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The involvement of the monocytes/macrophages in chronic inflammation associated with atherosclerosis

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ABSTRACT

Atherosclerosis is a progressive chronic disease of large and medium arteries, characterized by the formation of atherosclerotic plaques. Monocytes and macrophages are key factors in lesion development, participating to the processes that mediate the progression of the atherosclerotic plaque (lipid accumulation, secretion of pro-inflammatory and cytotoxic factors, extracellular matrix remodeling). The recruitment of the monocytes in the vascular wall represents a hallmark in the pathology of the atherosclerotic lesion. Monocyte adhesion and transmigration are dependent on the complementary adhesion molecules expressed on the endothelial surface, whose expression is modulated by chemical mediators. The atherosclerotic plaque is characterized by a heterogeneous population of macrophages reflecting the complexity and diversity of the micro-environment to which cells are exposed after entering the arterial wall. Within the atherosclerotic lesions, macrophages differentiate, proliferate and undergo apoptosis. Taking into account that their behavior has a direct and critical influence on all lesional stages, the development of therapeutic approaches to target monocytes/macrophages in the atherosclerotic plaque became a focal interest point for researchers in the field.

Introduction

Atherosclerosis is a progressive chronic disease of the large and medium arteries, characterized by the formation of atherosclerotic plaques. Lesions occur mainly in large and medium elastic and muscular arteries and may cause ischemia of the heart, brain, and extremities, or stroke. Lesions may be present throughout the entire life of a person. The term *atheroma* was first proposed by Albrecht von Haller in 1755 to designate the degenerative process observed in the intima of arteries. The scientific hypothesis of atherosclerosis evolved from the exclusive involvement of hyperlipidemia (lipid hypothesis), to a complex pathological process, which includes in addition to dyslipidemia, activation and injury of endothelial cells (ECs), accompanied by an inflammatory process which modulates the initiation and progression of the lesions (Tedgui and Mallat 2006; Simionescu 2009; Manduteanu and Simionescu 2012).

Several factors causing endothelial dysfunction have been proposed: increased levels of modified low density lipoproteins, free radicals generated in diabetes, hypertension and smoking, genetic factors, elevated homocysteinemia, infections with microorganisms (herpes viruses, *Chlamydia pneumoniae*, cytomegalovirus), *etc.* Regardless of the process responsible for initiating the atherosclerotic lesions, each stage of its development represents a chronic inflammatory process in the arterial wall (Ross 1999; Libby et al. 2002; Packard and Libby 2008). Cycles of accumulation of mononuclear cells, migration and proliferation of smooth muscle cells (SMCs), and fibrous tissue formation determine the enlargement and restructuring of the lesion until when a fibrous head covers the necrotic lipid core, turning it into a complicated lesion. At one point, the artery can no longer compensate through dilation and the lesion occupies the lumen altering blood flow. However, some processes involving the macrophages may have a beneficial potential for the plaque steady state (promoting proliferation of SMCs, apoptosis, efferocytosis) or regression of atherosclerotic lesion. The balance between the protective or harmful effects of these processes depends on the lesional stage.

Elevated plasma cholesterol and especially LDL cholesterol is a major risk factor for atherosclerosis. Low density lipoproteins (LDL) modified by oxidation, glycation (in diabetes), aggregation,

Abbreviations: LDL, low density lipoproteins; oxLDL, oxidized LDL; SMCs, smooth muscle cells; ECs, endothelial cells; TNF, tumor necrosis factor; IL, interleukin; M-CSF, macrophage colony stimulating factor; GM-CSF, granulocyte/macrophage colony stimulating factor; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, intercellular adhesion molecule 1; MCP-1, monocyte chemoattractant protein-1; TGF, transforming growth factor; PKC, protein kinase C; apoE, apolipoprotein E; ROS, reactive oxygen species.

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association with proteoglycans, or incorporation into immune complexes, is a major cause of injury to ECs and impairment of SMCs (Khoo et al. 1992; Steinberg 1997; Sima et al. 2009). LDL particles sequestered in the arterial wall may undergo progressive oxidation and may be internalized bound to scavenger receptors expressed on macrophages. The LDL internalization facilitates lipid peroxides and cholesterol esters accumulation, leading to foam cells formation (Moore and Freeman 2006). Modified LDL uptake and removal are important processes for the protective role that macrophages have in the first phase of the inflammatory response in atherosclerosis. In this way, the macrophages minimize the effect of modified LDL on ECs and SMCs. Modified LDL have chemotactic effect for monocytes and induce expression of monocyte chemoattractant protein-1 (MCP-1), granulocyte and macrophage colony-stimulating factors, endothelial adhesion molecules (Quinn et al. 1987; Leonard and Yoshimura 1990; Rajavashisth et al. 1990). In this way, modified LDL stimulate the recruitment of new monocytes at the lesion site and induce proliferation of monocytes-derived macrophages, contributing to the extension of the inflammatory response (Hansson 2001; Gui et al. 2012). The inflammatory response influences the lipoprotein movement within the lesion. Inflammatory mediators such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1) and macrophage colony stimulating factor (M-CSF) increase the binding of LDL to the endothelium and to SMCs and enhance the LDL receptor gene transcription (Stopeck et al. 1993). The binding of LDL to scavenger receptors initiates intracellular pathways leading to pro-inflammatory cytokines production, such as IL-1 (Hajjar and Haberland 1997). Thus, inflammation is amplified by the inflammatory response itself and it is maintained by the presence of modified lipids in the arterial wall (Ross 1999).

