Accumulating evidence suggests that atherosclerosis should be regarded as a complex set of interactions among endothelium, circulating cells and particles, soluble mediators, and chemotactic agents. The processes leading to the creation of atherosclerotic lesions may also be seen as imbalances between pro- and anti-atherogenic factors, pro- and anti-inflammatory agents, and vasoconstrictors and vasodilators. However, irrespective of the pathogenesis, multiple cross-reacting compounds influence both the structure and function of vessels, resulting in their dysfunction and damage. The endothelium thus seems to be a target and a focus for all the reactions mentioned above. Therefore, clarifying the mechanisms responsible for atherosclerosis seems to be the key target for future investigations (Adv Clin Exp Med 2006, 15, 6, 971–978).

Key words: adhesion molecules, cytokines, nitric oxide, reactive oxygen species, endothelium.
The Natural History of Atherosclerosis

Atherosclerotic lesions, although asymptomatic, start already in childhood. Atherogenesis covers a wide range of abnormalities, including fatty streaks, lipid cores, and fibrous caps. Early lesions, complicated by thrombi, calcifications, and ulcerations, develop into plaques that change the geometry of vessels and lead to their stenosis or even occlusion. All these disturbances are conditioned by microscopic changes within the endothelium caused by inadequate responses to various stimuli from the serum and surrounding tissues.

Classification of Atherosclerotic Lesions

In the early stages of atherosclerosis, leukocyte recruitment and lipid deposition dominate (Table 1). Lipid-laden macrophages (foam cells) accumulate in the intima and form regions of thickening, called fatty streaks [1]. The next components of atherosclerotic lesions are lipid-containing vascular smooth muscle cells (VSMCs) and pools of extracellular lipids. The migration and proliferation of VSMCs coexists with extracellular matrix synthesis [1]. Until this moment, the changes are potentially reversible and elimination of the factors destroying the endothelium, as well as lipid-lowering therapy, may prevent plaque formation.

Further lesion progression is characterized by continuous lipid accumulation, leading to the formation of lipid cores. These appear due to the fusion of lipid deposits, necrotic foam cells, and cellular debris [2]. Initially, the layer surrounding the lipid core consists only of the intima. Then the lipid core becomes encapsulated by collagen. The fibrous cap may also contain calcifications [2]. Extensive VSMC proliferation may result in the overproduction of collagen fibers and lipid core destruction. Shear stress disturbs blood flow in the regions of the modified vessel geometry and may break down the fibrous caps [2]. Erosive atherosclerotic plaques are places of thrombi formation (Table 1).

The initial lesion and plaque formation results in stenosis of the vessel lumen and hypertrophy of the entire vessel wall. Such remodeling aims at maintaining the ability of the vessel wall to contract and relax. When compensatory mechanisms are depleted, the vessel stenosis becomes irreversible and the final occlusion progresses.

Adhesion Molecules in Atherosclerosis

Current data emphasize the role of chronic inflammation in the pathogenesis of atheroscler-

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Table 1. Classification of atherosclerotic lesions

