The functional role of dendritic cells in atherogenesis (Review)

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Abstract. Accumulating evidence suggests that dendritic cells (DCs) play a crucial role in the generation and progression of atherosclerosis (ATS), a lipid-related immuno-inflammatory disease. DCs have the ability to process and present antigens (mainly oxidized low-density lipoproteins, heat shock proteins and fragments of necrotic or apoptotic cells) to naïve T cells, and the activation of T cells is a key step for the progression of atherosclerotic disease. The existence of some distinct DC subtypes has now become evident. The main categories of DC subsets are the 'conventional or myeloid' and the 'plasmacytoid', which differ in toll-like receptor type and site of expression, pathogens and antigens recognized, and effector cytokines and functions. Studies on the potential impact of DCs in the pathogenesis of ATS may lead to novel therapies to regulate the immunoreactions occurring in atherogenesis. In particular, diltiazem, peroxisome proliferator activated receptor agonists and statins have been shown to protect endothelial cell function by inhibiting DCs, a mechanism that may play a significant role in the prevention of ATS.

Contents

1. Introduction

It is now well accepted that atherosclerosis (ATS) is a chronic disease of the arterial wall involving both innate and adaptive immuno-inflammatory mechanisms (1-3). In fact, endogenously modified molecules such as oxidized and glycated lipoproteins are known to stimulate the inflammatory and immune responses that promote ATS development, progression and complications (1,4). The main cellular components of atherosclerotic lesions are macrophages, T cells and smooth muscle cells (SMCs) (5-7). Previous studies have also reported the presence of dendritic cells (DCs) in plaques (8-11). DCs are considered powerful components of the immune system with a markedly elevated capacity to stimulate T and B cells and to control the differentiation of T cells (12).

Experimental and clinical studies have suggested that DCs may be involved in atherogenesis (13). This review summarizes the main recent advances in determining the functional role of DCs in the development, progression and complications of ATS.

2. Dendritic cells: origin and functions

DCs are a heterogeneous population of bone marrow-derived immune cells that specialize in capturing, processing and presenting antigens to T lymphocytes in order to induce and control immunity (14). DCs are morphologically characterized by the presence of several thin cytoplasmic processes (dendrites) and by large cytoplasmic veils that are continuously extended and retracted (15). DCs are very efficient at internalizing antigens either by phagocytosis or by receptor-mediated endocytosis, subsequently displaying a fragment of the antigen, bound to a class II major histocompatibility complex (MHC), on their membrane. T cells recognize the antigen-class II MHC molecule complex and interact with it on the DC membrane. An additional co-stimulatory signal is then produced by the DCs, such as B7-1, B7-2, leading to the activation of T cells.

It is now widely accepted that DCs are a part of the mononuclear phagocytic system, and comprise several subpopulations of migratory and lymphoid organ resident types derived from a common $CD34^+$ precursor (12,16). From the bone marrow, DC progenitors migrate through the bloodstream to reach sites of potential antigen entry, particularly along epithelial and body cavity surfaces (12,15,17,18). From these sites, 'immature' or 'processing' DCs migrate into lymphoid tissues to complete their maturation. However, some of them also maturate in the non-lymphoid peripheral tissues and present antigenic proteins to circulatory T cells. The maturation process of DCs is known to be modulated by

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Table I. Characterization of conventional myeloid and plasmacytoid dendritic cells (DCs).

Feature	Conventional myeloid DCs	Plasmacytoid DCs
Preferential TLR expression	TLR2, TLR4	TLR7, TLR8, TLR9
Site of TLR expression	Cell surface	Cytoplasm
Pathogens recognized	Bacterial fragments	Viral DNA and RNA
Autoantigens recognized	Oxidized LDL, heat shock protein 60	Nucleotides from dying cells
Effector function	Activation of T cells	TRAIL up-regulation on cytotoxic T cells in the plaque
Effector cytokines	TNFα, IL-6, IL-12	Type I interferon

IL, interleukin; TLR, toll-like receptor; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand. Modified from Niessner and Weyand (23).

varying combinations of cytokine growth factors, including tumor necrosis factor (TNF)-α, granulocyte macrophage colony stimulating factor (GM-CSF), interleukin (IL)-4, stem cell factors, transforming growth factor β and flt-3 ligands (12,15,17,18). The precise nature of the DC precursor that migrates from the bone marrow via the blood stream to the target tissues is not yet known. Furthermore, it remains to be fully elucidated at which stage the DC lineage diverges from that of monocytes (19).

