GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Activity of the Inflammatory Process in Different Types of Unstable Atherosclerotic Plaques

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Inflammatory biomarkers and chemoattractants characteristic and important for different types (lipid; inflammatory erosive; degenerative necrotic) of unstable plaques in coronary arteries were identified and studied in male patients with coronary atherosclerosis without acute coronary syndrome we studied. Among the three types of unstable plaques, elevated concentrations of IL-1β, IL-6, IL-8, IL-18 and monocyte chemotactic protein-1 were characteristic of not only inflammatory erosive type, but also lipid type compared with degenerative necrotic type. Thus, intensification of the inflammatory process plays an important role in the development of not only inflammatory and destructive, but also of lipid type of unstable atherosclerotic plaques.

Key Words: *atherosclerotic plaques of coronary arteries; lipid, inflammatory erosive, and degenerative necrotic types of unstable plaques; inflammatory cytokines; chemoattractants*

Disintegration of the endothelium at the site of ulceration/destruction of the fibrous cap of unstable atherosclerotic plaque or vulnerable atherosclerotic plaque liable to ulceration/destruction triggers thrombus formation on the surface of the atherosclerotic plaque resulting in artery occlusion and myocardial infarction [1,4,11]. The critical stage in the development of atherosclerotic focus [7,13] is the formation of unstable plague with thin/thinned or locally thinned cap, local destruction of the endothelium, inflammatory infiltration, and loose lipid core (sometimes with foci of necrosis and calcification) [5,8,9,14].

It is now proved that the inflammatory process plays an important role in destabilization of the atherosclerotic plaque. Many studies have revealed pronounced infiltration of unstable/vulnerable atherosclerotic plaques with activated macrophages, T cells and leukocytes [5,8,11], secreting proinflammatory cytokines including IL-1β, IL-6, IL-8, IL-18, TNF-α, and others [12,13]. IL-1β и TNF-α induce synthesis of adhesion molecules by endothelial cells, which impairs antiadhesive and anticoagulant properties of the endothelium. IL-6, a cytotoxic T cells differentiation factor, stimulates the synthesis and secretion of the major acute phase proteins. IL-8 stimulates production of factors that activate neutrophil and T-cell chemotaxis to the atherosclerotic lesions by monocytes/macrophages. IL-18 secreted by T cells activates monocytes/macrophages and initiates apoptosis. IL-2 is also secreted by T cells and after their activation potentiates their proliferation [3,7,11,12,15].

Along with inflammatory cytokines, activated macrophages of atherosclerotic foci secrete a number

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of chemoattractants including monocyte chemotactic protein (MCP-1), endothelial-monocyte activating polypeptide (EMAP-II), adhesion molecules (intercellular adhesion molecule sICAM-1, endothelial cell adhesion molecule sVCAM-1). MCP-1 expressed by macrophages in response to their exposure to cytokines TNF-α, IL-1β и IL-6 is a monocyte- and T cell-specific chemoattractant [3,11]. EMAP-II, secreted by macrophages and T cells is both pro-inflammatory cytokine and chemoattractant inducing the formation of procoagulant tissue factor on the surface of endothelial cells, neutrophil activation, apoptosis, and inhibition of neovascularization of arterial intima-media [3,10,15].

There are several types of unstability/vulnerability of atherosclerotic foci. Most often they are classified according to some common, unifying feature into 3 types [5,6,14]: 1) lipid (fibroatheroma), 2) inflammatory erosive and 3) destructive (degenerative necrotic). The first type is characterized by large atheromatous core (circularly or eccentrically located), thinned fibrous cap; the second type plaques have small lipid core, fibrous cap abundantly infiltrated with inflammatory cells (macrophages and T cells) or foam cells, dying endothelial cells on the surface of inflammatory or lipid erosion of the cap; and in the third type plaques, pronounced degenerative changes and necrosis in the cap, foci of calcification of various sizes under the thinning cap [2,5,6,14].

To date, few studies have examined directly arterial vascular wall as well as local factors and mechanisms underlying development of different types of plaque instability. Here we studied the role of inflammatory process in the development of different types of instability in atherosclerotic foci of coronary arteries.

MATERIALS AND METHODS

The study was conducted within the framework of joint research projects of Institute of Therapy, Siberian Division of RAMS and Federal Agency for High-Technological Medical Care. The study was approved by the ethics committees of both institutions. The study included men suffering from coronary atherosclerosis documented by coronary angiography, without acute coronary atherosclerosis and with stable angina (II-IV functional class). The patients entered the clinic of Federal Agency for High-Technological Medical Care for coronary bypass surgery. All patients completed informed consent form.

During surgery, coronary artery endarterectomy was performed in 54 patients in case intraoperative inications. Each of the 54 endarterectomy specimens containing coronary artery intima-media was visually symmetrically divided longitudinally and transversely into 3-4 fragments for histological and biochemical studies.