<table>
<thead>
<tr>
<th>Histological type (Typ histologiczny)</th>
<th>Cells and structures involved (Zaangażowane komórki i struktury)</th>
<th>Functional changes (Zmiany czynnościowe)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I; intima</td>
<td>monocyte recruitment</td>
<td>clinically silent</td>
</tr>
<tr>
<td></td>
<td>macrophage formation</td>
<td>reversible</td>
</tr>
<tr>
<td></td>
<td>lipid deposition</td>
<td></td>
</tr>
<tr>
<td>II; intima, media</td>
<td>foam cell accumulation → fatty streaks</td>
<td>clinically silent</td>
</tr>
<tr>
<td></td>
<td>lipid-containing vascular smooth muscle cells (VSMCs)</td>
<td>reversible</td>
</tr>
<tr>
<td>III; intima, media</td>
<td>VSMC proliferation and migration</td>
<td>clinically silent</td>
</tr>
<tr>
<td></td>
<td>extracellular matrix synthesis</td>
<td>potentially reversible</td>
</tr>
<tr>
<td></td>
<td>small pools of extracellular lipids</td>
<td></td>
</tr>
<tr>
<td>IV; intima, media</td>
<td>fusion of lipid deposits, necrotic foam cells and cellular debris, lipid cores</td>
<td>vessel wall hypertrophy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>vascular remodeling</td>
</tr>
<tr>
<td>Va; intima, media</td>
<td>collagen-containing fibrous cap</td>
<td>modified vessel geometry</td>
</tr>
<tr>
<td></td>
<td></td>
<td>vessel stenosis</td>
</tr>
<tr>
<td>Vb; intima, media</td>
<td>calcifications in fibrous cap</td>
<td>modified vessel geometry</td>
</tr>
<tr>
<td></td>
<td></td>
<td>vessel stenosis</td>
</tr>
<tr>
<td>Vc; intima, media, lumen</td>
<td>collagen fiber overproduction</td>
<td>vessel stenosis, occlusion</td>
</tr>
<tr>
<td></td>
<td>lipid core destruction</td>
<td>clinical manifestations</td>
</tr>
<tr>
<td>VI; intima, media, lumen</td>
<td>plaque rupture, formation of thrombi, erosive plaque</td>
<td>vessel stenosis, occlusion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>clinical manifestations</td>
</tr>
</tbody>
</table>
sis. Endothelial response to injury is a prerequisite for initiating this reaction. Although laminar shear stress is an atheroprotective factor, even slight changes in blood flow may stimulate defense mechanisms [3]. Endothelial activation may also be provoked by other unspecific stimuli, such as hypoxia, hypercholesterolemia, histamine or thrombin, or specific agents, such as immune complexes [4]. Endothelial activation causes structural changes within the innermost layer of the endothelium and enables the rolling, activation, firm adhesion, and migration of cells from the vessels to the inflamed or injured tissues. In particular it allows cells to migrate towards atherosclerotic lesions, thus aggravating the inflammatory reactions in situ. The sequence of these reactions, defined as the leukocyte adhesion cascade, is regulated by adhesion molecules, such as immunoglobulin superfamily members (ICAM-1, VCAM-1, platelet-endothelial cell adhesion molecule (PECAM)-1), selectins (E-, L-, P-selectin), and integrins [4].

Circulating cells do not adhere to endothelium under normal conditions. However, unfavorable hemorheology in lesion-prone areas of vessels (e.g. flow turbulence in the regions of bifurcations) may stimulate the adhesion cascade by increasing ICAM-1 and inducing VCAM-1 expression on the endothelium [4]. Ox-LDL, IL-1, IL-4, TNF-α, and monocyte chemoattractant protein (MCP)-1 are other potent ICAM-1 and VCAM-1 activators, whereas HDL, transforming growth factor (TGF)-β, fibroblast growth factor (FGF)-2, and IL-10 inhibit their cytokine-induced expression (Fig. 1). Meanwhile, P-selectin is released upon activation from Weibel-Palade bodies in endothelial cells and translocated to the plasma membrane. Then it can react with a counter-receptor on leukocytes, i.e. sialyl Lewis x antigen [4]. Simultaneously, L-selectin, constitutively expressed on leukocytes, binds to endothelial receptors [4]. During the phase of selectin ligation, cells transiently adhere and detach, depending on local shear forces and the variable endothelial distribution of P-selectin [5]. These reactions diminish the velocity of rolling and facilitate cell contact with chemotactic agents and other cytokines [4]. The subsequent firm adhesion of leukocytes to the endothelium, conditioned by the local expressions of ICAM-1 and VCAM-1, is a prerequisite for monocyte transmigration through the endothelial layer into the intima.

