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Atherosclerosis: a chronic inflammatory disease mediated by mast cells

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Abstract

Inflammation is a process that plays an important role in the initiation and progression of atherosclerosis and immune disease, involving multiple cell types, including macrophages, T-lymphocytes, endothelial cells, smooth muscle cells and mast cells. The fundamental damage of atherosclerosis is the atheromatous or fibro-fatty plaque which is a lesion that causes several diseases. In atherosclerosis the innate immune response, which involves macrophages, is initiated by the arterial endothelial cells which respond to modified lipoproteins and lead to Th1 cell subset activation and generation of inflammatory cytokines and chemoattractant chemokines. Other immune cells, such as CD4+ T inflammatory cells, which play a critical role in the development and progression of atherosclerosis, and regulatory T cells [Treg], which have a protective effect on the development of atherosclerosis are involved. Considerable evidence indicates that mast cells and their products play a key role in inflammation and atherosclerosis. Activated mast cells can have detrimental effects, provoking matrix degradation, apoptosis, and enhancement as well as recruitment of inflammatory cells, which actively contributes to atherosclerosis and plaque formation. Here we discuss the relationship between atherosclerosis, inflammation and mast cells.

Key words: *leukocytes, inflammation, mast cells, athersclerosis.*

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The discovery of Toll-like receptors (TLRs) in mammalian innate immune cells at the end of the last century demonstrates once again that the innate immune response is crucial in the inflammatory process [1]. Inflammation is a process that plays an important role in the initiation and progression of atherosclerosis and circulatory diseases [2]. In fact, atherosclerosis and inflammation present similar impairment with a decrease in vasoreactivity. The fundamental lesion of atherosclerosis is the atheromatous or fibro-fatty plaque which is a lesion that causes narrowing of the artery, predisposes to thrombosis, calcifies, and causes weakening of the muscle resulting in aneurismal dilation [3]. Atherosclerosis is a chronic inflammatory and immune disease involving multiple cell types, including monocytes, macrophages, T-lymphocytes, endothelial cells, smooth muscle cells and mast cells (MCs) [4]. The importance of monocytes and macrophages in atherosclerosis was noted in the early ultra-structural studies of Still and O'Neal in 1962 [5]; while the presence of leukocytes within atherosclerotic arteries was described in the early 1980s [6]. Macrophages, however, were the first inflammatory cells to be associated with atherosclerosis. Great effort has been devoted to elucidate the molecular mechanisms by which immune cells contribute to atherosclerosis [7]. The innate and adaptive immune systems have evolved to protect humans from pathogens, such as bacteria, virus, parasites and fungi, and the presence of infection is detected by specialized cells [8], such as macrophages, mast cells, monocytes, natural killer (NK) cells, and dendritic cells, as well as non-specialized immune cells, such as fibroblasts and others [9]. Immune-inflammatory cells of atherosclerosis can be part of innate immune response involving monocytes and macrophages that respond to the excessive uptake of lipoproteins, while adaptive immune response involves antigen-specific T cells [10]. In atherosclerosis, the innate immune response is initiated by the arterial endothelial cells which respond to modified lipoproteins and lead to the generation of inflammatory cytokines and chemoattractant chemokines [11]. Initially, researchers thought that only macrophages were present in atherosclerotic plaques, but later studies noted that other immune cells, such as CD4+ T inflammatory cells (which play a critical role in the development and progression of atherosclerosis and are the most abundant T cells present in atherosclerotic lesions), regulatory T cells (Treg) (which have a protective effect on the development of atherosclerosis) [12], myeloid cells (that comprise immature macrophages and granulocytes), and mast cells (which accu-

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mulate in the human arterial intima and adventitia during atherosclerosis and actively participate in plaque destabilization) are present [13]. It is well established that Th1 cell subset activation promotes inflammatory response and plays an important role in atherosclerosis [14]; while Th2 cells mediate both atherogenic and anti-atherogenic [and anti-inflammatory] effects by generating certain cytokines such as interleukins (IL), including IL-10, IL-4, IL-13 and IL-37 [11, 15]. These interleukins inhibit the activation of macrophage and dendritic cells and therefore the over-expression of inflammatory cytokines. IL-38 is a member of the IL-1 cytokine family which binds to IL-36 receptor (IL-36R) and also has anti-inflammatory properties exerting a protective effect in some autoimmune diseases [11].

Interestingly, oxidized LDL-specific Tregs not only reduce the initiation, but also the progression of atherosclerosis and plaque formation [16, 17]. These effects can also be mediated by statin drugs, which regulate T_H1/T_H2 imbalance both *in vitro* and *in vivo* [18]. Tregs and their main subsets $CD4^+CD25^+$, $Foxp3^+$, T cells (natural Tregs) are crucial in mediating immune homeostasis and promoting the establishment and maintenance of peripheral tolerance [18]. They are also associated with an increased risk of atherosclerosis, diabetes [19, 20], fatty liver disease, autoimmune diseases and cancer [20].

