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—Reviews—

Risk factors and pathogenesis of atherosclerotic lesion

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Introduction

Vascular pathological changes exhibit variable features as a result of the functional and morphological differences between blood vessel types. Endothelial dysfunction induces vascular changes associated with an imbalance between vasoactive substances and vessel structure[1]. Various pathological and physiological conditions induce disturbances in the contractility and permeability associated with morphological changes[2]. The abnormal intraendothelial and interendothelial transports of circulating macromolecules may be the first step in the development of atherosclerosis[1-3]. Atherosclerosis is a major cause of the morbidity and mortality associated with myocardial infarction and cerebral bleeding[4]. Intimal thickening with lipid deposition affects the elastic properties and contributes to the hydrophobic changes that occur in the intima of arteries. The anisotropic elasticity induced by microelastic changes is associated with functional disturbances of the vascular wall[5].

In the atheromatous lesion, infiltrated macrophages, lymphocytes and platelets produce various kinds of cytokines and growth factors[6]. These cytokines and growth factors are synthesized by vascular cells such as the endothelial and smooth muscle cells and induce the inflammatory-fibroproliferative response that is associated with cell proliferation and the production of extracellular matrix (ECM)[7]. Proliferated smooth muscle cells induce local changes in the production of extracellular matrix components, and may contribute to local weakening of the fibrous cap[8]. In the review presented here we discuss the mechanisms underlying the devolopment and fragility of atherosclerosis.

1. The role of endothelial dysfunction in atherosclerosis

Normal endothelial cells synthesize prostacyclin, plasminogen activator, factor VIII including the von Willebrand factor and various other components[1]. The endothelium forms a barrier to the passage of blood constituents into the vascular wall and reveals various functional properties (**Table 1**)[1,9]. Endothelin, which is also found in the endothelium, is a potent vasoconstrictive peptide[10]. ET (endothelin)$_A$ receptors predominate in the media of both normal and diseased blood vessels, and ET_B receptors have been found on endothelial cells and macrophages[11]. Acetylcholine is known to increase the plasma endothelin concentration of patients with endothelial dysfunction, and is associated with vasoconstriction[12].

Vasoconstrictors acting on aortic smooth muscle cell receptors evoke a depolarization in the endothelial membrane potential[13]. And also increases in ET-converting enzyme-1, ET-1, and its receptor expression play an active role in the pathogenesis of neointimal thickening. Vascular endothelial growth factor (VEGF) and ET-1 have stimulatory interactions on each expression, which may play an important role in concomitant proliferation of endothelial and smooth muscle cells in the vascular wall[14].

On the other hand, nitric oxide (NO) formation by endothelial cells is enhanced by a variety of physical and chemical stimuli, among which the most important factor may be flow-induced shear stress. The in-

Table 1 Functional properties of endothelium

1. Regulation of vascular permeability
2. Anticoagulation of vascular lumen
3. Regulation of vascular tonus
4. Control of wandering and growth of vascular smooth muscle cells

tracellular cytoskeleton of the endothelium exhibits bundles in the high-shear regions, and chronic shear may induce cell injury[15]. The biological activity of NO is also decreased in early hypercholesterolemia, and oxide low-density lipoproteins have been shown to reduce the expression of endothelial NO synthase (NOS)[16].

Decreased NO activity and increased endothelial expression of the vascular cell adhesion molecule-1 gene in the vessel wall are characterisitic features of atherosclerosis[17]. The excessive production of NO by Ca^{2+}-independent enzyme in response to endotoxin may contribute to the loss of peripheral vascular tone and hypotension[18]. And NO may mediate the inflammatory events involved in the pathogenesis of atherosclerosis, and it exhibits physiological functions such as the induction of leukocyte adhesion, mitogenesis, and the proliferation of vascular smooth muscle cells[19]. NO production is generated by cytokines, and inducible NOS (iNOS) is expressed in endothelial and vascular smooth muscle cells[20,21]. Endothelial cells express intercellular adhesion molecule-1 (ICAM-1), and P-and E-selectins by the stimulatory action of the cytokines. P- and E-selectins are expressed on activated endothelium and platelets at sites of vascular injury and inflammation[22].

