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Recent views on cytohistological characteristics and pathogenic mechanisms of atherosclerotic lesions types I, II and III

Savremeno shvatanje citoloških karakteristika i patogenih mehanizama aterosklerotskih bolesti tipa I, II i III

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Introduction

Contemporary model of the pathogenesis of atherosclerosis is based on the hypothesis that local damage of the endothelium together with systemic factors such as hypercholesterolemia, hyperglycemia, hypertension, chronically infections, genetic factors, *diabetes mellitus*, initiate a cascade of processes which eventually cause atherosclerotic lesion development [1, 2].

Although various hypotheses are already known about the pathogenesis of atherosclerosis and different factors of predisposition have been studied, from the point of molecular and vascular biology to clinical presentations and therapy methods, in modern literature there are still opposing views about the initial moment in the pathogenesis of this condition, regarding its functional and morphological changes.

The stage of the initial lesion - early lesion (type I)

Activation of endothelium

The initial stage, which precedes the development of atherosclerosis, is endothelial dysfunction, namely, activation of endothelial cells as a specific response to the action of harmful agents. Acute response of endothelium results in inflammation, coagulation disorders and vasomotor changes. The release of inflammation mediators deposited in the Weibel Palade bodies represents a very quick response of endothelial cells [3]. One of the most important consequences of endothelial activation is a decreased NO production, which causes the absence of its antiatherogenic, antiproliferative and vasodilatatory effects and, also, causes an increased production of endothelin-1 (ET-1) which in turn, through its mitogenic effect, stimulates proliferation of smooth muscle cells (SMCs) in the intima, and consequently initiate atherosclerosis [4, 5].

During activation of endothelial cells, changes in the process of coagulation have also been observed. Endothelial cells modulate their phenotype from anticoagulative to procoagulative, through an increased expression of tissue factors, or increased release of tissue plasminogen [6, 7].

Besides alterations in tonus and coagulation, endothelial activation includes increased expression of adhesive proteins (P-selectin, integrins) which promote the adhesion of leukocytes on endothelium and their infiltration in the subendothelial connective tissue [8]. This results in the release of free radicals, proteases and elastases which lead to further damage of endothelial cells [7].

Accumulation of lipids in the intima

The first step in the development of atherosclerosis is the accumulation of lipid droplets in the intima of the vessel

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wall, as a consequence of hypercholesterolemia, coupled with systemic hypertension. Lipid droplets bind to proteoglycans in the intimal connective tissue and have a tendency to form aggregates [9]. Lipoproteins in the lipid droplets may oxidize or become chemically modified in other ways [10, 11].

Hypothesis of oxidative modification

Oxidative modification is the best researched study about the modification of lipoproteins (LDL) in atherosclerosis. Studies showed that oxidized LDL (ox-LDL) has been intensely absorbed in the culture of macrophages, partially through SR-A receptor, but in a larger part through other scavenger receptors, especially CD36. Circulating monocytes express SR-A and CD36, but at a lower degree. However, when monocyte enters into vascular wall and transforms to macrophage, during this transformation the expression of scavenger receptors is increased, and this allows absorption of modified LDL forms [12].

The rate of production of ox-LDL in the arterial intima is related to the concentration of native LDL. Concentration of native LDL in the intima depends on its concentration in plasma. These results have shown that possible predilected places for pathogenesis of atherosclerotic lesions could be thickenings of the intima on the proximal parts of the artery, after their branching or bifurcation [13].

Furthermore, it has been experimentally approved that the concentration of LDL in the vascular intima is higher in places where atherosclerosis will develop, compared to the places which are resistant [14]. It is interesting that permeability of the overlying endothelium is not increased, which means that LDL somehow selectively accumulates in those places. It is proven that LDL in these predilection places binds to proteoglycans of extracellular matrix [9]. Lipid droplets binded to proteoglycans in this way oxidize much more easily [15–17]. Also according to the literature, genetically engineered LDL does not bind to proteoglycans, which means it is less pathogenic than native LDL [18]. The presence of SMCs of synthetic phenotype contributes to the accumulation of LDL in intima. The number of these cells is increased in initial lesions; they act as fibroblasts and synthesize large amounts of proteoglycans. Previously presented facts suggest that the accumulation of LDL in intima could be the initial step in the pathogenesis of atherosclerotic lesion [16, 17].

