REVIEWS

Atherogenesis and Immune Inflammation

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Modern concepts on the role of inflammation in atherogenesis are reviewed. The evidence indicating that changes in cellular composition of the vascular wall are associated with the expression of mediators of inflammation and lead to the development of the protective-compensatory reaction under conditions of autoantigen production is presented. The hypotheses on the pathogenesis of atherosclerosis are discussed that associate the causes and conditions for emergence of leukocytes in the arterial intima against the background of apoprotein B-containing lipoprotein deposition.

Key Words: *atherogenesis; autoantigens; cytokines; immune inflammation*

The key concepts on the pathogenesis of atherosclerosis have been recently reconsidered on the basis of the following findings: 1) modified (oxidized) low density lipoproteins (mLDL) formed in the blood and vascular wall are the major factor responsible for the formation of arterial atherosclerotic lesions, 2) autoimmune complexes containing mLDL as antigen were detected in the blood and vascular wall of atherosclerotic patients [19,23,24], and 3) cellular composition changes and mediators of inflammation appear in the foci of atherogenesis [3,4,13].

In a number of reviews the term "atherosclerotic arterial lesion" is discussed in the context of immune inflammation. Is this a revival of Virchow's ideas of atherogenesis, return to the hypothesis put forward by German scientists in the 1940-s that serous-fibrous edema of arterial intima triggers the development of atherosclerosis (arteriosclerosis), or there exists a principally new approach to the assessment of key events in atherogenesis?

Role of Autoantigens in Atherogenesis. Our previous studies [7,8] showed that apoB-containing lipoproteins (total fraction of low density and very low density lipoproteins), immunoglobulin G, and C_3 component of the complement are deposited in the walls of the aorta and coronary arteries of humans and rabbits with experimental atherosclerosis. Based on these findings, we have hypothesized that there exists an immune complex whose components can be identified by immunomorphological methods. A tolerance to experimental atherosclerosis was then demonstrated in rabbits neonatally immunized with homologous apoB-containing lipoproteins from rabbits with experimental atherosclerosis and probably possessing autoantigenic activity [2]. The formation of autoimmune complexes in human and animal blood in atherosclerosis has been reported [9].

On the basis of these data an autoimmune theory of atherogenesis was formulated [20], which was recognized in the 1990-s. It was shown that mLDL acquire autoantigenic properties as a result of peroxidative modification occurring in the vascular wall. Anti-mLDL antibodies were detected in patients with ischemic heart disease [23]. The dynamics of circulating anti-mLDL antibodies was assumed to be associated with the progression of atherosclerosis of the carotid arteries [24].

It was demonstrated that 5.7% mLDL in atherosclerotic arterial wall are aggregated into immune

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complexes (*vs.* 0.2-0.5% in healthy arteries) [19]. A hypothesis of antiatherosclerotic vaccination was put forward [1], which has been assessed in the context of atherosclerosis prevention.

An atherosclerotic plaque has much in common with a developing immune inflammation: formation of neoantigens (mLDL, products of plaque degradation), focal accumulation of agranulocytes (monocytes/macrophages, T cells), emergence of granulocytes and mast cells, and outgrowth of connective tissue. A similarity of the cell population in atherosclerotic plaques and the foci of immune inflammation observed in various diseases implies that atherogenesis is a chronic inflammatory response similar to the delayed hypersensitivity reaction [13].

A complex immunomorphological study of arterial atherosclerotic lesions and immunocompetent tissues of atherosclerotic patients and sudden death victims (acute cardiovascular insufficiency) has been carried out for a decade. It showed that profound atherosclerosis-related structural and functional changes occur not only in the vascular wall but also in the immunocompetent tissues [1,10]. For example, at the early stages of atherogenesis the number of lymphoblasts in the T zone of the spleen white pulp increases 2.5-fold and mitotic activity of lymphoid cells increases 2.3-fold compared with those in health. In the B zone, the content of IgG- and IgM-producing cells increases 2.2- and 1.8-fold, respectively. Similar cellular perturbations were observed in the paraaortic lymph nodes irrespective of their localization (aortic arch, thoracic, or abdominal aorta). The lymph node cortex contained numerous lymphoid follicles with developed germinal centers.

In progressing and complicated atherosclerosis, most pronounced changes occurred in the spleen: the number of lymphoblasts increased 1.5-fold, while the number of T suppressors decreased and that of T helpers increased 1.3-fold. In the B zone of white pulp, the number of plasmablasts and immature plasma cells increased 1.8-fold, that of mature plasma cells 2-fold, and of IgG- and IgM-producing cells 4.5-fold. In contrast to the spleen, in visceral and somatic lymph nodes immunomorphological modifications occurred only in the B zone. Most pronounced changes were observed in both the T and B zones of regional lymph nodes (relative to the aorta): their medullary sinusoids and cords were packed with plasma cells of various degree of maturity. This is regarded as an immune response to plaque degradation products brought into regional lymph nodes by lymph outflow.

