Dendritic cells in atherosclerosis: current status of the problem and clinical relevance

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Dendritic cells (DCs) are the most potent antigen-presenting cells. DCs were identified in arteries in 1995 and, since then, further knowledge has been gained indicating the importance of DCs in atherosclerosis. Vascular DCs have been shown to become activated from a very early stage of atherosclerosis. Some DCs cluster with T cells directly within atherosclerotic lesions, while others migrate to lymphoid organs to activate T cells. Dyslipidaemia systemically alters DC function and recent findings suggest that DCs play a role in plaque destabilization. This review summarizes the current status of the problem.

KEYWORDS
Dendritic cells; Arteries; Atherosclerosis; Plaque destabilization

Introduction

Initially described in the skin by Langerhans in 1868, dendritic cells (DCs) were identified as antigen-presenting cells in 1973 by the pioneering work of Steinman and Cohn.1 In all tissues and all pathological conditions, DCs are minor cell populations but they are the most potent antigen-presenting cells.2

In arteries, DCs were originally identified in 1995.3 Since then, knowledge has been gained indicating the importance of DCs in atherogenesis. This review summarizes the current status of the problem.

DCs and immunity

In recent years, the biology and pathophysiology of DCs have been highlighted in a number of reviews and monographs.2,4–9 DCs are members of both innate and adaptive immune systems.4–8 As members of the innate immune system, DCs can respond to danger signals by immediately generating protective cytokines.4–9 As the key element of the adaptive immune system, DCs respond to danger signals by the acquisition of the capacity to direct the development of primary immune responses appropriate to the type of danger.4–9 DCs have a potent antigen-presenting capacity for the stimulation of naive, memory, and effector T cells.5–9 DCs are capable of activating not only conventional T cells but also natural killer T (NKT) cells.9 During the development of an adaptive immune response, T cells form direct contacts with DCs and respond to peptide antigen displayed on major histocompatibility complex (MHC) class II and class I molecules present on DC surfaces.4–9 In DC/T cell interactions, the presence of co-stimulatory molecules on DCs is required for T cell activation and the differentiation into effector cells. In the absence of sufficient co-stimulation, T cells contacting DCs exhibit anergy or undergo apoptosis.4–9 In DC/T cell contacts, the secretion or lack of secretion of cytokines, in particular interleukin-12 (IL-12), by DCs is instrumental in the differentiation of T cells into type 1 (Th1) or type 2 (Th2) effector T cells, respectively.4–9

The growing interest in DC physiology reflects the importance of DCs in the initiation and progression of various diseases, including tumour, viral and bacterial infections, and various autoimmune diseases.2,4–9 The ability of DCs to elicit immune responses and the availability of DC culture systems has led to their use in cancer immunotherapy and therapeutic intervention in autoimmunity and transplantation.2,4,9,10

Origin, migratory routes, and histological nomenclature of DCs

DCs originate from a common CD34+ progenitor in the bone marrow.4–9 Their development involves three stages for which the terms, ‘precursors’, ‘immature’, and ‘mature’ are used.2,4–9 DC precursors circulate via the bloodstream to reach their target tissues where they take up residence at sites of potential antigen entry.5–4–6 In this stage, DCs can be identified in essentially all tissues but are mostly concentrated along epithelial and body cavity surfaces.5–4–6 In these locations, DCs continuously and efficiently sample the antigenic content of their microenvironment by phagocytosis, by high-volume fluid phase macropinocytosis, by receptor-mediated endocytosis, or by direct contact with necrotic, apoptotic, or virally infected cells.5–4–6 Internalized antigens are degraded into short peptides that...
are loaded onto nascent class I and class II MHC or CD1 molecules for subsequent display on the cell surface.\textsuperscript{2,4–6} Because, in this stage, DCs are not yet able to stimulate T cells, these DCs are termed 'immature' or 'processing' DCs.\textsuperscript{2,4–6} Processing DCs exit the non-lymphoid tissues and migrate via the afferent lymph into lymphoid tissues, such as the spleen and lymph nodes, where DCs then complete their maturation.\textsuperscript{2,4–6} Maturation of DCs involves the down-regulation of endocytic activity and the up-regulation of the adhesion molecules (CD11a, CD50, CD54, and CD58), co-stimulatory molecules (CD40, CD80/87.1, CD86/87.2) and antigen-presenting molecules, including the class I and class II of MHC proteins and CD1 molecules.\textsuperscript{2,4–6} Co-expression of the adhesion, co-stimulatory, and antigen-presenting molecules enables DCs to form contacts with and activate T cells.\textsuperscript{2,4–6} It has been recognized also that the maturation of some DCs can occur in the peripheral non-lymphoid tissues\textsuperscript{4–6} and that DCs can present antigenic peptides to circulating T cells.\textsuperscript{4} The trafficking of DCs into tissue sites is regulated by chemokines.\textsuperscript{2,4–6} At different stages of their differentiation, DCs produce distinct compositions of cytokines which affect neighbouring cells.\textsuperscript{2,4–6}