The migration of monocytes has been implicated as a key player in atherogenesis. Their recruitment is mainly governed by MCP-1, a member of the CC family of chemokines (Fig. 1). The pro-atherogenic activity of MCP-1 has been observed in apoE–/– mice [6], whereas an investigation on rats revealed a growth-promoting effect of MCP-1 on VSMCs, which may suggest

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**Fig. 1.** Network of interactions among adhesion molecules, cytokines, and ROS in atherogenesis; stimulation ⇒, suppression ⊳

**Ryc. 1.** Sieć interakcji między molekulami adhezyjnymi, cytokinami i wolnymi rodnikami tlenowymi w procesie tworzenia zmian miażdżycowych; aktywacja ⇒, supresja ⊳
a potent role of this chemokine in vessel wall remodeling [7]. Another potent chemoattractant for monocytes is the soluble form of fractalkine (FK), the only member of the CX3C chemokine family, found in atherosclerotic plaques [8]. The next chemokine with well-established pro-atherogenic activity is IL-8. Its expression is stimulated by oxLDL and its chemoattractant action mainly concerns monocytes [9]. Platelet-derived RANTES, delivered to the endothelium by platelet microparticles, is also known for its chemotactic impact on monocytes [10]. However, their activity may be suppressed by TGF-β, down-regulating MCP-1 and IL-8. Macrophage colony-stimulating factor (M-CSF) also plays an important role in cell migration. M-CSF influences monocyte differentiation into macrophages, which is the next step to foam cell formation (Fig. 1).

Cytokine stimulation activates membrane-bound adhesion molecules and induces their proteolytic shedding from cells into the circulation [11]. Soluble forms (s) appear to be biologically active and influence leukocyte attachment to the vascular endothelium, thus playing an important role in atherogenesis. Additional support for this notion is the fact that the levels of circulating adhesion molecules correlate with intima-media thickness and plaque score [12]. Moreover, sICAM-1 serum concentration has been established as a risk factor for future myocardial infarction in apparently healthy men and might predict the progression of carotid atherosclerosis independently of traditional risk factors [13]. sVCAM-1 and sE-selectin are also potential serum markers of atherosclerosis [14].

### Cytokine Activity in Atherosclerosis

Most of the cytokines have been proposed to be involved in atherogenesis, and all cells found in the atherosclerotic plaques produce cytokines and respond to cytokine mediators. Migrating cells, such as monocytes, neutrophils, T cells, and platelets, accumulate due to the adhesion cascade on the vessel wall and release numerous interleukins (IL), proteins, growth factors, and enzymes upon activation.

The globally accepted classification of interleukins as either pro- or anti-inflammatory, or rather pro- or anti-atherogenic, can only partly systematize this heterogeneous and ever-expanding group of molecules. Apart from those with well-established pro-atherogenic (IL-1α/β, IL-2, IL-6, IL-8, IL-12) and anti-atherogenic (IL-1ra, IL-9, IL-10) roles, there are still those with unknown impact on atherogenesis (IL-19 to IL-27). Certain cytokines may also exert pro- and anti-inflammatory activity simultaneously. For example, IL-13 can induce VCAM-1, but has no impact on ICAM-1 or E-selectin [15]. Disturbed balance among different cytokines may represent another candidate mechanism underlying atherogenesis. Such interactions have been noted between IL-10 and IL-12, IL-4 and IL-12, and IL-6 and IL-10 [16]. In vitro studies have also shown that different combinations of several cytokines may act either synergistically or antagonistically towards adhesion molecule expression [11]. The changes in gene expression induced by certain cytokines are attracting increasing attention due to their potent role in atherosclerotic lesion formation. Up-regulation of genes encoding VCAM-1, MCP-1, and IL-6 in human vascular endothelial cells treated with IL-4 may be an example of pleiotropic cytokine activity [17]. Therefore, the cytokine contribution to atherosclerosis seems to be a set of actions among various signals, creating a self-perpetuating mechanism of lesion formation. Its complexity is aggravated by the fact that migrating cells are not the only source of cytokines.

Adipose tissue produces a recently discovered group of cytokines called adipocytokines. Apart from the well-characterized representatives IL-6 and TNF-α, there are new proteins, leptin and adiponectin, of great interest due to their role in atherogenesis. In vitro studies have demonstrated that leptin stimulates MCP-1 expression and ROS production in cultured endothelial cells, thus acting pro-atherogenically [18]. Adiponectin has been suggested to have anti-atherogenic properties. Together with a decrease in oxLDL uptake by macrophages and inhibition of VCAM-1, ICAM-1, and E-selectin expression, it also stimulates NO production [19]. Therefore it is connected with major pathways of atherosclerotic disturbances, i.e. dyslipidemia, inflammation, and endothelial dysfunction.