At the initial lesions beside the increased expression of the vascular cell adhesion molecule-1 (VCAM-1), which selectively promotes the adhesion of lymphocytes and monocytes, ox-LDL itself contributes to the adhesion of these cells on the endothelial layer [18]. Oxidized LDL has cytotoxic effect on endothelial cells, acts as a mitogen of macrophages and SMCs and stimulates the release of monocyte chemoattractant protein-1 (MCP-1) and monocyte colony-stimulated factor (M-CSF) from endothelial cells, which directly starts chemotaxis and migration of monocytes into the subendothelial layer [19].

Degradation of polyunsaturated fatty acids in sn-2 place of phospholipids in LDL generates ox-LDL. After generation, ox-LDL binds to proteoglycans and becomes uptaken by macrophages through scavenger receptors [20].

Expression of adhesion molecules in atherosclerosis

After initiation by hypercholesterolemia, leukocytes (at first, monocytes and T lymphocytes) adhere to the endothelium and actively migrate both, between and through endothelial cells into the intima [21]. In the subendothelium of the intima monocytes begin to accumulate lipids and begin transforming into foam cells. In that manner, increased expression of adhesion molecules, as a consequence of endothelial dysfunction and the accumulation of lipid droplets in the intima, promote adhesion of leukocytes to the endothelial layer and their migration into the subendothelium, in the initial and advanced stages of atherosclerosis [22–24].

A variety of adhesion molecules have been identified as important for this process. These include immunoglobulin superfamily members intercellular adhesion molecules 1 and 2 (ICAM-1 and ICAM-2), vascular cell adhesion molecule 1 (VCAM-1), platelet-endothelial cell adhesion molecule-1 (PECAM-1), as well as E-, P- and L-selectin [21, 25, 26].

Increased expression of VCAM-1 is a characteristic of an initial stage of atherosclerosis. This molecule interacts with specific integrin, very late antigen-4 (VLA-4), which is expressed only on monocytes and lymphocytes [25, 27]. As a result of this fact, at the initial stage of atherosclerosis, monocytes and T cells adhere in a significantly higher percentage than other types of leukocytes [28, 29]. On the other hand, ICAM-1 allows adhesion of a number of different leukocytes, because it interacts with CD11a (LFA-1) and CD11b (Mac-1) integrins on their membranes, and its expression is a characteristic of advanced atherosclerotic lesions [25, 27].

E-selectin (previously ELAM-1) is a typical representative of this group of adhesion molecules. Its name derives from a specific expression only on endothelial cells. Its increased expression is a characteristic of the advanced atherosclerotic lesions. This molecule interacts with Sialyl-Lewis X ligand on the membrane of granulocytes and memory T cells and due to this fact, at the advanced atherosclerotic lesions, these cells adhere to endothelial layer in a significantly higher percentage [25, 27].

P-selectin (previously GMP-140) is expressed primarily in thrombocytes, but also in overlying endothelium above atherosclerotic lesions. This molecule interacts with P–selectin glycoprotein ligand-1 (PSGL-1) on monocytes, lymphocytes and granulocytes. It has a significant role in „rolling“ of leukocytes on endothelial layer above the lesion [30–32].

L-selectin is expressed on leukocytes and interacts with PSGL-1 ligand on monocytes, lymphocytes and granulocytes, but also with mucosal vascular addressin cell adhesion molecular link-1 (MAdCAM-1) [25, 27, 31].

In the endothelium above an atherosclerotic plaque, in the sites of infiltration of macrophages, there is a noticeable increase of expression of ICAM-1 and P-selectin [21, 23, 30]. These results indicate that the accumulation of monocytes which will later transform into macrophages partially dependes on synergic action of P-selectin and ICAM-1 on the endothelial layer [31, 32].

Many factors combined can induce an expression of endothelial adhesion molecules in atherosclerosis [31]. These

factors are ox-LDL, inflammatory cytokines and biomechanical strength of blood shear stress. In the tissue culture, it is noticeable that the treatment of endothelial cells with ox-LDL increases the expression of P-selectin, ICAM-1 and VCAM-1 [32, 33]. Cytokines, including interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), increase the infiltration of leukocytes through adhesion molecules [2]. Regarding biomechanical stimuli, it is shown that the regulation of ICAM-1 expression is under influence of blood flow [34].