Endothelial cells act as a barrier to anionic substances such as albumin, lipoprotein and other substances[2,3]. In particular, the permeability of high-molecular-weight substances is enhanced when the intercellular space is widened following contraction of the endothelium, and a change in the molecular structure of the plasma membrane is induced by plasma lipids, platelets, and hemodynamic stress (**Fig 1**)[1,3].

The physical and morphological integrity of the plasma membrane, and the proteoglycans in the extracellular matrix may be influenced by hypercholesterolemia[23]. After the administration of cholesterol, pinocytotic vesicles and cytosomes are increased in both endothelial and smooth muscle cells. Tracers of various molecules may be incorporated into the vesicular system through the plasma membrane at intercellular spaces and via pinocytotic vesicles, and eventually form cytosomes[24].

Na^{+}-K^{+}-and Ca^{++}-ATPase activities have been clearly identified in the cytomembrane and mitochondria of vascular endothelial and smooth muscle cells[25-27]. As mentioned previously, the endothelial plasma membrane controls various endothelial functions, and the physical condition of the plasma membrane is influenced by its chemical composition, such as the polar head groups, fatty acids and acidic gly-

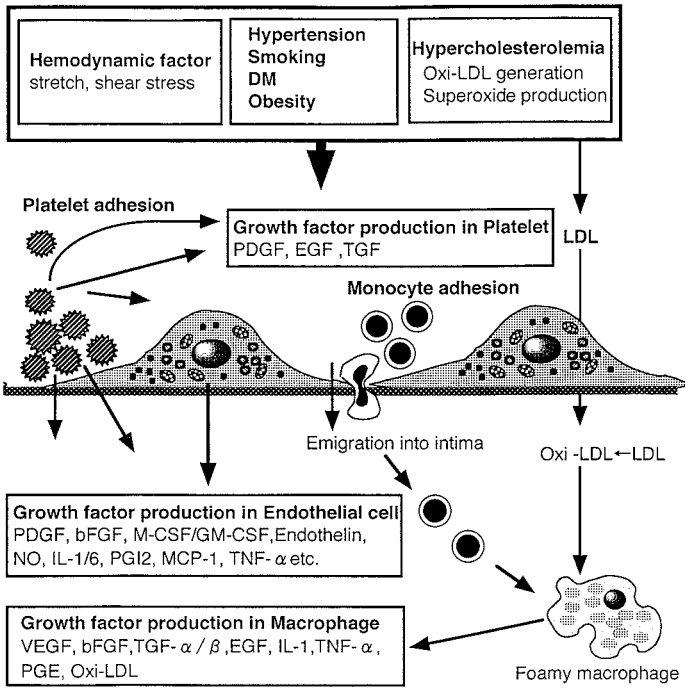

Fig. 1 Risk factors of endothelial injury and dysfunction

Table 2 Control factors in proliferation of smooth muscle cell

Adrenaline/Noradrenaline
Advanced glycosylation end product (AGE)
Angiotensin Ⅱ (A-Ⅱ)
Atrial natriuretic polypeptide (ANP)
Basic fibroblast growth factor (bFGF)
C-type natriuretic peptide (CNP)
Endothelial-derived relaxing factor-nitric oxide (EDRF-NO)
Endothelin-1 (ET-1)
Epidermal growth factor (EGF)
Fibronectin (FN)
Heparin-binding EGF-like growth factor (HB-EGF)
Heparin
Insulin-like growth factor-I (IGF-I)
Interferon-γ (IFN-γ)
Interleukin-1 (IL-1)
Interleukin-6 (IL-6)
Keratinocyte growth factor (KGF)
Laminin
Leukotriene B4 (LTB4)
Monocyte chemotactic protein 1 (MCP-1)
Osteonectin (SPARC)
Platelet-derived growth factor (PDGF)
Platelet-activating factor (PAF)
Prostacyclin (PG12)
Prostaglandin E1 (PGE1)
Serotonin
Tenascin (TN)
Thrombin
Thromboxane A sub 2 (TXA sub 2)
Transforming growth factor α (TGF-α)
Transforming growth factor β (TGF-β)
Tumor necrosis factor α (TNF-α)
Thrombospondin (TSP)
TSG-6
Type I collagen
Vasopressin

coproteins[23,28].

