SCIENTIFIC SECTION

Review Article

Smooth muscle and endothelial cell function in the pathogenesis of atherosclerosis

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Although clinical studies have been very useful in identifying factors that accelerate the development of atherosclerotic vascular disease, the pathogenesis of the vascular lesions remains unknown. Studies carried out in the last 10 years have shown that smooth muscle and endothelial cells of the vascular wall play a very important role in atherogenesis. It has become apparent that these cells are very active metabolically during the initiation and subsequent growth of the plaques, and that the materials that these cells produce and secrete are important in the composition and growth of the plaques. In addition, there are important interactions at the vessel wall–blood interface that involve endothelial cells, hemodynamic forces and many constituents of the blood, including platelets, lipoproteins, coagulation factors, fibrinolytic agents and leukocytes. In this article the numerous functions of both smooth muscle and endothelial cells are discussed and the effects of known atherogenic agents on these cellular functions are reviewed. Emphasis is placed on the important interactions that take place both within the vessel wall and at the vessel wall–blood interface. Understanding of the regulation of smooth muscle and endothelial cell function during the development and subsequent growth of fibrofatty plaques may be useful in designing appropriate therapeutic interventions to control atherosclerotic disease.

Bien que les études cliniques aient été fort utiles pour identifier les facteurs qui accélèrent le développement de l'athérosclérose vasculaire, la pathogénèse des lésions vasculaires demeure inconnue. Des études menées au cours des 10 dernières années ont montré que les cellules musculaires lisses et endothéliales de la paroi vasculaire jouent un rôle très important dans l'athérogenèse. Il est devenu manifeste que le métabolisme de ces cellules est très actif pendant la formation et la croissance subséquente des plaques, et que les matériaux produits et sécrétés par ces cellules entrent de façon importante dans la composition des plaques et participent à leur croissance. De plus, à l'interface de la paroi vasculaire et du sang il survient d'importantes interactions entre les cellules endothéliales, les forces hémodynamiques et plusieurs constituants du sang, dont les plaquettes, les lipoprotéines, les facteurs de coagulation, les agents fibrinolytiques et les leucocytes. Dans cet article on discute les nombreuses fonctions des cellules musculaires lisses et endothéliales, et on passe en revue les effets des agents athérogènes connus sur ces fonctions cellulaires. On souligne les importantes interactions qui s'exercent à l'intérieur de la paroi vasculaire tout comme à l'interface de la paroi vasculaire et du sang. La connaissance de la régulation des fonctions des cellules musculaires lisses et endothéliales durant la formation et la croissance subséquente des plaques fibro-lipidiques peut être utile à l'élaboration d'interventions thérapeutiques appropriées destinées à juguler l'athérosclérose.

Atherosclerosis is the leading cause of death in the Western World. Fibrofatty plaques (atheromas) result in serious damage to the arterial wall and severe narrowing of the vascular lumen (Fig. 1). These pathologic changes in the aortic and arterial wall of large-sized and medium-sized elastic and muscular arteries cause several disease entities, including ischemic heart disease, cerebral vascular disease, peripheral vascular disease, and aortic aneurysm and rupture.

Although much investigative work has been carried out on the effects of blood constituents, particularly platelets, and hemodynamic forces on atherosclerosis, the pathogenesis of the fibrofatty plaque is unknown. Clinical, epidemiologic and experimental studies have been helpful in identifying factors that predispose individuals to accelerated or premature atherosclerosis. Thus, the mainstay of the treatment of atherosclerosis and its ensuing complications has involved efforts to reduce or eliminate risk factors by such maneuvers as the early identification and treatment of hypertension and the campaign to encourage people to stop smoking.

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Therapy has also been directed at agents in the blood that appeared to promote the complications of atherosclerosis; lipid-lowering regimens,[1] anticoagulants[2,3] and more recently antiplatelet drugs have been used.[4,5] The benefits have not been dramatic, probably because the mechanisms involved in the initiation and growth of fibrofatty plaques are much more complex than initial studies indicated. Studies using in vivo animal model systems and, more recently, tissue culture systems of vascular wall cells — pure cultures of endothelial[6] and smooth muscle cells[7] — are now providing insight into the complex activities that occur both within the vessel wall and at the interface between the blood and the vessel wall. This review will discuss some of the data in support of the hypothesis that the smooth muscle and endothelial cells of the vessel wall play a very important role in controlling many processes, both within the wall and in the blood, that act in the initiation and growth of the atherosclerotic fibrofatty plaque.

Smooth muscle cells

Structure of the noninjured medial smooth muscle cell

In a normal noninjured vessel the smooth muscle cell has a very slow mitotic rate. Since its prime function is contraction, such a cell (Fig. 2) has a highly developed contractile system made up of actin filaments (5 to 8 nm thick) surrounding myosin-containing filaments (12 to 18 nm thick).[7] Although the way in which these cells contract has not been defined, as it has been for skeletal muscle cells, the small patches of electron density, called dense bodies, associated with the bundles of myofilaments are thought to be part of the contractile system, analogous to the thin filaments inserted into the Z disk of striated muscle. Small amounts of rough endoplasmic reticulum, free ribosomes and Golgi complexes are located primarily at the nuclear poles of the cells[8] and are associated with the synthesis of extracellular matrix, including collagen, elastin and glycosaminoglycans.[7] This synthesis occurs at very low levels in the media of noninjured vessels. Along the plasmalemma of the medial smooth muscle cell are vesicles that may function as calcium-accumulating structures. In addition, coated vesicles containing mildly electron-dense material are present in the plasma membrane and have been associated with the internalization of low-density lipoproteins.[9] A basement membrane surrounds each smooth muscle cell, and the cells communicate with their neighbours via gap junctions.[7]