### Lipid Disturbances in Atherosclerosis

Major lipid changes in atherosclerosis result from disturbed lipoprotein transport, mainly involving LDL (low-density lipoprotein). Under normal conditions, intra- and extra-cellular concentrations of LDL are balanced by the LDL receptor density [20]. When cholesterol levels in cells are high, de novo LDL synthesis is stopped and the LDL receptor density decreases. In the case of intracellular cholesterol deficiency, the LDL receptor density increases and LDL transport from outside the cell is intensified [20]. In particu-
ular, when LDL concentrations in the blood are increased, cholesterol synthesis in situ is blocked and only LDL influx takes place, thus playing a defensive role against hyperlipidemia [20]. Additional protection results from HDL-related cholesterol efflux. However, even slight modifications in LDL structure, e.g. caused by oxidative stress or hyperglycemia, render LDL receptors on the endothelium unable to recognize the lipoprotein particles. Therefore, oxidized, glycated, or glycoxidized LDLs are caught by scavenger receptors present on endothelial cells and macrophages. The density of these receptors is independent of intracellular cholesterol concentrations. Moreover, glycoxidized LDLs induce their specific class A scavenger receptors (SR-A) and suppress class B type I scavenger receptors (SR-BI) for HDL, thus acting pro-atherogenically [21]. Due to these facts, macrophages and endothelial cells can be easily laden with cholesterol, thus transforming them into foam cells and initiating early atherosclerotic lesions. Their subsequent development into atherosclerotic plaques requires immune system activation.

Endothelial Dysfunction in Atherosclerosis

Impairment of endothelium-dependent vasorelaxation is a characteristic feature of atherosclerosis. It strongly depends on the bioactivity of the endothelium-derived relaxing factor nitric oxide (NO’). Decreased NO’ bioavailability, typical of atherosclerosis, may result from a decline in its synthesis by endothelial NO’ synthase (eNOS) or accelerated degradation by reactive oxygen species (ROS).

ROS are a group of oxygen-containing molecules responsible for the oxidation of lipids, proteins, carbohydrates, and DNA. Some of these molecules, such as superoxide anion (O2−), hydroxyl radical (HO’), and NO’, are free radicals due to unpaired electrons (•), and such a chemical structure provokes chain reactions occurring at extremely fast rates. The end products of the initial reactions usually attack adjacent molecule side chains. This perpetual process causes the accumulation of free radicals. The aggravated regional toxic effects are partly suppressed by defense mechanisms which decrease the ROS concentration. These native protectors include anti-oxidative enzymes, such as copper/zinc-superoxide dismutase (Cu,Zn-SOD) and manganese-superoxide dismutase (Mn-SOD), metal-binding proteins (transferrin, ceruloplasmin, ferritin, metallothionein), and vitamins (A, C, E) [29]. Recent investigations have shown that imbalance between pro- and antioxidiant mechanisms is of paramount importance in the pathogenesis of lipid oxidation and NO’-related endothelial dysfunction.

ROS production is conditioned by the activity of enzyme complexes. The NADPH oxidase system, present in endothelium, neutrophils, and vascular smooth muscle cells, mainly produces O2•−. It may be activated by mechanical forces, such as shear stress, stretching, or disturbed flow at lesion-prone areas of the vasculature. NADPH oxidase is also responsive to angiotensin II and TNF-α stimulation. The xanthine oxidase system, implicated in ischemia-reperfusion injury, is another complex generating mainly O2•− and H2O2. Contrarily, nitric oxide synthases (eNOS) form a complex that generates excessive amounts of O2•− only in pathologic states and produce NO’ under normal conditions. Recent investigations indicate that eNOS
Gene polymorphism should be regarded as a risk factor for endothelial dysfunction [30].