Histochemically, animals that were administered cholesterol exhibit a reduction in the glycocalyx on the endothelial surface and an increased distribution of proteoglycans, such as chondroitin, dermatan, and heparan sulfates, in the intima[23]. Hypercholesterolemia, which results in superoxide production, may contribute to endothelial damage and increased permeability, and is associated with an irregular distribution of proteoglycans and Na^+-K^+-ATPase[3].

2. Endothelial cell injury and proliferation of macrophages and vascular smooth muscle cells

The earliest lesion of an atherosclerotic plaque is observed as a fatty streak. In early atherosclerosis, foamy cells proliferate in the intima and systemic leukocyte activation are correlated with age, smoking and hypertensive conditions. Monocyte chemoatractic protein-1 (MCP-1) is a soluble protein that is implicated in the acute and chronic inflammatory processes of atherosclerosis[29]. MCP-1 does not merely have a chemotactic function, but also has a mitogenic effect on cultured rat vascular smooth muscle cells[30].

On the other hand, inflammatory cytokines induce hyperconstrictive responses and cause the smooth muscle cells to move toward dedifferentiation[31,32]. Depending on the anatomic location, the development and progression of atherosclerotic lesions are heterogeneous. But smooth muscle cells contribute to the formation and progression of intimal thickening in restenosis after angioplasty and in the early phase of atherosclerosis[25]. This event is due to both the proliferation and migration of SMC. And the proliferation and migration of SMC are known to be controlled by large numbers of molecules (**Table 2**). The absence of MCP-1 provides dramatic protection from macrophage recruitment and atherosclerotic lesion formation in apo B transgenic mice, without altering lipoprotein metabolism[29]. MCP-1 plays a critical role in the initiation of atherosclerosis. The invasion of phenotypically modified smooth muscle cells into the intima is the result of active migration through the internal elastic lamina (**Fig 2**)[30].

In human atherosclerotic lesions, immunohistochemical studies have revealed that smooth muscle cell myosin occurs in the endothelium, myointimal cells and foam cells[33].

Smooth muscle myosin heavy chains isoforms are important molecular markers for studying vascular smooth muscle cell differentiation[34]. At least two different phenotypes have been described for smooth muscle cells based on the distribution of myosin filaments and large amounts of secretory protein apparatus, such as rough endoplasmic reticulum (**Fig 3**)[35]. The phenotypic modulation of the vascular smooth muscle cell may induce responsiveness to growth