The histological nomenclature of DCs is rather complex. In peripheral blood, circulating DCs are known as blood DCs.\textsuperscript{4} In peripheral blood, circulating DCs represent the migratory form of DC precursors. Immature DCs are known as Langerhans cells in the skin and other epithelial surfaces and as interstitial DCs in interstitial spaces of organs such as the heart, kidney, and lung.\textsuperscript{2,4–6} The migratory form of processing DCs in the afferent lymph is known as veiled cells. Interdigitating cells in the lymphoid organs represent mature DCs.\textsuperscript{2,4–6}

The morphological appearance of DCs varies, but some members of the DC family possess distinctive cytoplasmic structures.\textsuperscript{4} Langerhans cells contain Birbeck granules, while the tubulovesicular system is well developed in interdigitating cells.\textsuperscript{4} Accumulating evidence suggests that both Birbeck granules and the tubulovesicular system are involved in antigen processing and antigen presentation.\textsuperscript{4} DCs are characterized by the presence of numerous thin cytoplasmic processes (dendrites) as well as large cytoplasmic veils that are continuously extended and retracted.\textsuperscript{4} The surface molecular content of DCs varies depending on the tissue location and the stage of cell maturation, and DCs display either the molecules essential for antigen capture and antigen processing or the molecules essential for their specialized interactions with lymphocytes.\textsuperscript{2,4–6}

The expression of the CD83 molecule, a member of the immunoglobulin superfamily with unknown functions, as well as the expression of antigen-presenting CD1 molecules including CD1a, CD1b, CD1c, and CD1d are essentially restricted to DCs.\textsuperscript{4} Fascin (55 kDa actin-bundling protein) is a reliable cytoplasmic marker for identifying different DC subtypes, while Lag-antigen and Langerin are uniquely present in Birbeck granules and Birbeck granule-associated structures in Langerhans cells.\textsuperscript{4} S100 (S100A1 and S100B) proteins, the function of which in DCs are poorly understood, are expressed intensely by both mature and immature DCs. The cell adhesion molecule E-cadherin is expressed by only some subsets of DCs.\textsuperscript{4}

Three pathways by which the CD34\textsuperscript{+} DC precursor can develop into immature DCs have been recognized.\textsuperscript{2,4–6} The first pathway leads to the differentiation of typical Langerhans cells which express E-cadherin and contain Birbeck granules with Lag-antigen/Langerin. This pathway is regulated by granulocyte macrophage colony stimulating factor (GM-CSF) and tumour necrosis factor \(\alpha\) (TNF \(\alpha\)). The second is a monocyte-related pathway.\textsuperscript{2,4–6} This pathway does not yield typical Langerhans cells in the presence of GM-CSF and TNF \(\alpha\) but leads to interstitial DCs displaying high levels of CD14 and CD68 during their development. The third is a lymphoid pathway which provides DCs for the thymus medulla and the T cell areas of lymphoid tissues. This subset of DCs lacks the myeloid marker CD11b. ‘Myeloid’ DCs are required for T cell activation whereas ‘lymphoid’ DCs induce T cell tolerance.\textsuperscript{2,4} The development of different DC subsets is influenced by varying combinations of cytokine growth factors, including TNF family members (TNF-\(\alpha\), TRANCE/RANK), GM-CSF, IL-4, stem cell factor, transforming growth factor-\(\beta\), and flt-3 ligand.\textsuperscript{2,4–6}

### DCs in the non-diseased arterial wall

Small numbers of DCs, predominantly localized along the endothelium in the subendothelial layer, are present in the intima of apparently normal, non-diseased arteries.\textsuperscript{3,11} Electron microscopy revealed that the normal arterial intima harbours a distinct subtype of the DC family displaying unique ultrastructural features.\textsuperscript{3,11,12} Vascular DCs, through their processes, form focal networks in some intimal areas.\textsuperscript{13} Wick et al.\textsuperscript{14,15} have suggested that in the normal intima, DCs are a key element of the so-called vascular-associated lymphoid tissue (VALT). VALT is analogous to the mucosa-associated lymphoid tissue of the respiratory and gastrointestinal tracts.\textsuperscript{14} VALT consists of aggregates of vascular DCs, T cells, and resident macrophages distributed throughout the subendothelial layer of the arterial intima along the luminal endothelial monolayer.\textsuperscript{15} It has been suggested that VALT screens ‘vascular tissue’ for potentially harmful antigens.\textsuperscript{14,15} Besides their intimal location, small numbers of resident vascular DCs are present also in the adventitia, usually in close proximity to capillaries forming the vas avarsum.\textsuperscript{16}