TH17 cells contribute to the atherogenesis process and are involved in plaque formation [15]. In addition, vascular arterial dendritic cells which are similar to Langerhans cells of the skin, are involved in atherosclerotic lesions [21]. $CD11b^+$ cells are myeloid cells in early differential stages that include dendritic cells, immature macrophages, and granulocytes [22]. $CD11b^+$ cells are critical to atherogenesis but their inhibition does not reduce atherogenic plaque [22, 23].

The blood vessel reaction is one of the most striking events in inflammation. It consists of vasodilation and vasoconstriction as well as in an increase in vascular permeability in the inflamed area [24]. The impairment of vasoreactivity found with edema, resembles the impairment of vasoreactivity found in pre-arteriosclerotic and arteriosclerotic animal models [24]. In arteriosclerosis, injury to the vessel wall leads to intimal smooth muscle cell proliferation and luminal occlusion [25, 26]. Low-density lipoprotein (LDL) remains the most important risk factor for this inflammatory disease process. Studies on the cell culture indicate that LDL has numerous direct actions on vascular smooth muscle cells (VSMC) [26]. One action is to decrease VSMC prostacyclin production, and down-regulation of cyclooxygenase expression [27, 28]. Apoptosis of endothelial cells can occur on exposure to circulating factors and inflammatory cells, leading to disruption of endothelial glycocalyx monolayer integrity [29]. In fact, endothelial cell apoptosis contributes to the pathogenesis of atherosclerosis and other vascular and circulatory disorders. Many pathogenic factors may promote apoptosis in endothelium, and these include oxidized LDL (oxLDL) and certain cytokines such as IL-1 and TNF [30]. OxLDL provokes a delayed, but sustained, increase in intracellular calcium in endothelial cells, which causes cell death, an effect that can be reversed by preventing the calcium increase [30]. Some authors reported that oxLDL provokes depletion of cholesterol in endothelial cells via the scavenger receptor CD36, leading to ineffective e-NOS targeting to cholesterol and an attenuated capacity to activate the enzyme [31, 32].

Macrophages have two sub-populations: M1 and M2. Both take part in the atherosclerotic process, although with opposite roles [33]. M1 macrophages generate high levels of IL-12, IL-23, IL-6, IL-1, and TNF; while activated M2 macrophages produce IL-4, IL-13, and IL-10 which can deactivate M1 macrophages, and all contribute to atherogenesis [34].

TNF is an important pro-inflammatory cytokine, capable of classical activation of macrophages to the M1 phenotype, thereby inducing the production of other pro-inflammatory Th1 cytokines.

We know that IL-1 induces TNF and activates endothelial cell apoptosis along with growth factor deprivation [35]. Platelet-derived growth factor (PDGF) is another cytokine, generated by a number of cell types, including macrophages, platelets, endothelial cells and smooth muscle cells, which induces both smooth muscle migration and proliferation [36]. It also participates in atherogenesis.

Levels of PDGF mRNA in intact arteries *in vivo* are very low, while certain stimuli, such as hypercholesterolemic serum, may activate the synthesis and release of inflammatory cytokines, including PDGF [37]. It is possible that high levels of pro-inflammatory cytokines lead to imbalance of effector/regulatory T-cells in atherosclerosis and other chronic diseases, forming an attractive new immunotherapy [38].

A monoclonal antibody that targets inflammatory cytokines, such as IL-1 and TNF-α receptors, may alleviate symptoms in patients with inflammatory diseases such as arteriosclerosis [38]. Expression and generation of IL-8 and IL-6 are also correlated with plaque formation in human atherosclerosis, an effect that is likely mediated by local activation of perivascular mast cells, since these cells increase vascular leakage, induce plaque hemorrhage, provoke macrophage apoptosis, and result in leukocyte infiltration through the CXCR2, an IL-8 receptor [39, 40]. LDL and β-very low density lipoprotein (β-VLDL) have an effect on the incidence of coronary heart disease and together are, with others, an important risk factor [41]. Lipids, mostly in form of cholesterol and cholesteryl ester, are a serious problem for the circulatory system, heart failure [41], and the overload cholesterol in macrophage foam cells of the arterial wall [42]. This leads to the development of atherosclerotic plaque and accumulation of cholesteryl esters in the cytoplasm of macrophages, trans-

forming them into foam cells [43, 44]. However, the precise mechanisms by which the increased levels of lipids induce atherosclerotic lesions are still unclear.