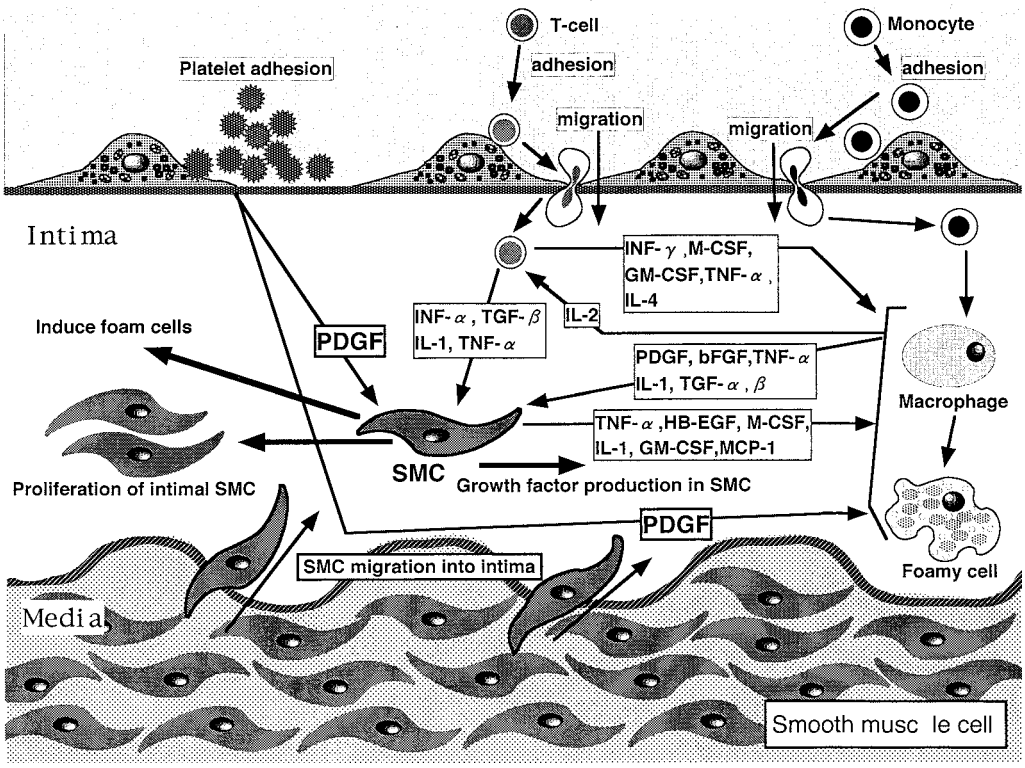

Fig. 2 Pathogenesis of atherosclerosis

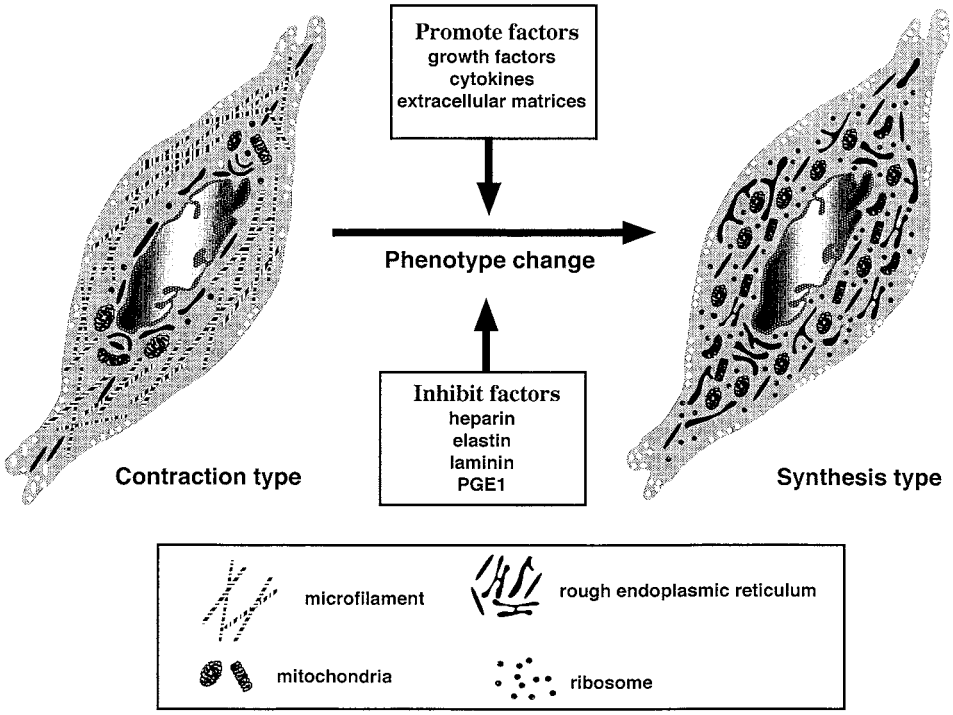

Fig. 3 Phenotypic modulation of vascular smooth muscle cell

stimulation and the synthesis of abundant extracellular matrix. Smooth muscle cells have receptors that interact with extracellular matrix components, including two elastin-binding proteins and several integrins[36]. Recent studies have focused on the regulation mechanism of smooth muscle cell-specific genes at the levels of transcription and/or alternative splicing in a phenotype-dependent manner. Typical examples of such genes include caldesmon, alpha-tropomyosin, myosin heavy chain, SM 22, calponin

and alpha 1 integrin[37]. Cell adhesion molecules and growth factors also play a critical role for controlling phenotype of smooth muscle cells via signal transduction pathways such as phosphoinositide 3-kinase and mitogen-activated protein kinases[37].