### Vascular DCs in pre-atherosclerotic stage

The numbers of vascular DCs in the intima of atherosclerosis-prone and atherosclerosis-resistant areas of the non-diseased aorta have been assessed and it has been found that, in atherosclerosis-prone areas, there were more DCs than in atherosclerosis-resistant areas.\textsuperscript{17} In atherosclerosis-prone areas, DCs were found to form cell clusters.\textsuperscript{17} The accumulation of DCs has also been identified in the intima of the carotid arteries of children, 8 weeks to 10 years of age, at sites subjected to major haemodynamic stress and predisposed to the development of atherosclerosis.\textsuperscript{18} In other pathological conditions, the accumulation of clustering DCs is thought to be an indicator of autoimmune processes.\textsuperscript{2,4} According to the autoimmune hypothesis of atherosclerosis proposed by Wick et al.,\textsuperscript{14} VALT activation by auto-antigens is responsible for initiating immune responses in the arterial wall which lead to atherosclerotic alteration. The observations of DCs accumulating and clustering in atherosclerosis-prone areas of the normal aorta and carotid arteries\textsuperscript{3,15,17,18} support the concept that
immune mechanisms are involved in the formation of atherosclerotic lesions from the very early stages of the disease.19–21

DCs in atherosclerotic lesions

In atherosclerotic arteries, the number of DCs increase.11,22–24 Vascular DCs residing in the intima become activated in the earlier stages of atherosclerosis.17,22,25 Advanced atherosclerotic plaques become enriched by DCs invading the atheromatous plaques from the adventitia, along with neovascularization associated with vasa vaso rum.26 In addition to these originally resident vascular DCs, atheromatous plaques may be invaded by blood DCs via inflamed neovessels.12,24 Monocytes that infiltrate the intima from the very early stages of atherosclerosis27 may contribute to an increased DC population, as well. Depending on the micro-environmental conditions such as the content and composition of cytokines, monocytes migrating through the luminal endothelial monolayer from the blood may differentiate into macrophages or into DCs.28 DC adhesion and migration are modulated by changes in endothelial function.29 In vitro experiments have revealed that DC adhesion and transmigration are markedly increased after exposing endothelial cells to hypoxia, oxidized low density lipoproteins, and TNF-α.29 The inhibition of endothelial NO synthase increases DC binding and transmigration, while the augmentation of endothelial NO synthase activity has been found to prevent DC adhesion.29 These findings suggest that the adhesion and migration of DCs are increased by stimuli known to accelerate atherogenesis.29

Accumulating evidence supports a view that, in the arterial wall, DCs are involved in antigen capture and antigen processing, as are DCs in other peripheral tissues.22,26,30 It has been suggested that the migratory routes of vascular-associated DCs are similar to those known for Langerhans cells in the skin and DCs in other peripheral tissues.22,26 After engulfing antigen in the arterial wall, vascular DCs likely migrate as veiled cells via the afferent lymph into regional lymph nodes where they activate T cells. Supporting this possibility are the immunohistochemical observations showing that the number of interdigitating cells in para-aortic and jugulodigastric lymph nodes attached to atherosclerotic arterial wall segments exceed those in the lymph nodes attached to non-atherosclerotic arterial segments.22 Besides their migration to lymph tissues, it has been suggested that some DCs emigrate to the blood stream.30