Mast cells participate in both innate and adaptive immunity and play an important role in maintaining a healthy physiology in wound healing and angiogenesis [45]. Considerable evidence indicates that mast cells play a key role in inflammation, and their products modulate inflammatory mediator production [45]. Upon activation, mast cells release a broad spectrum of pro-inflammatory cytokines, growth factors, vasoactive substances, and proteolytic enzymes [46]. Human mast cells are derived from a common myeloid progenitor and have high-affinity IgE receptors (FcεRI) [47]. They are predominantly localized in mucosal and connective tissues and are distributed along blood vessels [48, 49]. Activated mast cells can have detrimental effects on their immediate surroundings in the vessel wall, provoking matrix degradation, apoptosis and enhancement as well as recruitment of inflammatory cells, which actively contributes to atherosclerosis and plaque formation [47, 48, 50]. In recent years, the mast cell has been implicated in inflammatory disease processes, such as cardiovascular disease and atherosclerotic plaque progression [51-53]. Mast cells are the major effector cell in inflammation, allergy and asthma [54]. They have been shown to accumulate in atherosclerotic plaque and in the perivascular tissue during this disease [55]. Their numbers increase within the arterial wall during atherosclerosis, and they are found in the human arterial intima and adventitia during atherosclerotic plaque progression, as well as participating in plaque destabilization [56]. Although vascular mast cells are rare, they are found within lesions of atherosclerotic plaques, especially in the location of rupture-prone shoulder regions [56]. Mast cells generate proteases such as tryptase and chymase [57]. The activation of these enzymes can cause intra-plaque hemorrhage, macrophage and endothelial cell apoptosis, vascular leakage, and cytokine/chemokine production, which lead to the recruitment of leukocytes to the plaque [58-60]. Mast cells release angiogenic compounds, which induce not only growth of microvessels, but also result in leakiness and rupture of the fragile neo-vessels [48]. This may result in intraplaque hemorrhage. Mast cells are present in human arterial intima where they can degranulate after stimulation and secrete chymase, which inhibits HDL apolipoprotein and may retard the efflux of cellular cholesterol. In fact, it has been reported by Lee and colleagues that mast cell chymase provokes the degradation and lowers the levels of ApoA-I, the main HDL apolipoprotein, altering lipid metabolism [61].

MCs express toll like receptors (TLR) including TLR-9 and TLR-3, which can be activated by infections and lead to the generation of several cytokines and chemokines such as TNF, IFN-γ, IL-6, and IL-8 [62]. They accumulate in the stroma of a number of inflamed tissues in response to locally produced chemotactic factors for monocytes/mast cells, such as RANTES and MCP-1 [63, 64]. Since mast cells express CCR3 receptor, it is possible that they are recruited in the atherosclerotic plaque by certain chemokines, including eotaxin [63]. The inhibition of this receptor prevents mast cell migration into the inflammatory site. In 1997, we reported for the first time that injection of chemokines RANTES and MCP-1 under the rat skin produced inflammation and mast cell recruitment [63-66]. It was also reported that MCP-1 and macrophage accumulation in the atheromatous plaque may play a pivotal role in restenosis after percutaneous trans-luminal coronary angioplasty and induce luminal renarrowing, at least in part, by inducing O_2^- release in monocytes [67]. Further understanding of the mechanism[s] by which MCP-1 is generated and acts after arterial injury may provide insights into therapies to limit the progression of atherosclerosis and restenosis.

IL-1 and TNF are macrophage products capable of inducing increased mast cell adhesion, along with macrophages, which also produce chemotactic factors such as LTB4 [68, 69]. Chemokines and C5a for leukocytes, including T cells, participate in a process that may form an amplification mechanism for the recruitment of further immune cells into atheromatous plaque [69].

Mast cells increase local inflammation with an augmentation of immune cells such as T lymphocytes and macrophages, which increase atherosclerosis, an effect abolished in mast cell-deficient $Kit^{W\text{-}sh/W\text{-}sh}Ldlr^{-/-}$ mice [70].

The inhibition of mast cell diminishes the generation of the inflammatory products, and as a result, atherosclerosis.

Below we report a few examples of the inhibitory mast cell compounds:

Syk is an inflammatory receptor located in immune cells, including mast cells, which mediates atherosclerosis [70, 71]. Therefore, this receptor can be a potential target not only for the treatment of allergic reactions, but also to alleviate atherosclerotic and cardiovascular diseases [71].