The directional character of the migration of smooth muscle cells is chemotactic rather than chemokinetic in nature. In the early stages of neointima development, vascular smooth muscle cells appear to be predominantly immature, and have the ability to shift to a more differentiated phenotype, as shown by the increased content of α-smooth muscle actin[25]. Vascular smooth muscle cell proliferation is associated with a proportional increase in the early expression of the c-fos nuclear proto-oncogene[38]. Acute hypertension and angioplasty rapidly induced MAP kinase activation in the arterial wall. Kinase activation is followed by an increase in c-fos and c-jun gene expression and enhance transcription factor AP-1 DNA-binding activity[39].

The fibrous plaque is a representative lesion for advanced atherosclerosis and is made up of increased intimal smooth muscle cells surrounded by extracellular matrix and containing variable amounts of intracellular and extracellular lipid[40,41]. For example, in patients with hyperlipidemia, monocytes will easily adhere to vascular endothelial cells, with the aid of ICAM-1 and MCP-1 produced by the endothelial cells, and the invading monocytes will undergo transformation into macrophages[42]. Macrophages that incorporate lipids will convert to foam cells. In this process, macrophages produce growth factors and cytokines such as platelet derived growth factor (PDGF) and transforming growth factor beta$_1$ (TGF-β_1)[43]. PDGF induces the migration and proliferation of vascular smooth muscle cells, and TGF-β_1 stimulates the production of extracellular matrices such as collagen, tenascin and proteoglycans[43,44].

The coronary arteries, when affected in combination by both diabetes mellitus and myocardial infarction, exhibit a reduction in the number of actin-positive smooth muscle cells together with an increase in the level of tenascin in the thickened intima[44]. These findings suggest that the expressions of tenascin in the intima of coronary arteries is a specific pathological finding that contributes to the development of myocardial infarction in diabetes mellitus[44].

Extracellular growth stimuli are transmitted into the cells via a specific receptor on the cell membrane following the induction of the relevant gene expression. Various compounds such as growth factors, cytokines, and the extracellular matrix promote smooth muscle cells growth via some receptors in the intima of the aorta[45]. Protein phosphorylation is known to play an integral role in this intracellular signal transduction process. Receptors that are involved in cell growth are categorized into two types. One type is associated with tyrosine phosphorylation, while the other is associated with G-protein coupling[36]. Conditioned medium obtained from injured vascular smooth muscle cells is known to be highly mitogenic, and this activity is due to multiple growth factors interacting synergistically[46]. Growth factor-stimulated up-regulation of IGF-1 receptors is critical in mediating the proliferative responses to multiple growth factors[45].

The mesenchymal components, comprising elastic and collagen fibers, vary depending upon the blood vessel type. Atherosclerosis shows organ specificity and its effect is different according to the angiospastic potential of each artery[4]. Within the same species, there are variations in endothelial cell function, depending not only on the vascular bed of origin, but also on the size of the vessel within the same vascular territory. Endothelial differences have been observed in phenotype, antigen expression, cell size and growth, secretory function, and G-protein expression[47].

3. The role of advanced glycosylation end products and morphological specificity in diabetic angiopathy

Blood serum from streptozotocin (STZ)-treated rats promotes vascular smooth muscle cell proliferation, and the protein synthesis of bFGFR, and IGF-I are increased in the vascular smooth muscle cells. These results suggest that the receptors of growth factors induced by STZ-treated rat serum contribute to the cell proliferation of vascular smooth muscle cells in autocrine and paracrine patterns[45].

In advanced hyperglycemia, proteins that have undergone Maillard's reaction, in vivo, are called ad-

vanced glycosylation end-products (AGE). AGE are found not only in the extracellular matrix but also in the foam cells originating from macrophages, endothelial and smooth muscle cells in sclerotic lesions. Immunohistochemically, iNOS and AGE are localized in the coronary artery of STZ-treated Mongolian Gerbils[48]. High glucose treatment leads to severe changes in endothelial and medial cells, and also increases ecNOS gene expression in vascular cells. The interaction of AGE with RAGE in the endothelial cell membrane produces active oxygen during glycosylation, and this reaction is thought to enhance injury to smooth muscle cells in the media[49]. STZ treatment induces the expression of AGE and their receptor (RAGE) mRNA in the endothelial and smooth muscle cells of aorta[50].