Changes in Cellular Composition of the Vascular Wall in Atherogenesis. The cap of a fibrous plaque contains 18% T cells and 24% macrophages [18]. The shares of these cells in plaque shoulders are almost the same (22% T cells and 18% macrophages), while the lipid nucleus (atheroma) contains 9% T cells and 60% macrophages. $CD8^+$ T cells predominate in lipid spots and lipid plaques, while $CD4^+$ macrophages are the major cell type in advanced lesions [14,22].

In order to find out whether T cells identified in plaques are immunologically active, it is necessary to analyze their surface proteins, which are not expressed by nonactivated cells. Localized tissue distribution of class II antigen is important for the immune response control. However, in inflammation and autoimmune states these antigens are produced by various cell types.

In healthy subjects, the vascular wall cells do not express class II antigens, but can express it in atherosclerosis. In atherosclerotic plaques, HLA-DR, and -DQ antigens (activation markers) are expressed by the vast majority of macrophages and about 1/3 of smooth muscle cells (SMC) [17,18]. These data are important for the analysis of the mechanisms responsible for the development of immune inflammation in the vascular wall during atherogenesis.

Thus, together with LDL accumulation and modification, migration of agranulocytes into the intimal foci of lipid deposition is a necessary condition of atherogenesis. This idea is based on the analysis of the pathogenic mechanisms of atherogenesis [3,4] with emphasis laid upon the fact that the interactions between mLDL infiltrating the vascular wall and intimal cells are crucial for the development of atherosclerosis.

Although the mechanisms of lipid peroxidation in the vascular wall remain obscure, *in vitro* studies showed that endothelial cells, monocytes/macrophages, and SMC are involved in the formation of mLDL [21,25]. Based on analysis of the mechanisms of mLDL formation in the vascular wall, it was hypothesized that locally activated intimal macrophages produce considerable amounts of reactive oxygen radicals and cytokines providing for lipoprotein peroxidation: superoxide anion radical ($O_2^{\cdot-}$) and hydroxyl radical ($HO^{\cdot}$) [27]. In addition, such cytokines as interferon and tumor necrosis factor-α (TNF-α) induce an arginine-dependent production of nitric oxide (NO) due to blockade of mitochondrial respiration. This may also facilitate lipid peroxidation of LDL [26].

In line with activation of vascular cells, their intensive proliferation has been observed at the early and advanced stages of atherogenesis. A complex histo- and electron microscopic and radioautography study of the structure and function of cells participating in proliferation (^{3}H-thymidine incorpora-

tion) and collagen synthesis (^{3}H-proline and ^{14}C-hydroxyproline) was performed in rabbits with experimental atherosclerosis [5,6]. Radioautography showed that the primary foci of proliferation consisting of 3-5 dividing cells are formed in the proximal region of the aorta during the second week of atherogenesis and coincide in localization and time of emergence with ^{125}I-LDL deposits in the vascular wall.

The dividing cells are of different origin. In the proliferation loci, an intensive incorporation of ^{3}H-thymidine in endothelial cells was recorded, and their proliferation index increased 7-fold after 3-4 weeks of experiment. In lipid spots 20% of cells divided. Proliferation of SMC is accompanied by phenotypic modification and participation in the desmoplastic reactions. Quantitative photometry showed that the synthesis of fibrillar proteins in lipid spots is 10-fold as intensive as in uninvolved intima. We have discovered that ^{3}H-thymidine is incorporated not only in endothelial cells and SMC, but also in monocytes/macrophages, which do not turn into foam cells.

Atherogenesis and Macrophages. In the cap of atherosclerotic plaques, 12% of macrophages were found to divide. Macrophages are highly determined cells, and their proliferation in tissues and particularly in the vascular wall seems questionable. Special conditions are necessary to induce macrophage division *in situ*. At first, our discovery of macrophagal proliferation in atherosclerotic foci seemed doubtful. Meanwhile, subsequent studies showed that generation of superoxide anions by macrophages in the foci of mLDL formation and expression of macrophagal colony-stimulating factor by activated intimal cells [12] are probably the conditions providing for cell differentiation and proliferation in atherosclerotic plaques. Macrophagal proliferation in atherosclerotic foci was confirmed by experiments with monoclonal antibodies to nuclear proteins of dividing macrophages [16].

Electron microscopic and immunohistochemical investigation of atherogenesis in patients and rabbits with experimental atherosclerosis demonstrated heterogeneity of macrophagal population in atherosclerotic plaques. This argues against the universally accepted concept that in an atherosclerotic plaque macrophages are responsible only for the scavenger uptake of mLDL and formation of foam cells.

We have identified 3 populations (phenotypes) of macrophages which fulfill different functions in atherosclerotic plaques. The first phenotype is represented by monocytes/macrophages migrating via the endothelium into the zone of deposition and/or *in situ* formation of mLDL, participate in scavenger-uptake of these LDL, and change into foam cells. This cell type was well investigated, so no more comments are necessary. The second phenotype is represented by macrophages located in the superficial and profound layers of atherosclerotic plaques and do not change into foam cells, although are surrounded by them. These macrophages have no lipid vacuoles in the cytoplasm and have a well-developed endoplasmic reticulum, considerable numbers of free ribosomes, and small optically dense granules resembling secretory vacuoles.