The cells involved in atherogenesis

Atherosclerotic lesions represent the effect of a series of highly specific cellular and molecular responses. The earliest lesion called "fatty streak", is common in young children and is a pure inflammatory lesion, strictly consisting of monocytes-derived macrophages and T lymphocytes. According to the original theory "response to injury", focal injury to the arterial endothelium followed by the adherence and aggregation of platelets represent the first steps in the development of atherosclerotic lesions (Ross and Glomset 1976a, 1976b).

The theory was improved, emphasizing the effect of endothelial dysfunction that determines compensatory responses in the arteries. These responses alter normal homeostatic properties of the endothelium, increasing the leukocytes and platelets adhesion to its surface and enhancing its permeability. All these modifications are translated into phenotypic changes from "anticoagulant" in "pro-coagulant", determining the secretory phenotype necessary for the production of vasoactive molecules, cytokines and growth factors (Simionescu 2007). If arterial inflammatory response fails to neutralize or remove the injuring agent, inflammation continues indefinitely, resulting in migration and proliferation of SMCs at the site of the inflammation causing the apparition of the intermediate lesions. Subsequently, the perpetuating response leads to arterial wall thickening compensated by gradual dilation, therefore, up to a certain point, the diameter of the lumen remains unaltered. This phenomenon is called vascular remodeling (Ross 1999).

During each phase of atherogenesis, the inflammatory response is mediated by monocytes-derived macrophages and specific subtypes of T lymphocytes, while granulocytes and neutrophils are rarely present during any phase of atherosclerosis (Galkina and Ley 2007; Soehnlein and Weber 2009; Soehnlein 2012). Monocytes and macrophages participate in all the stages of the lesion development, contributing to the processes that mediate the progression of the atherosclerotic plaque (lipid accumulation, secretion of pro-inflammatory and cytotoxic factors, extracellular matrix remodeling). The continuous inflammatory state leads to an increase in macrophages and lymphocytes migration from the blood and multiplication within in the plaque. At this level, under the influence of cytokines, growth factors and lipoproteins, the monocytes differentiate into dendritic cells, macrophages and foam cells (Bobryshev 2006). The foam cells may undergo necrosis, forming the lipid core of the advanced atherosclerotic plaques. Following the rupture or erosion of the atherosclerotic plaques, acute thrombotic events are triggered, producing myocardial infarction and stroke (Lusis 2000).

Monocyte recruitment and transmigration in the atherosclerotic plaque

The recruitment of circulating monocytes in the vascular wall represents a key factor in the pathology of the atherosclerotic lesion. The monocytes enter the subendotelial space through a process named transmigration or diapedesis, that requires a complex system of interactions between adhesion molecules and chemotactic factors.

As depicted in Fig. 1, the accumulation of macrophages in the atherosclerotic plaque is mediated by different mechanisms: (i) monocyte recruitment from circulation due to the expression of adhesion molecules and chemotactic factors; (ii) differentiation, activation and proliferation of local macrophages after their migration from blood; (iii) immobilization of macrophages in the active inflammatory site, induced by some cytokines and oxidized lipids.

In normal states, the vascular endothelium does not allow the adhesion of leucocytes and prevents their passage. When hemodynamic conditions are altered, white cells adopt a peripheral position along the endothelial surface. This marginalization process is followed by rolling and adhesion of monocytes to the activated endothelium. Adhered monocytes extravasate through EC junctions into the subendothelial space (Osterud and Bjorklid 2003; Simionescu 2007).

Monocyte adhesion and transmigration are dependent on the complementary adhesion molecules present on the endothelial surface, whose expression is modulated by chemical mediators. The adhesion molecules involved in the recruitment of monocytes to the endothelium belong to four families: selectins, the immunoglobulin superfamily, intengrins and mucin-like glycoproteins.

Selectins are characterized by an extracellular N-terminal domain related with lectins that recognizes and binds sialylated oligosaccharides covalently bound to different mucin-like glycoproteins (GlyCAM-1, PSGL-1, ESL-1 and CD34). Monocytes and most types of leukocytes express L-selectin (CD62L, LAM-1), that appears to be involved in lymphocyte homing to mouse aorta but not in monocyte recruitment (Galkina and Ley 2007). The main selectin responsible for monocyte rolling in atherosclerotic mouse arteries appears to be P-selectin (Ramos et al. 1999).

Immunoglobulin superfamily members vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) expressed on the endothelium are ligands for the integrins expressed on leukocytes. VCAM-1 is highly expressed in ECs near atherosclerotic lesions, playing a major role in early atherosclerosis (Cybulsky et al. 2001).

Integrins are transmembrane glycoproteins present as heterodimers formed by α and β subunits, expressed on many cell types, which bind ligands from ECs, other leukocytes and extracellular matrix. Monocytes express β2 integrins, LFA-1 and Mac-1 (CD11a/CD18 and CD11b/CD18), which bind ICAM-1, and β1