The activation of macrophages and smooth muscle cells is thought to be associated with AGE. The accumulation of AGE might initiate diabetic macroangiopathy through RAGE, and the increase in RAGE expression by endothelial cells could be a reason that diabetes mellitus causes a rapid acceleration of atherosclerosis[50]. The interaction of cellular RAGE with its ligands could be a factor contributing to a range of important chronic disorders. AGE are usually found in proteins with a relatively longer half-life, such as collagen, lens crystallin, and myelin, in patients with diabetes mellitus[51]. Cell proliferation and collagen gene expression may be under separate biological controls during the development and evolution of human atherosclerosis[52]. The balance between proteolysis and matrix synthesis may influence both the stability of atheromatous plaques and the development of restenotic lesions[53]. Collagens with AGE show alterations in ligand binding and increased resistance to the actions of proteinase such as pepsin[54].

On the other hand, AGE is characterized by autofluorescence, and ceroid is a known autofluorescent substance that is present in atherosclerotic lesions. When viewed with the aid of fluorescence microscopy, extracellular autofluorescence materials in the intima are arranged into ring-shaped structures[55]. These materials are mostly encountered in atheromatous plaques. Foamy cells aggregate in the peripheral region of atheroma and usually have multiple fine autofluorescent granules in their cytoplasm. Tissues taken from patients with diabetes mellitus show a high ratio of autofluorescence area compared with that of non-diabetic groups. The heterogeneous localization of AGEs in atherosclerotic lesions depends on their different epitopes, and a close link, exists between the peroxidation of LDL and the formation of AGEs in atherosclerotic lesions[56].

4. Risk factors of atheromatous plaque rupture for acute myocardial infarction

Atherosclerosis develops via a complex interaction between blood elements and vessel wall abnormalities. Atheromatous plaques consist of the intraarterial accumulation of varying quantities of intracellular and extracellular lipids, macrophages, T lymphocytes, smooth muscle cells, extracellular matrix, calcium and necrotic debris[57]. Inflammatory changes with increased endothelial permeability, endothelial activation, and monocyte recruitment are confirmed in this lesion[29,58]. In addition, cell growth, with vascular smooth muscle cell proliferation and matrix synthesis is noted along with lipid accumulation[59]. Abnormal proliferation of vascular smooth muscle cells is thought to contribute to neointimal thickening during spontaneous atheroscleosis and vessel narrowing. Advanced atherosclerotic plaques contain a necrotic core and extracellular matrix proteins including fibrin, with lipids and some calcification[5].Tissue necrosis factor α (TNFα) is also a prominent constituent that is associated with the foamy macrophages found adjacent to cholesterol clefts[60].

The surface of the atherosclerotic plaque is strongly associated with the frequency of superficial inflammation, and results either in mural thrombosis with subsequent growth of the plaque or in a thrombotic occlusion as in unstable angina[57]. Deep plaque ulceration expose collagen and TNFα, leading to thrombotic occlusion and myocardial infarction[60]. The development of an intraluminal thrombus is the important dynamic process unifying angina pectoris, acute myocardial infarction, and sudden ischemic death[61].

Angiotensin-type-I-receptor-positive vascular smooth muscle cells with exerberant inflammation of the atheromatous plaque may enhance vasoconstriction by the vascular smooth muscle cells and may induce the rupture of the fibrous cap[62]. On the other hand, a local dynamic process of plasminogen activator-