Migration of leukocytes through endothelial layer

When leukocytes adhere to endothelium, they must receive a signal in order to go through endothelium into the deeper layers of vascular wall. Two characteristic processes, selectin-mediated rolling and integrin-mediated adhesion, cooperate to promote active migration of leukocytes, both across endothelial cells (transendothelial migration) and by diapedesis. Rolling of leukocytes and migration into the vascular wall are the crucial first steps in the development of inflammation and atherosclerosis [21].

Contemporary concepts regarding direct migration of leukocytes include activation of special cytokines-chemokines. At the early stage of atherosclerosis, one of those cytokines, MCP-1 is being produced in the endothelial cells, as a response to ox-LDL and other stimuli [34, 35]. This cytokine directly starts chemotaxis and the migration of monocytes into the subendothelial layer. At this stage, subpopulations of intimal SMCs and endothelial cells also express M-CSF which also promotes chemotaxis of monocytes, their adhesion and differentiation to macrophages, regulate proliferation of macrophages and other types of cells, which is implicated in inflammatory-fibroproliferative response at the advanced stages of atherosclerosis. Oxidized LDL also stimulates endothelial cells to express granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF). These growth factors affect differentiation, survival, proliferation, migration and metabolism of macrophages/granulocytes, and G–CSF and GM–CSF also affect migration and proliferation of endothelial cells [32–35].

Other types of chemokines may also increase lymphocyte accumulation in plaque. In atherosclerotic lesion, overlying endothelium expresses three types of lymphocyte-selective chemokines: interferon-inducible protein-10 (IP-10), interferon-inducible T-cell alpha chemoattractant (I-TAC) and monokine induced by interferon-γ (MIG). Interferon-γ which is present in the atherosclerotic lesion induces genes which code T cell hemoattractant protein family of chemokine, and subsequently, accumulation of T cells in plaque [36, 37].

Foam cells of monocyte-macrophage lineage

When monocytes enter the intima of a vessel wall, they undergo phenotype modification to macrophages. Macrophages then accumulate lipid droplets and become foam cells. The mechanism of cholesterol uptaking in macrophages is especially interesting.

Most of the cells use a plasma membrane receptor to uptake and release cholesterol. When a cell uptakes cholesterol for its own metabolic needs, a mechanism of the transcription control of this receptor become activated, which in turn shuts down the receptor expression and completely shuts down further absorption of cholesterol. On the contrary, macrophages in the atherosclerotic lesion (future foam cells) do not posses this kind of negative feedback, so it is obvious that they uptake cholesterol through some other kind of receptors [38].

Some investigations have shown that in the culture of macrophages in *in vitro* conditions, cells rapidly uptake modified, acetylated LDL, not through the known LDL receptor, but through the special "acetyl-LDL" receptor. Later, this receptor is cloned and called the scavenger receptor A (SR-A). Contrary to LDL receptor, expression of SR-A is not down-regulated as a response to the increased intracellular concentration of cholesterol. Theoretically, this kind of receptor might have a role in forming foam cells, because there is no data that acetyl-LDL has been generated in *in vivo* conditions [39, 40]. According to literature, the various modified forms of LDL also could have a role in the creation of foam cells, which brings out the question of the existence of various receptors [41, 42].

Other studies [42–44] have shown that some foam cells originate from SMCs, probably due to the fact that SMCs can also express scavenger receptors if they are properly activated, which will be discussed later.

Cytohistological characteristics of an initial lesion – early lesion (type I)

The results of a large number of recent studies on cytohistological characteristics of atherosclerotic lesions have shown that at the stage of initial lesion (early lesion – type I) there are no visible morphological changes in the structure of vascular wall, which is in accordance with the previously stated pathogenic mechanisms. According to these results, at the initial stage endothelial continuity is preserved, with the presence of individual foam cells and T cells in the intima. At initial phases of lesions circulating monocytes are the main precursors to foam cells. Smooth muscle cells in the intima and media show contractile phenotype (Figure 1). Their phenotype modulation from the contractile to synthetic phenotype can be noticed during the fatty streak stage [28, 45, 46].