After macroscopic examination (plaque size, degree of stenosis, plaque hemorrhages, foci of calcification, and thrombosis) and standard staining with hematoxylin and eosin and by van Gieson's method, histological analysis of intima-media fragments of the coronary arteries was carried out under Axiostar Plus binocular microscope (Carl Zeiss). Of 162 examined fragments, relatively unchanged intima was detected in 19, lipid spot/strip in 23, stable young atherosclerotic plaque in 34, stable plaque with fibrosis/calcinosis in 41, and unstable plaque liable to ulceration or rupture in 45 samples. The types of unstable plaques were identified: fibroatheroma with massive lipid core and thin fibrous cap (lipid type) in 14 (Fig. 1), plaque with increased content of proteoglycans and inflammation leading to erosion with inflammatory or lipid

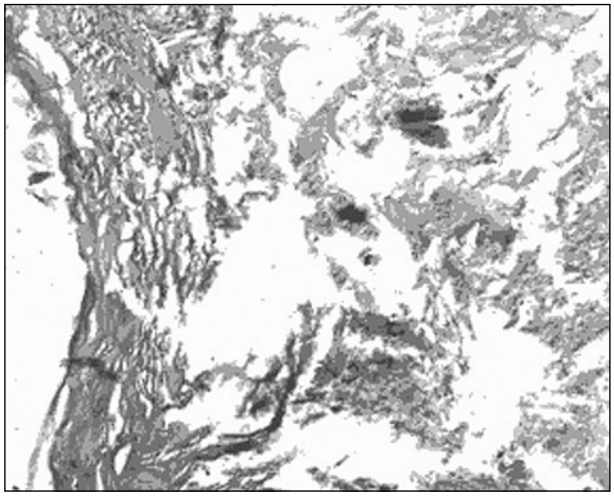

Fig. 1. Unstable atherosclerotic plaque. Fibroateroma; thinning and ruptures of the cap. Staining with hematoxylin and eosin and after van Gieson method. ×240.

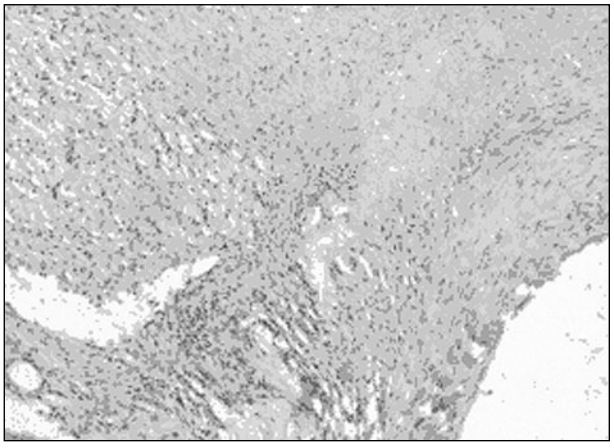

Fig. 2. Unstable atherosclerotic plaque of inflammatory type. Lymphocytic infiltration of the core and thinning and ruptures of the cap. Hematoxylin and eosin staining. ×240.

erosive lesions in the caps (inflammatory and lipid erosion of the plaque surface) in 16 (Fig. 2), and plaque with calcified core, severe degenerative changes, and necrosis in the caps (degenerative necrotic type) in 15 samples (Fig. 3).

For biochemical studies, samples frozen in liquid nitrogen were homogenized at 4°C in phosphate-buffered saline (pH 7.4). Protein content in the homogenates was measured by the method of Lowry, biochemical parameters were standardized by protein content. Using ELISA method and ELISAs kits, we determined the levels of TNF-α, IL-1β, IL-6, IL-8, IL-18, IL-2 (BCM Diagnostics kits), C-reactive protein (high-sensitivity, ELISA, HS-CRP; Bender Medsystems kits), MCP-1, EMAP-II (BCM Diagnostics kits), sICAM-1 and sVCAM-1 (Bender Medsystems kits) in homogenates.

Statistical analysis was performed with the program SPSS 11.5 at 5% statistical significance.

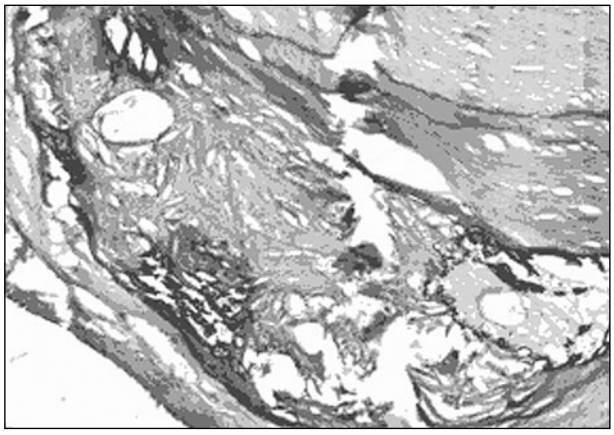

Fig. 3. Unstable atherosclerotic plaque of degenerative necrotic type. Calcinosis, degeneration, foci of necrosis, ruptures of the cap. Staining with hematoxylin and eosin and by van Gieson method. ×240.