L-arginine is the substrate for NO synthesis by eNOS and tetrahydrobiopterin (BH4) is the cofactor of this reaction. In the absence of one of these, eNOS becomes uncoupled, thus producing superoxide anion (O2−) and hydrogen peroxide (H2O2) instead of NO. eNOS activity can also be suppressed by hypercholesterolemia and, in particular, by oxLDL [31]. The latter inhibits dimethylarginine dimethylaminohydrolase, which is the enzyme inactivating asymmetric dimethylarginine (ADMA) [32]. Subsequently, increased concentrations of ADMA, which is in competition with L-arginine, inhibit NO synthesis and increase O2− production via eNOS uncoupling. When NO and O2− are produced simultaneously, they react with each other. The reaction rate for NO and O2− is three times faster than that for O2− and superoxide dismutase (SOD), which can stabilize NO. Therefore, the first reaction dominates and NO becomes inactivated. Moreover, other free radicals are generated. One of them is peroxynitrite (ONOO−), which at low concentrations has a mildly relaxing activity, but at high levels destroys the vessel wall. NO can also react with hydroxyl radical (HO•) and products of lipid oxidation [33].

Oxidized lipids can be generated in the vasculature by several enzymatic and non-enzymatic mechanisms. Animal experiments have provided evidence that lipoxygenases (LOXs) play a major role in atherosclerosis progression [34]. Enzyme-catalyzed oxidation takes place in leukocytes (isoform 5-LOX), platelets (isoform 12-LOX), and reticulocytes (isoform 15-LOX). During these reactions, enzyme-bound radical intermediates are generated, such as lipid alkyl (L'), alkoxyl (LO•), and peroxy (LOO•). All of these react with NO and decrease its bioavailability. Moreover, 15-LOX is cytokine inducible and may show pro-atherogenic activity [35]. Another enzyme prone to cytokine stimulation is prostaglandin endoperoxide H synthe (PGHS), the expression of which is raised in the presence of IL-1 and TNF-α. It acts similarly to LOX, generating lipid radicals reacting with NO [36]. Increased activity of myeloperoxidase (MPO), the protease which catalyzes hypochlorous acid (HOCl) synthesis, may also play a causative role in lipid oxidation. MPO is produced by foam cells and its expression has been demonstrated in human atherosclerotic plaques. Nitrating and chlorinating products of reactions catalyzed by MPO damage tissues due to their toxic properties [37]. Sub-endothelial matrix destruction further increases ROS production and initiates smooth muscle cell migration, thus propagating the formation of atherosclerotic lesions. Moreover, MPO influences NO' bioavailability, thus showing additional pro-athergenic properties [37]. A non-enzymatic candidate mechanism for lipid oxidation is the Fenton reaction, in which free metal ions (Fe2+ and Fe3+) react with lipid hydroperoxide (LOOH), thus producing LO' and LOO'.

ROS overproduction and lipid disturbances are tightly connected with macrophage activity. Oxidative stress is responsible for impaired macrophage phagocytosis of apoptotic cells in atherosclerotic plaques. Apart from the already mentioned LOX and MPO, there are other macrophage-derived enzymes and substances influencing atherogenesis. Recently developed animal models revealed pro-atherogenic features of lipoprotein lipase (LPL) and anti-atherogenic activities of lysophospholipase 3 and lysophosphatidylcholine [38, 39]. Macrophages are also a source of resistin, a newly discovered adipocytokine inducing insulin resistance, correlating with inflammatory markers and predicting atherosclerosis [40].

The involvement of adhesion molecules, interleukins, chemokines, growth factors, and reactive oxygen species in atherogenesis has recently been revealed. Great progress has been made in the understanding of their activity when examined individually. However, there are still many questions about the interactions among these components within the "endothelial network". Both in the circulation and inside the vascular wall, the balance between protective and destructive mechanisms seems to be tipped towards the latter. Although the agents affecting this equilibrium are under investigation, our knowledge of the subject is limited. It is still under consideration whether pro-atherogenic over-activity is the key phenomenon or whether the impairment of anti-atherogenic mechanisms triggers the vicious circle, thus propagating lesion formation. The consequences of pleiotropic disturbances are inflammation and endothelial dysfunction that lead to plaque rupture, vessel occlusion, and clinical complications. Therefore, delineating both pro- and anti-atherogenic pathways is a prerequisite for introducing effective therapies against atherosclerosis and may be a challenging objective of future research.
References


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