Immunomorphological studies showed that these cells produce mediators of inflammation, predominantly, interleukin-1β (IL-1β) and smaller amounts of IL-1α and TNF-α and may participate in antigen presentation by T cells.

The third phenotype is represented by a small population of cells that do not change into foam cells, but intensively produce TNF-α and exhibit cytopathogenic activity towards the surrounding tissues.

The nature of macrophages localized within atherosclerotic plaques is unknown. However, the following hypothesis can be put forward. The monocytes/macrophages that migrate from the circulation into the intima have an entire set of scavenger receptors and participate in the internalization and catabolism of mLDL, converting into foam cells. However, the macrophages involved in immune inflammatory response originate from cells proliferating in the intima. Presumably, the genes responsible for cytokine production are primarily expressed in macrophages proliferating in lipid spots and plaques, since these cells form tight contacts with T cells.

The reactions in which participate the three macrophagal phenotypes are probably aimed at the same target: to rid the vascular wall of foreign substances and antigens. So far, it remains unclear why this sometimes is not achieved.

Leukocyte Adhesion and Mediators of Inflammation in Atherogenesis. Considerable attention has been recently focused on the role of chemoadhesive molecules, growth factors, and cytokines in atherogenesis [13,26]. Most experiments, however, were carried out *in vitro*, and changes in cell composition occurring in the foci of atherogenesis were not taken into consideration. We studied cytokines in human coronary arteries during the development of atherosclerotic lesions, taking into account adhesion and migration of agranulocytes into the vascular wall.

Scanning electron microscopy of lipid spots and unaltered segments of coronary arteries revealed a number of specific features in the leukocyte adhesion, which are typical of the early stages of atherogenesis. These peculiarities stem primarily from the fact that adhesion and migration of agranulocytes are confined to the zones where IL-1β is produced by endothelial cells.

Active synthesis of IL-1β by endothelial cells, which is probably induced by mLDL occurring in the subendothelial layer, may trigger their proliferation and enhance adhesive properties of the endothelial monolayer. In the first case, IL-1 may act both as an activator of proliferation [22] and as an autocrine factor maintaining prolonged proliferation of endothelial cells. This was confirmed by analysis of ^{3}H-thymidine incorporation in the nuclei of aortic endothelial cells of rabbits fed an atherogenic diet. After 2 weeks of experimental hypercholesterolemia, endothelial cell proliferation increased 3- to 4-fold, and after 6 weeks the endothelium proliferation index was 18.8±1.8% *vs.* 0.5±0.1% in the health.

Disruption of tight cell-to-cell contacts in the endothelium during cell division facilitates migration of large amounts of monocytes, lymphocytes, and probably granulocytes into the intima. Production of active oxygen radicals and proteolytic enzymes of various specificities and directionalities by leukocytes leads to disintegration not only of the intima but also of the media. As a result, proliferation of SMC and probably modification of their phenotype occur.

In the second case, IL-1 activates the expression of chemoadhesive molecules and cytokines by endothelial cells; the following substances are generated: endothelial leukocyte-adhesive molecule-1, intercellular adhesive molocule-1, vascular adhesive molecule-1, monocyte chemotaxic protein-1, and monocyte colony stimulating factor. It was hypothesized that focal leukocyte-endothelial cell interactions in atherogenesis are similar to those occurring in acute and chronic inflammation [15]. *In vitro* experiments with cytokine-activated endothelial cells showed that E-selectin provides adhesion of monocytes and T cells [11].

Adhesion of monocytes to the inner arterial surface is determined not only by the above-mentioned interactions between endothelial adhesion molecules and the corresponding ligand on the surface of mononuclear cells. Monocytes/macrophages also bind to damaged endothelium. This reaction involves cellular Fc-receptor and IgG adsorbed on exposed cytoskeletal components. Binding of IgG to these components also activates the complement cascade, generating C5a anaphylatoxin, an important chemoattractant for monocytes and granulocytes [18].

Interleukin-1 is not produced by all monocytes/macrophages migrating in the intima. Its expression is suppressed in macrophages transformed into foam cells, while the production of small amounts of TNF-α is preserved.

Smooth muscle cells, which migrate into the intima from the media, are also involved in the development of arterial atherosclerotic lesions. Immunocytochemical analysis showed that the vast majority of presumably functionally active SMC produces considerable amounts of IL-1β and does not express IL-1α and TNF-α.

Thus, it was demonstrated that at the early stages of atherogenesis intimal cells located in the zones of mLDL deposition express mediators of inflammation that largely determine the development of the process. Considerable evidence has been accumulated indicating that the pathogenesis of atherosclerosis is associated both with mLDL and immune inflammatory reactions occurring in the vascular wall.

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