RESULTS

Investigating the complex inflammatory biomarkers and chemoattractants, we have detected increased concentrations of IL-6, IL-8, IL-18, HS-CRP, MCP-1, and EMAP-II which were relevant for the development of unstable atherosclerotic plaque (compared with other stages of the development of atherosclerotic lesions: lipid spot, stable young and fibrous plaques) (Table 1).

Comparative study of inflammatory biomarkers and chemoattractants in different types of instability showed that in the plaques of inflammatory erosive type, as expected, the levels of inflammatory biomarkers IL-1β, IL-6, IL-8, IL-18 and chemoattractants MCP-1 and EMAP-II were higher than in degenerative necrotic plaques by 3.2, 2.2, 2.7, 2.8, 3.7 and 1.3 times, respectively.

Increased levels of inflammatory cytokines IL-6 and IL-8 and chemoattractants MCP-1 and EMAP-II in unstable plaques of inflammatory erosive type reflect the most marked activity of acute inflammatory process and reactions of tissue damage associated with increased chemotaxis of monocytes and macrophages, neutrophils and T cells.

However, we revealed increased inflammatory activity in unstable plaques of lipid type. So, the levels

TABLE 1. Content of Inflammatory Biomarkers and Chemoattractants in Unstable Atherosclerotic Plaques of Various Types (*M*±*m*)

Parameter	Unstable plaque, joint group (*n*=45)	Type 1: lipid (*n*=14)	Type 2: inflammatory erosive (*n*=16)	Type 3: degenerative necrotic (*n*=15)
IL-1β, pg/mg protein	4.3±1.2	8.1±1.3*	7.6±1.2*	2.4±1.1
TNF-α, pg/mg protein	3.1±0.4	2.3±0.2	2.6±0.2	3.9±0.4+
IL-6, ng/mg protein	15.9±1.1	27.6±2.4*	14.2±1.3*	6.3±0.6
IL-8, pg/mg protein	37.1±4.8	58.9±5.4*	56.1±5.4*	20.9±2.1
IL-18, pg/mg protein	7.8±0.9	9.4±1.3*	8.6±1.2*	3.1±1.1
IL-2, pg/mg protein	20.8±3.1	18.2±2.9	19.0±2.3	20.4±3.6
HS-CRP, ng/mg protein	2.3±0.3	2.2±0.2	2.2±0.2	2.5±0.3
MCP-1, pg/mg protein	401.7±39.8	470.6±41.6*	598.0±54.1*	159.7±13.8
EMAP-II, pg/mg protein	2007.8±189.5	2099.3±197.0	2180.4±200.1*	1701.1±165.5
sICAM-1, ng/mg protein	85.7±8.7	85.4±8.3	88.7±8.5	82.4±8.9
sVCAM-1, ng/mg protein	148.5±12.9	145.2±10.3	152.2±12.4	149.1±13.3

Note. *$p<0.05$ in comparison with type 3; +$p<0.05$ in comparison with type 1 and type 2.

of inflammatory cytokines IL-1β, IL-6, IL-8, IL-18 and chemoattractant MCP-1 in these plaques were also higher than in degenerative necrotic plaques by 3.4, 4.4, 2.8, 3.0 and 2.9 times, respectively. In that case, the levels of cytokines IL-1β, IL-6, IL-8, IL-18 in unstable plaques of the lipid type were even slightly higher (although insignificantly) than in inflammatory erosive plaques (Table 1). These data attest to the presence of inflammatory activity in unstable plaques not only of inflammatory erosive type, but also of lipid type.

A distinctive feature of unstable plaques of degenerative necrotic type compared with lipid or inflammatory erosive types is elevated levels (by 1.7 and 1.5 times, respectively) of TNF-α, a cytokine that exerts both proinflammatory and destructive effects [2,15], which indirectly indicates the important role of destructive activity along with inflammation in unstable plaques of this type.

In general, the results of the comparative study of inflammatory biomarkers in atherosclerotic foci with different types of instability revealed its features characteristic for different types of unstable plaques. Thus, the increased inflammatory activity (elevated levels of IL-1β, IL-6, IL-8, IL-18 and MCP-1) is characteristic not only for inflammatory erosive type of unstable plaques, but also for the lipid compared with degenerative necrotic type.

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