The fatty streak stage - early lesion (type II)

Cytohistological characteristics of the fatty streak stage – early lesion (type II)

With further development of atherosclerotic lesion, at the fatty streak stage (early lesion – type II) increase in the number of foam cells (Figure 2) and intense infiltration of T cells occurs. At this stage, lipid accumulation in foam cells forms fatty streaks. Endothelial layer is still morphologically preserved, although it is functionally damaged [46, 47].

Foam cells at this stage originate from macrophages (Figure 3) [46, 47]. The fatty streak stage is also characterized by the presence of SMCs of synthetic phenotype, but they are not foam cells yet [29, 47].

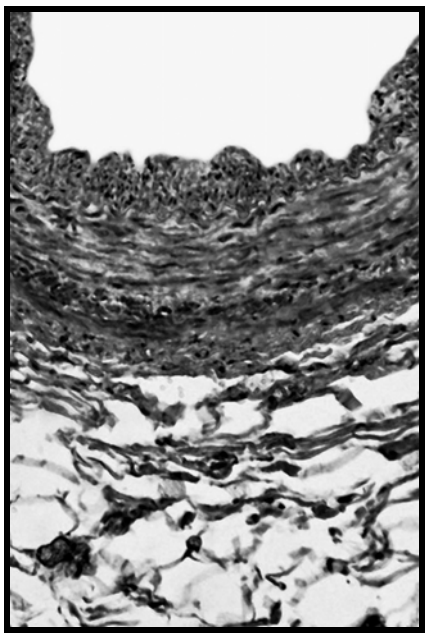

Fig. 1 – Coronary atherosclerosis at the initial stage. Endothelial layer is well-preserved without visible morphological changes. Smooth muscle cells in intima and media show contractyle phenotype (immunohistochemical staining of MHC, original magnification ×64)

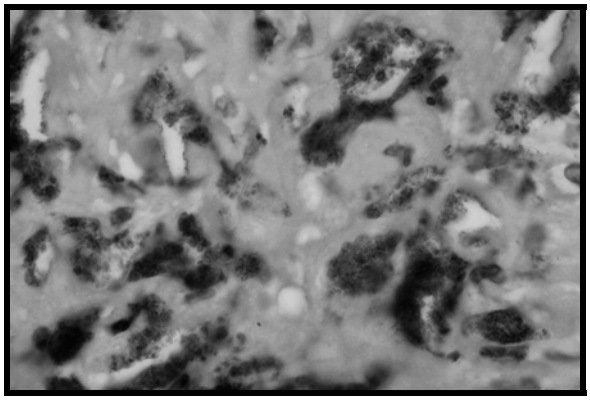

Fig. 2 – Coronary atherosclerosis at the fatty streak stage. Foam cells in subendothelial layer (immunohistochemical staining of CD68, original magnification ×256)

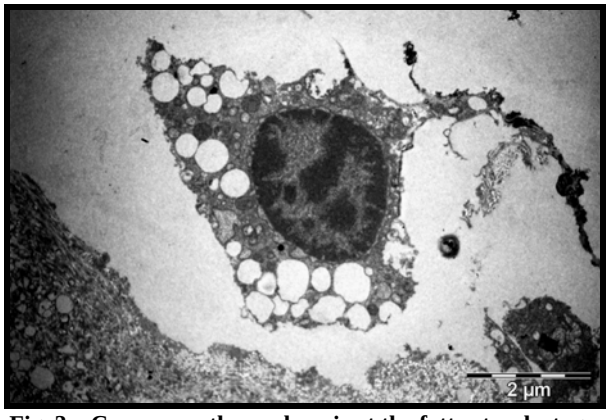

Fig. 3 – Coronary atherosclerosis at the fatty streak stage. Macrophage with a large number of lipid inclusions in cytoplasm, *ie* foam cell that originate from the monocyte-macrophage lineage (TEM, original magnification ×16875)

Smooth muscle cells (SMCs)

The results of testing phenotypical status of SMCs of atherosclerotic lesions showed that from a fatty streak stage, the modified SMCs of synthetic phenotype form the dominant cell population [28]. The development of synthetic phenotype is followed by a reduction in myofilaments [29]. Smooth muscle cells of synthetic phenotype expressed α-smooth muscle actin (α-SMA) and vimentin, with a lack of expression of desmin [43, 48]. The loss of desmin expression with concurrent vimentin expression is the first sign in the process of switching from contractile to synthetic phenotype [43, 49]. According to the existing literature, vimentin is an intermediary filament that can be found in differentiated SMCs as well, but it is coexpressed there with desmin. With the loss of contractile phenotype and characteristic desmin expression (*i.e.* with switching of cells to synthetic phenotype), the expression of vimentin filaments can be noticed [28, 45, 50].