The existence of many distinct DC subtypes has now become evident. They differ in surface markers, migratory patterns, localization and dependence on various infectious and inflammatory stimuli for their generation (20-22). The main categories of DC subsets are 'conventional or myeloid' (cDCs) and the 'plasmacytoid' (pDCs). Table I summarizes the functional properties of cDCs and pDCs (23).

cDCs are further divided into distinct subcategories on the basis of their origin, location and different expression of surface markers (24). These categories comprise the Langherans cells and dermal cells present in the skin, as well as various subsets of epithelial DCs, including pulmonary DCs (25).

pDCs are preferentially located in the shoulder region of the ATS plaque and have the ability to secrete large amounts of type I interferon (IFN-I). The IFN-I α produced by pDCs is capable of inducing marked up-regulation of the TRAIL molecule (TNF-related apoptosis-inducing ligand) in CD4 T cells (26). T cell-mediated apoptosis of plaque-residing cells, such as endothelial cells (ECs) and VSMCs, is considered an important mechanism in plaque destabilization and rupture (26,27).

Recent research readdressing the role of each subset in antigen presentation '*in vivo*' has focused on the complexity of the DC network (25). The unique ability of DCs to activate 'naïve' T cells is dependent on their stage of maturation and on their capacity to express high levels of class I and II MHC molecules, human leukocyte antigen-DR, and CDIa and its co-stimulatory molecules, CD40 and CD80/CD86.

CD40 is a member of the TNF superfamily, which is activated by the CD40 ligand (CD40L). CD40-CD40L are present in human ATS lesions and are expressed by macrophages, SMCs and T cells. CD40-CD40L signaling induces the release of cytokines and the expression of chemokines. It has been shown that DCs, upon constant CD40L stimulation, provide long-lasting IL-12 responses (28-30). For T cell activation, co-stimulation by the ligands CD80/CD86 and their CD28 receptor on T cells are also required (9,12,31,32).

In conclusion, upon migration and maturation, DCs develop not only the ability to capture and process antigens, but also the necessary molecules to engage lymphocytes and initiate immune programs. DCs may therefore be regarded as the 'sentinels' that orchestrate the immune system (24).

3. Dendritic cells and atherogenesis

DCs are present in healthy arteries and have been documented in the subendothelial space and at the media-adventitia junction (33,34). The localization of DCs adjacent to the vasa vasorum allows them to monitor the most important access pathways to the vessel wall, to present autoantigens to T cells, and to locally initiate inflammatory responses (23). Of the modified self-antigens expressed in ATS plaques, the best characterized is oxidized LDL. Stress-induced heat shock proteins represent a second category of autoantigens, while a third category consists of dying cells in both apoptotic and necrotic processes.

Inflammatory DCs (e.g., TNF or iNOS producing DCs) (22,35) are not present in the steady state. Weber *et al* (4) suggested that inflammatory DCs may differentiate from the $Ly6C^{low}$ monocyte and promote ATS lesions. However, further investigation is required to clarify both the development of DC precursors into each subpopulation and the classification of DC subgroups regarding ATS disease (36).

It has been demonstrated that DCs accumulate in the arterial intima in the regions that are subjected to major haemodynamic stress by turbulent flow conditions, which results in a predisposition to the development of ATS (37). DCs have been identified in atherosclerotic plaques, where they co-localize with T cells and are located in the neovascularization areas associated with inflammatory substrates.

The relationship between DCs and ATS has been assessed by several studies under experimental conditions and in humans. Studies on diet-induced hypercholesterolaemia demonstrated the presence of DCs in the aortic ATS lesions of apoE-deficient mice (11). In rats, DCs were also found during arterial neo-intima formation after balloon injury (38). DCs are present in human unstable ATS plaques (26,39), and higher DC densities were found in carotid plaques from symptomatic

patients as compared to those from asymptomatic patients (40). DCs were also described in human symptomatic in-stent restenosis (41) and in aortic aneurysms (42).