An experimental in vivo study revealed trafficking of monocyte-derived cells from atherosclerotic plaques during lesion regression but little emigration was detected from progressive plaques, suggesting that the progression of atherosclerotic plaques may result not only from robust monocyte recruitment into arterial walls but also from reduced emigration of these cells from lesions.10 This is in agreement with a histopathological study of human arterial tissue which indicated that only some DCs may migrate to the lymph nodes while other DCs may activate T cells directly within the intima.26 Mapping of the distribution of DCs in human atherosclerotic lesions revealed that DCs are most frequent in areas enriched with T cells and, particularly so, within inflammatory infiltrates where DCs are found to cluster with T cells.26 DCs clustering with T cells display the intercellular cell adhesion molecule-1 and vascular cell adhesion molecule-1 interactions of which with leukocyte function-associated antigen-1 and very late activation antigen-4, respectively, are essential for T cell activation.31 In DC–T cell interactions, DCs display CD40L and express high levels of HLA-DR.26 Vascular DCs also express DC-SIGN33 and some DCs display CD83, a marker of DC activation.22 In atherosclerotic lesions, DCs express not only high levels of class II MHC molecules but also display on their surfaces CD1 molecules,1,2,25,26,34 which have been recognized as antigen-presenting molecules in the same sense as the classical MHC class I and II molecules.35,26 It has been found also that CD1d, an antigen-presenting molecule responsible for the presentation of lipid antigens,36 is expressed in atherosclerotic lesions25,34,37 and that its expression is restricted to DCs.25 The effects of the atherosclerotic environment on DCs are still poorly studied but it has been shown that in atherosclerotic lesions, DCs are activated and display an abundance of heat shock protein-70.25 In contrast to other members of the DC family, the pathophysiologival significance of DCs as pro-inflammatory and auto-immune cells in atherosclerosis is poorly understood.

Atherosclerosis displays features of immune activation both locally and systemically.19–21 Angeli et al.38 demonstrated that dyslipidemia associated with atherosclerotic disease systemically alters the DC function. In different pathophysiological conditions, inflammatory cytokines and chemokines are known to be responsible for DC activation and migration,2,4–9 but the peculiarities of the effects of cytokines and chemokines on DCs in atherosclerosis have not yet been investigated. Nicotine, which is known to accelerate atherosclerosis development, activates DCs and augments their capacity to stimulate T cell proliferation and cytokine secretion.39 Modified lipoproteins accumulating in atherosclerotic lesions likely trigger DC activation, especially as in in vitro studies, oxidized low density lipoproteins have been shown to promote mature DC transition from monocytes40 and induce prominent DC clustering.41 C1q, which is thought to be involved in capturing immune complexes in the lymphoid tissue, has been found to be expressed by vascular DCs.42

DCs populating atherosclerotic lesions might be involved in the regulation of innate immunity in atherosclerosis, especially as the presence of Chlamydia pneumoniae has been identified in the cytoplasm of vascular DCs.43 Spanbroek et al.46 reported the expression of 5-lipoxygenase by DCs in atherosclerotic lesions. 5-lipoxygenase pathway is the major source of potent pro-inflammatory leukotrienes issued from the metabolism of arachidonic acid.47 These lipid mediators released by vascular DCs may act as physiological autocrine and paracrine signalling molecules and play a central role in regulating the interaction between innate and adaptive immunity.46 The expression of C1q by vascular DCs may also relate to innate immunity in atherosclerosis.

DCs and plaque destabilization

In atherosclerotic plaques, the number of DCs markedly increases with more than 90% of DCs accumulating in plaque shoulders, which represent plaque rupture-prone
DCs and therapeutic intervention in atherosclerosis

DCs may be used for the suppression of damaging immune responses in atherosclerosis. Approaches in atherosclerosis immunotherapy might be similar to those employed nowadays for cancer immunotherapy. One of these is based on a technique in which DCs, isolated from the peripheral blood, are activated (‘pulsed’) with the appropriate antigen ex vivo and then the ‘pulsed’ cells are returned to the bloodstream.

In atherosclerosis, the nature of the antigen(s) responsible for the activation of T cells is still not well understood. For atherosclerosis immunotherapy, DCs obtained from the peripheral blood can be pulsed ex vivo by cultivating them in a medium containing a total extract or suspension of atherosclerotic plaque material. Such cultivation would imitate the activation of DCs occurring in plaques in vivo. In contrast to cancer immunotherapy, immune responses in atherosclerosis need to be suppressed. As the presentation of peptide–MHC complexes to T cells by DCs in the absence of co-stimulatory signals leads to anergy and apoptosis of T cells, co-stimulatory molecules on pulsed DCs should be blocked ex vivo prior to returning them to the bloodstream. Recent achievements in developing the techniques for the suppression (ligation) of co-stimulatory molecules and for the loading of DCs with antigens have made it possible to generate DCs with desirable properties.

Recent studies indicate that NKT cells aggravate atherosclerosis. DCs may represent a useful tool for the regulation of NKT cell function in atherosclerosis. In one cancer immunotherapy technique, DCs pulsed with βGalCer ex vivo are returned to the bloodstream.

Concluding remarks

DCs are present in their immature forms in non-diseased arteries and become activated during atherogenesis. Some DCs cluster with T cells directly within atherosclerotic lesions, while others migrate to lymphoid organs to activate T cells. The identification of activated DCs in atherosclerosis-like lesions in animal models may facilitate the investigation of the functions of vascular DCs and their use for atherosclerosis immunotherapy.

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References