Switching of SMCs to synthetic phenotype is a main characteristic of vascular remodeling during the pathogenesis of atherosclerosis [48, 51]. Vascular remodeling is an adaptive process involving the adjustment of structure and function of blood vessels to long-term changes in hemodynamic conditions, especially to hypertension [45, 52]. Scientific reports prove that reactive – adaptive intimal proliferation ("early response" of the wall) during the process of vascular remodeling, does not have a mechanism of negative feedback and that it can be continued in the form of intimal hyperplasia. In the primary response to the conditions of increased pressure, there is a proliferation of endothelial cells in vascular wall. After endothelial proliferation, during a rather long period of time it is characterized by SMCs proliferation accompanied by vascular wall thickening. The increased synthesis of collagen and elastin, which is an alteration of gene expression for the synthesis of these proteins, is the explanation for rapid enlargement and irreversible thickening in atherosclerotic vascular remodeling, in spite of apoptosis which is activated after proliferation of SMCs [52, 53].

Under the influence of chemotactic factors, SMCs migrate through fenestrae of the internal elastic lamina. As they express specific integrins (α2β1 which binds collagen and α3β1 and α5β1 which bind fibronectin) and elastin binding proteins on plasma membrane, they come in contact with connective tissue components and start to proliferate as a response to the stimuli of platelet-derived growth factor (PDGF) and fibroblast growth factors (FGFs) [55]. At the same time, under the influence of TGF-β, SMCs begin to secrete extracellular matrix components, thereby increasing the amount of collagen IV and collagen V. These changes were associated with up regulation of transforming growth factor β (TGF- β) [52].

Also, at this stage, adventitial myofibroblasts start to show the characteristics of contractile SMCs [29, 47, 48]. According to the same data, the increased expression of TGF-β in adventitia is the signal for differentiation of fibroblasts (which start to express α-SMA and the other markers of SMCs differentiation as well) and their migration, which makes it hard to differentiate from medial SMCs (Figure 4).

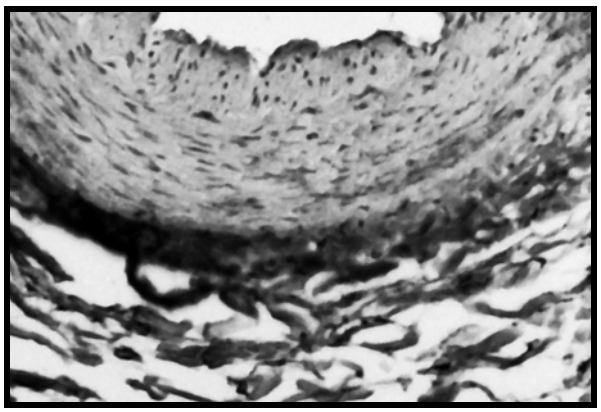

Fig. 4 – Coronary atherosclerosis at the fatty streak stage. Myofibroblasts in adventitia start to show the characteristics of contractile smooth muscle cells (immunohistochemical staining of MHC, original magnification ×64)

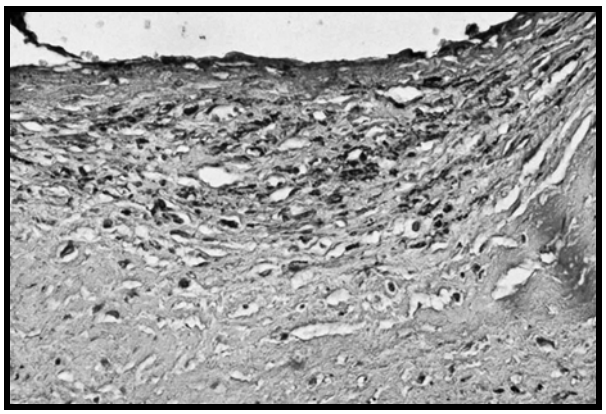

Fig. 5 – Coronary atherosclerosis at the preatheroma stage. Smooth muscle cells of the synthetic phenotype (immunohistochemical staining of vimentin, original magnification ×128)