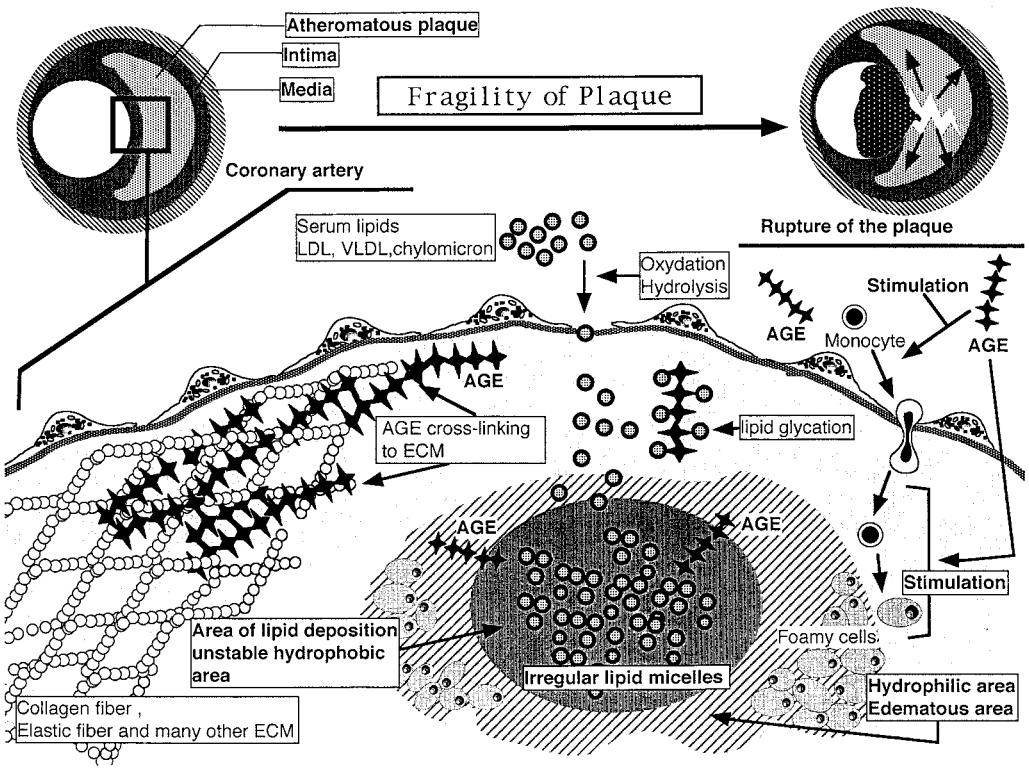

Fig. 4 Pathologic basis of atherosclerotic plaque rupture

dependent proteolysis has been noted in atheromatous lesions associated with macrophages and vascular smooth muscle cells[63].

Fukumoto reported that apoptosis exhibits nuclear and cytoplasmic condensation in vascular smooth muscle cells and macrophages and is an important mechanism in the regulation of the evolution of intimal thickening[44, 63].

Machida used fluorescence spectroscopic and histochemical techniques to evaluate the specificity of atherosclerotic lesions in the human aorta. In atherosclerotic lesions, the emission spectrum of the fluorescent dye hematoporphyrin (Hp) exhibits a sharp peak at around 620 nm, and a second peak in the range 640 ～750 nm. HP fluorescence is localized in the region of cell proliferation, around cholesterol micelles and damaged cells. Where the intima is thickened, the second peaks are increased in damaged cells, degenerative fibers and the extracellular matrix[64].

Prior to the increase in the cholesterol content of the plaque, a change in the water content, the supersaturation of lipids in the cell membrane, and a decrease in the pH are observed. Under these conditions, the cell membrane may easily become dysfunctional and destroy[40, 65, 66]. It is suggested that an unstable hydrophobic interaction may promote the formation of irregular lipid micelles and induce the instability and fragility of the plaque in coronary arteries (**Fig 4**).

Intimal thickening with lipid deposition affects the elastic properties and may contribute to the hydrophobic changes that occur in the intima of coronary arteries. This hydrophobicity between water and lipid micelles in the plaque is disturbed. It is suggested that the unstable hydrophobic interaction may promote the formation of irregular lipid micelles and induce the instability and fragility of the plaque[41].

Recent studies using immunocytochemical techniques have shown that the unstable atherosclerotic plaque contains a dense inflammatory infiltrate, composed mainly of macrophages, T lymphocytes, and, mast cells. The plaque complications, such as plaque ruptures and surface erosions, may always relate to inflammatory changes[43, 44, 67, 68].

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