HYPERLIPIDEMIA → ENDOTHELIAL AND MAST CELLS → CYTOKINES/CHEMOKINES → MONOCYTES ADHESION → FOAM CELLS → ATHEROMATOUS PLAQUE

Fig. 1. Schematic representation of a possible sequence of events in atherogenesis

Kit ligand is a stem cell factor for mast cell growth and differentiation; the inhibition of this factor may be a good and valid approach to prevent inflammatory reactions mediated by mast cells, including atherosclerosis [72, 73]. Therefore, inhibition of mast cell through kit ligand activation may be of interest for future therapeutic interventions.

CD40L: mast cells, along with other immune/inflammatory cells also express CD40L, which binds its receptor CD40 and initiates the inflammatory process contributing to the pathophysiology of atherosclerosis [73]. Therefore, the inhibition of CD40L levels can have a beneficial effect on patients with atherosclerosis and myocardial infarction, making this ligand a novel target for the treatment of atherosclerosis and other inflammatory diseases.

STATINS: It is interesting that statins can inhibit the levels of IgE and IgG-oxLDL. This effect may provide a mechanism for statins as potential anti-atherosclerotic drugs [72-74].

Statins are a class of cholesterol-lowering drugs and it has been reported that they exert beneficial effects in atherosclerosis along with TH2 cytokines such as IL-10 and IL-4, which have anti-atherogenic properties [72]. In addition, statins, like simvastatin, are capable of stabilizing atherosclerotic plaques by increasing Treg cells [73].

Statins influence the generation of cytokines released by TH2 cells, and have an anti-atherogenic property; therefore, they have beneficial effects on atherosclerosis. Moreover, these drugs suppress the secretion of pro-inflammatory cytokines, including IL-1β and IL-6, but not TNF [74]. *e*NOS plays a crucial role in mediating this anti-inflammatory action [75]. Its over-expression reduces post-ischemic hyperpermeability of coronary microcirculation and the reperfusion injury of the heart in an experimental animal model [75-77]. These results support human studies suggesting that statins inhibit inflammatory cells in the atherosclerotic plaque [78, 79]. Monitoring atherosclerosis is very important and therapeutic interventions at different sites of the inflammatory process should be considered. Therapeutic targets could include statins, anti-cytokine/chemokine receptors, anti-growth factors, anti-transcription factors, specific monoclonal antibodies, and others. In addition, inhibition of mast cell activation may be of interest for future therapeutic interventions. One of the protective actions of statins is mediated by nitric oxide (NO) [79, 80]. Statins up-regulate eNOS, which can activate NF-κB and cause its translocation to the cell nucleus. We previously reported that statin treatment in atherosclerosis was able to reduce plaque, microcirculatory dysfunction and necrotic cell death, thereby ameliorating tissue ultrastructure damage and provoking endothelial protection, probably by inducing endothelial nitric oxide synthase (eNOS) over-expression and reducing hyperpermeability [79-81]. Moreover, addition of vitamin D to an atherosclerotic animal model resulted in an increase in NO, demonstrating that vitamin D has anti-atherosclerosis effects [82].

However, physical activity, diet, and stress reduction can successfully modify cardiovascular risk factors [83].

Therefore, future novel therapeutic targets may be the inhibition of specific pathways of atherosclerosis-induced mast cell activation with some anti-receptors, stabilizers, Tregs or natural compounds such as flavonoids [luteolin or quercetin], which have been found to have anti-oxidant and beneficial effects in reducing inflammatory diseases by inhibiting the release of histamine, leukotrienes, prostaglandin D2, IL-6, IL-8, TNF and tryptase release from human cultured MCs [84].

Accumulation of lipids in vascular endothelium activates mast cells to produce cytokines and chemokines, which recruit macrophages [85]. On the other hand, mast cells augment inflammatory response by secreting the vascular endothelial growth factor, a key cytokine that mediates angiogenesis and the inflammatory response. The concepts expressed in this article support the importance of inflammatory mechanisms in the pathogenesis of atherosclerosis and suggest a possible role for mast cells, in the control of VSMC proliferation and atheromatous plaque [85, 86].

In conclusion, MC activation certainly participates in the process of the plaque progression and destabilization by an increased lipid uptake, leukocyte influx, apoptosis, matrix degradation, and intraplaque hemorrhage [87– 89].

These studies are not only of interest for the basic research field, but may also have relevance for clinical atherosclerosis and cardiovascular diseases [90].

However, the precise mechanism by which the increased levels of cholesterol induce the plaque and the progression of atherosclerosis, including the precise pathogenesis of such a widespread disorder, is still far from established. Taken together, these findings might provide new insights to explore potential targets for immune therapeutic intervention in atherosclerosis. Further studies are needed to determine the role of mast cells in the development of atherosclerosis and in general in the inflammatory response. Identifying specific intervention strategies are urgently needed, because they may provide novel therapies in the prevention of atherosclerosis including the dynamic of plaque formation.

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