A number of important stimulators and inhibitors of both SMCs and endothelial cells in atherosclerotic lesions have now been purified and biochemically characterized. Included in the group of stimulators are FGFs, vascular endothelial growth factor (VEGF), PDGF, epidermal growth factor (EGF) like factors, angiogenin, also vasoconstrictor peptides (which induce proliferation of SMCs and the expression of TGF-β and PDGF), angiotensin converting enzyme (ACE) (which induces proliferation of SMCs in *in vivo* conditions), insulin-like growth factors (IGFs) (potential mitogen of SMCs), interleukin-1 (IL1) (induces expression of FGF in SMCs), lipoprotein A (stimulates proliferation of SMCs) and ET-1 [22, 54].

Bifunctional growth factors, TGF-β and TNF-α, also have an influence on the proliferation and migration of SMCs. A multifunctional cytokine is TGF-β, which in its inactive form is being synthesized by endothelial cells and SMCs. This cytokine becomes activated under the influence of plasmin and induces differentiation of endothelial cells and SMCs, but paradoxically inhibits their migration and proliferation. TNF-α is cytokine which inhibits proliferation of endothelial cells and SMCs but it stimulates the transcription of heparin-binding EGF-like growth factor (HB-EGF) in SMCs [31, 54]. Each of these factors may influence the growth of SMCs or endothelial cell, both as a stimulant or inhibitor.

The preatheroma stage – intermediary lesion (type III)

Cytohistological characteristics of the preatheroma stage – intermediary lesion (typeIII)

At the preatheroma stage – intermediary lesion (type III), lipid accumulation surpasses the accumulation by foam cells and subendothelial layer contain scattered collections of extracellular lipid droplets and particles in the form of small isolated pools of extracellular lipid. The preatheroma stage is characterized by the presence of intimal and medial proliferating SMCs of synthetic phenotype (Figure 5). The phenotype modulation of intimal and medial SMCs (based on the loss of desmin expression and the appearance of vimentin expression) begins at the fatty streak stage, and at the preatheroma stage these cells form the dominant cell population [28, 29, 45].

The intermediary lesion is also characterized by a large number of foam cells of various origin. Some of foam cells develop from monocyte-macrophage lineage (the same as at the fatty streak stage) and others originate from SMCs [28, 46, 47]. Foam cells that originate from SMCs are spindle-shaped or star-shaped and they have short, thick extensions with lipid inclusions in cytoplasm (Figure 6) [28, 48].

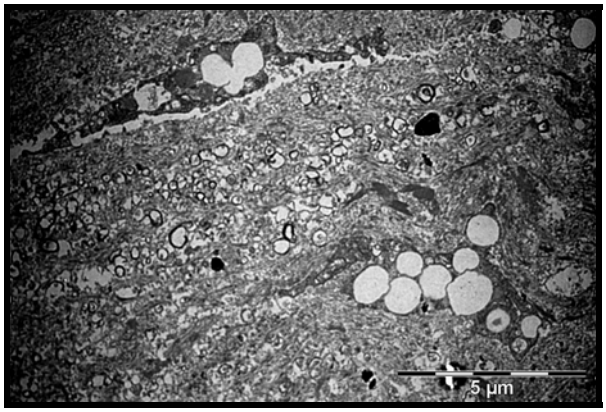

Fig. 6 – Coronary atherosclerosis at the preatheroma stage. We can notice the presence of foam cells that originate from smooth muscle cells. These lipid-laden smooth muscle cells are spindle-shaped or star-shaped, with lipid inclusions in the cytoplasm and they have short, thick extensions. The number of lipid inclusions in the cells varies, and therefore, they look as if they are at the different stages of phenotype transformations to foam cells (TEM, original magnification ×8250)

Foam cells of smooth muscle cells origin

The results of many studies have shown that SMCs of synthetic phenotype, proliferate and start to accumulate lipids from the fatty streak stage, but their largest number can be observed at the preatheroma stage [28, 29, 45]. According to the same data, some foam cells in atherosclerosis originate from SMCs of synthetic phenotype (express vimentin-immunoreactivity), probably due to the fact that some SMCs can also express scavenger receptors. Vimentin-immuno-

reactivity points out to their syntetic/proliferative activity and also to their mesenchymal origin [45, 46].

