Early seminal studies demonstrated that immunization of rabbits or mice with native or oxidized LDL resulted in considerable protection against atherosclerosis [70–72]. Considering the complex nature of the LDL particle, recent efforts are focused on identifying minimal LDL-related epitopes, both lipid and protein, that would elicit potent immune response against atherosclerosis [43].

Given the central role of DCs in the activation of cellular as well as humoral immunity, recent efforts are focused on developing DC-based vaccination strategies against atherosclerosis. For example, intravenous injection of exogenous oxidized LDL-loaded DCs resulted in attenuation of flow-induced atherosclerosis and stabilization of plaque phenotype in carotid arteries of mice [71]. In an interesting alternate approach, DCs loaded with apoB100 protein were treated with the immunosuppressive cytokine IL-10, which is known to induce a tolerogenic phenotype in DCs. The injection of these tolerogenic apoB100-containing DCs led to decreased atherosclerotic lesion progression, decreased systemic inflammation, and decreased lesional infiltration of CD4+ T cells in human apoB100-transgenic *Ldlr$^{−/−}$* mice [73]. These effects were likely due to induction of the protective Treg cell response. These pre-clinical studies suggest that DC-based vaccination may be a powerful approach for eliciting protective Treg cell response in the treatment of atherosclerosis. To accomplish this goal, new approaches are needed to identify the antigen specificity of reactive T cells in atherosclerotic lesions so that efficient immunosuppressive vaccines can be designed.

Acknowledgments

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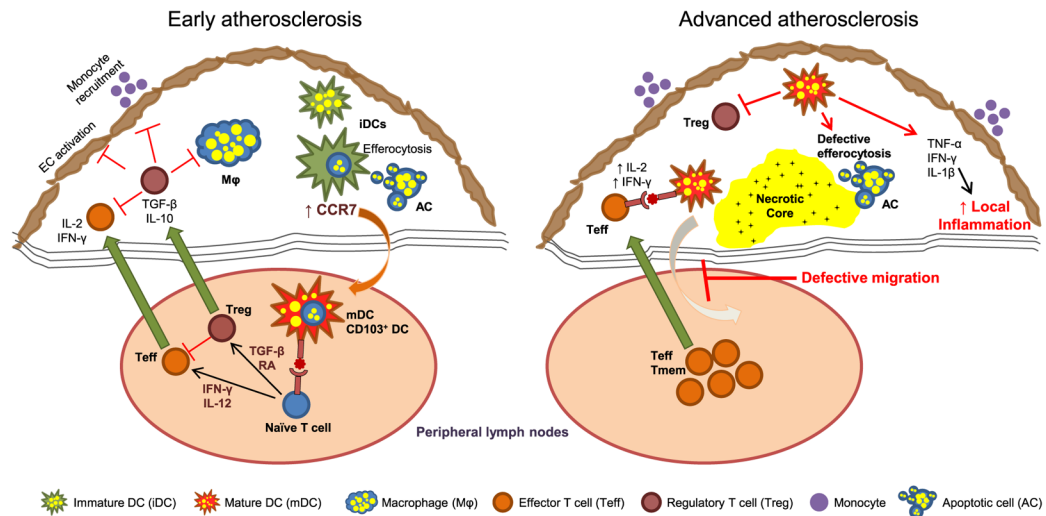
Fig. 1.

Proposed roles for DCs in the pathogenesis of early and advanced atherosclerosis. DCs in early atherosclerotic lesions become foam cells, most likely via the uptake of lipoproteins and lipid-laden apoptotic cells. Upon maturation and expression of CCR7, DCs emigrate from lesions into the draining lymph node to present antigen to and activate naïve T cells. The naïve T cells differentiate into Treg or Teff depending on the specific cytokine environment under which they are primed. Teff cells secrete pro-inflammatory cytokines and thereby have the capacity to promote atherogenesis, but this process is counteracted by Tregs. Tregs also inhibit the activation of endothelial cells and macrophages and block new monocyte recruitment via secretion of anti-inflammatory cytokines TGF-β and IL-10. However, in advanced atherosclerotic lesions, the emigration of DCs from the plaque is defective, perhaps because of the deficient upregulation of CCR7 or negative guidance cues. The retention of mature DCs in the plaque can lead to enhanced local inflammation via secretion of pro-inflammatory cytokines as well as activation of effector/ memory T cells. Moreover, as mature DCs are poor efferocytes, their accumulation in advanced atherosclerotic plaque could, in theory, lead to pockets of defective efferocytosis with consequent expansion of the necrotic core