A decrease in circulating DCs appears to be an independent predictor of the presence of coronary artery disease (43). The levels of circulating DC precursors were reduced in both acute and chronic coronary syndromes as compared to healthy controls, probably due to vascular recruitment. Moreover, the number of circulating DCs was inversely correlated with C-reactive protein and IL-6.

On the other hand, Gautier *et al* (44) found that the expansion of the DC population was not associated with an acceleration of ATS plaque progression in either LDL receptor-deficient or apoE-deficient mice. In these experimental situations, the expansion of the DC population was associated with an atheroprotective decrease in plasma cholesterol levels. These data suggest that DCs may be key players in ATS through their impact on not only the immune response, but also on cholesterol homeostasis regulation.

Overall, these studies support the hypothesis that DCs may be strongly involved in ATS generation and progression. However, the modulation of immune processes, which is maintained by the heterogeneous population of DCs with specialized function, requires further investigation. In particular, understanding the mechanisms involved in the homeostasis of DC subsets in steady state and inflammatory conditions is key to exploiting their therapeutic potential (45).

4. Dendritic cells as possible targets in the treatment of atherosclerosis

The novel *in vivo* evidence that DCs broadly impact the circulating levels of cholesterol and immune responses in ATS opens new therapeutic horizons in the treatment of ATS (44).

Bachetoni *et al* (46) showed that diltiazem impairs the maturation and function of DCs, with subsequent inhibition of T cell activation and possible prevention of ATS.

In 2004, Luo *et al* (47) suggested that the peroxisome proliferator activated receptor (PPAR)-γ agonist ciglitazone inhibited the ox-LDL-induced maturation and immune functions of DCs. More recently, Shi *et al* (48) observed that the PPAR-α agonist fenofibrate, which has favorable effects on the development of ATS, also inhibited the ox-LDL-induced immune maturation of DCs. These effects of PPARs may partially explain their ability to slow ATS progression and reduce the risk of coronary heart disease independently from their metabolic effects.

Statins, the inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase, are widely used as cholesterol-lowering agents, but have also been shown to possess immunomodulatory properties (49-51). Recently, Yilmaz *et al* (52) showed that pre-incubation with statins of lipolysaccharide-stimulated DCs significantly suppressed their endocytosis, basal secretion of proinflammatory cytokines and ability to induce T cell proliferation and activation. Furthermore, Kofler *et al* (53) investigated the role of statins in regulating DC/EC interactions. The exposure of human DCs to low-moderate atorvastatin concentrations decreased their invasion capability (adhesion/transmigration), probably through changes in cholesterol biosynthesis within DC membrane domains (54,55) and by the inhibition of protein geranyl-geranylation. Kajimoto *et al* (56) provided the first clinical evidence that statins (atorvastatin) at 20 mg/day for 4 weeks significantly reduce DCs, key inflammatory factors (including c-Jun IV-terminal kinase) and matrix metalloproteinase expression in the aortic wall of patients undergoing abdominal aorta replacement. These results partially contribute to the explanation of the beneficial effects of statins in the treatment of ATS.

5. Conclusions

Accumulating evidence suggests that DCs may play a key role in atherogenesis, as they are the most potent antigen-presenting cells and participate in the enhancement and regulation of cell-mediated immune reactions. Endothelial activation and injury by exposure to ox-LDL, TNFα, hypoxia and infection favour DC adhesion and transmigration. The maturation of DCs boosts their ability to present antigens to naïve cells. DC-induced T cell activation appears to be crucial in both the generation and progression of ATS, a chronic lipid-related immuno-inflammatory disease.

The experimental data on the potential impact of DCs in the pathogenesis of ATS may lead to new therapeutic strategies for regulating immunoreactions in atherogenesis. The atheroprotective effects of statins and PPAR agonists may be due, in part, to their ability to interfere with DC function and EC/DC interactions. However, further investigations and clinical trials are required to elucidate the possible use of DC-targeting drugs in the treatment of atherosclerotic disease.

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