The fact that SMCs express scavenger receptors and modulate to foam cells is possibly related to the embryonic origin of vascular SMCs [44]. All vascular SMCs have a mesenchymal origin, except for the fact that large arteries in the upper parts of the body can have the neuroectodermal origin [28, 56, 57]. Due to their mesenchymal origin, SMCs coexpress vimentin and desmin. During remodeling of vascular wall in atherosclerosis, there is a loss of contractile characteristics of SMCs and characteristic desmin expression so that SMCs of the synthetic phenotype express only vimentin [28, 29, 43]. After loss of contractile characteristics, SMCs proliferate and as a result of this proliferation start to intensively express S-100 protein. For both, SMCs of mesenchymal origin, as well as for SMCs of neuroectodermal origin, expression of this protein is a normal characteristic, but it is increased in processes which are Ca^{++} – mediated. This protein in increased expression shows a high affinity to bind unsaturated lipid acids [58]. It has been assumed that SMCs due to their synthetic and proliferative activity can accumulate lipids.

Pathogenesis of atherosclerosis as an inflammatory response of vascular wall

Earlier theories about the pathogenesis of atherosclerosis have assumed hypercholesterolemia as a necessary condition for the development of this disease. Only in the later decades, numerous researches have pointed out to inflammation as a basis for pathogenesis of atherosclerosis [27].

As mentioned above, beginning with the initial lesion stage, in the response to the presence of modified lipids in the subendothelial layer, endothelial cells increase the expression of adhesion molecules, at first VCAM-1, which selectively promotes the adhesion of monocytes and T cells on endothelial layer. In response to the same stimulus endothelial cells express monocyte-selective chemokine MCP-1 and three types of lymphocyte-selective chemokines (IP-10, I-TAC and MIG) which directly start chemotaxis and migration of monocytes and T cells into the subendothelial layer. The presence of macrophages and T cells in subendothelial layer show that the early stages of atherosclerotic lesion are an inflammatory response to exogenous pathogens [27, 51].

However, in addition to modified lipids, other exogenous pathogens, especially microorganisms, also promote inflammation. These antigens become presented by antigen-presenting cells (APCs), to Th CD4+ cells trough the MHC class II-dependent pathway. In response to immunological activation, mature forms of Th CD4+ cells begin to produce proinflammatory cytokines IL-2, IFN-γ and TNF-β. These cells also interreact with Tc CD8+ cells, NKT cells and macrophages, helping them to finish a started immune response against intracellural pathogens [27].

The role of APCs during atherosclerosis is played by macrophages, vascular dendritic cells (VDCs) and B cells, as a "professional APCs", but recent studies have show that low differentiated forms of SMCs also can process and present antigens [59, 60]. All APCs in vascular wall internalize antigens either by phagocytosis or by receptor-mediated endocytosis, and display a fragment of antigen bound to the class II MHC molecule on their plasma membrane. Expression of the antigen-class II MHC molecule complex which is a ligand for a T-cell receptor (TCR), on the membranes of APCs with additional co-stimulatory signals from APCs leads to immunological activation of T cells and manifestation of inflammatory reactions [27, 59, 60].

A great number of recent studies aimed to improve therapeutic procedures have been focused on the role of IFN-γ during atherosclerosis. Immediately after entrance into a subendothelial layer, T cells produce IFN-γ which induces genes that code T cell hemoattractant protein family of chemokine, and subsequently, further accumulation of T cells in the plaque. Besides, this cytokine activates macrophages immunoregulatory functions and simultaneously affects SMCs of a lesion. These literature data support the hypothesis that IFN-γ is a potent atherogenic cytokine, which significantly increases both, lesion size as well as the number of T lymphocytes within lesions, and also up regulates PDGF which causes an increased migration of smooth muscle cells [60, 61].

If pathogenesis of atherosclerosis is viewed, in the light of these previously stated facts, as an inflammation, it can be concluded that during the early stages, atherosclerosis, as a specialized form of inflammation, responds to an injury of vascular wall and the presence of different pathogens [51]. With further progression of inflammatory reaction, during the stages of advanced atherosclerotic lesions, development of a chronic inflammatory fibroproliferative response occurs, which is histologically manifested as atheroma (type IV), lesion with a lipid core; fibroatheroma (type V), lesion with a lipid core and prominent fibrous connective tissue above it, or complicated atherosclerotic lesion that is defined as type IV or V lesions with disruptions of lesion surface, hematoma or hemorrhage, and developed thrombotic deposits (Figures 7 and 8) [28, 45, 46, 48].

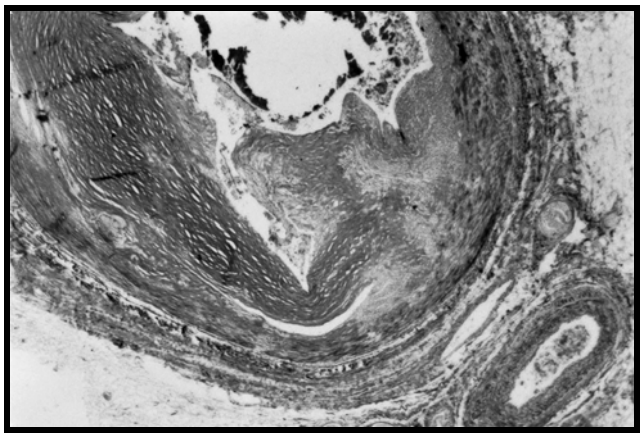

Fig. 7 – Advanced atherosclerotic lesion in the coronary artery – complicated, ulcerated atherosclerotic plaque with intraplaque hemorrhage (histochemical staining of Azzan-Heidenhain, original magnification ×32)

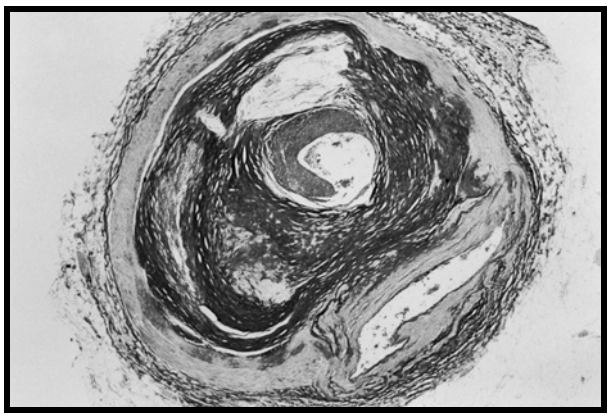

Fig. 8 – Advanced atherosclerotic lesion in the coronary artery – complicated atherosclerotic plaque with atheroma, fibroatheroma, fissure, intraplaque hemorrhage and thrombus (histochemical staining of Azzan-Heidenhain, original magnification ×8)

Conclusion

From all the previously presented facts we can conclude that the basic predisposing factor for development of atherosclerosis is hypercholesterolemia or infection, coupled with systemic hypertension.

The increased levels of plasma cholesterol promote the entrance of lipid droplets in subendothelial layer, their binding to proteoglycans and modification of LDL. Modified LDL stimulates increased expression of adhesion molecules and promotes the adhesion of leukocytes. Modified LDL also stimulates the production of cytokines-chemokines in endothelial cells which promotes the migration of leukocytes into the subendothelial layer, and also initiates synthesis of growth factors which affect differentiation, modulation, migration and proliferation of different cell types in the lesion. From the above presented facts we can conclude, too, that hypercholesterolemia starts *circulus vitiosus* in which many combined factors induce the development of inflammation in atherosclerosis. In addition to modified forms of LDL, the presence of other exogenous pathogens (especially microorganisms) initiates atherosclerosis through inflammatory response of vascular wall.

On the other hand, systemic hypertension promotes vascular remodeling. This process is characterized by smooth muscle cells phenotype modulation from contractile to synthetic phenotype, their migration from media into the subendothelial layer, their proliferation and collagen synthesis, as well as their transformation to foam cells in the presence of modified LDL, inflammatory cytokines and biomechanical strength of blood shear stress.

The previously analyzed facts suggest that atherosclerosis could be defined as inflammation which is a response to hypercholesterolemia or other exogenous pathogens, coupled with vascular remodeling, caused by systemic hypertension.

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