Smooth muscle cells in fibrofatty plaques

The many functions of medial smooth muscle cells are important in the pathogenesis of atherosclerosis. The pioneering studies of Movat and colleagues[10] and of Geer and Haust[8] in the late 1950s showed that the cells populating the atherosclerotic plaques were indeed smooth muscle cells derived from the underlying media. Since then it has been shown that these cells are responsible for organizing surface thrombi that form on atherosclerotic plaques and that they are one source of the foam cells seen in plaques.[8]

Ultrastructural observation of smooth muscle cells in developing atherosclerotic plaques showed that many of these cells had very well developed rough endoplasmic reticulum and Golgi complexes and few myofilaments (Fig. 3), unlike the typical medial smooth muscle cell of the uninjured wall, which contains mainly myofilaments and few organelles. This difference represents a change

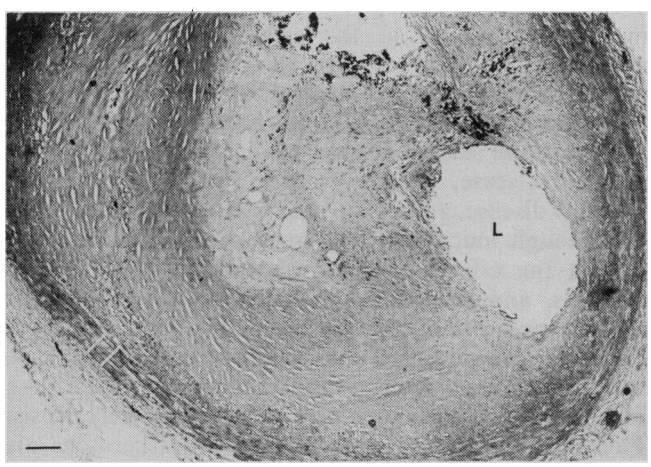

FIG. 1—Atherosclerotic plaque in coronary artery with severe narrowing of lumen (L). Lines in media indicate original wall thickness (hematoxylin–phloxine–saffron × 55). Bar is 0.18 mm long.

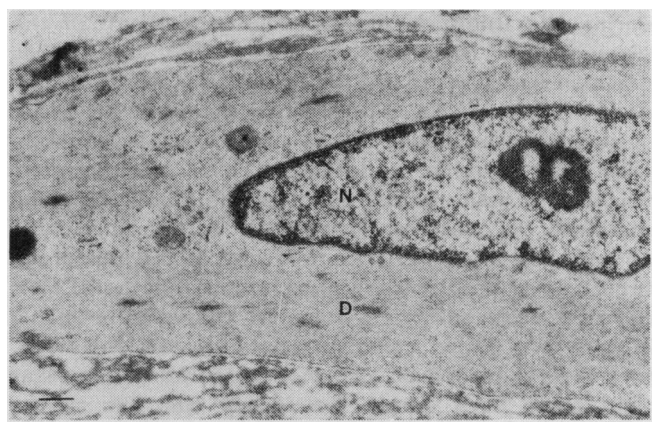

FIG. 2—Medial smooth muscle cell (N = nucleus) from noninjured aorta. This contractile cell contains numerous myofilaments (arrows) and dense bodies (D) (× 28 000). Bar is 0.036 μm long.

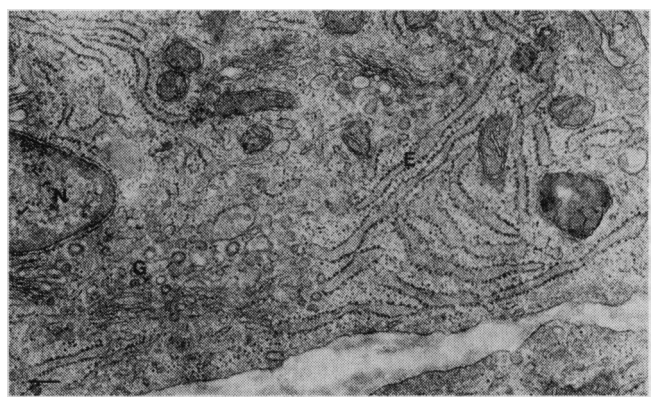

FIG. 3—Part of smooth muscle cell from atherosclerotic plaque that has undergone phenotypic change and contains numerous organelles, including endoplasmic reticulum (E) and Golgi complexes (G) (× 37 000). Bar is 0.027 μm long.

in the phenotype of the cell from one that contracts to one that readily undergoes proliferation and that synthesizes large amounts of connective tissue and glycosaminoglycans. In the contractile phenotypic state a smooth muscle cell is not stimulated to divide by mitogenic factors associated with vascular injury, such as platelet-derived growth factor (PDGF) or hyperlipemic low-density lipoprotein (LDL). However, once the cell loses its ability to contract it responds to these mitogens. Preliminary experiments suggest that neither plasma nor platelet-derived factors are involved in initiating this phenotypic change.[11]

Thus, medial smooth muscle cells respond to atherogenic injuries by undergoing a phenotypic change that results in the cells' being able to readily migrate and proliferate, and to synthesize and secrete many of the components of the fibrofatty plaque matrix.

Regulation of smooth muscle cell migration: Smooth muscle cells from the underlying media respond to vascular injury by migrating from the media into the intima.[8] Tissue culture systems are now being used to study the regulation of this migration.[12-14] In vitro studies have shown that although the movement of endothelial and smooth muscle cells takes place in the same general environment of the injured vessel wall the patterns of migration of these two types of cells are quite different: platelets that adhere to sites of injured, denuded endothelium may contain factors that influence the migration of endothelial and smooth muscle cells,[12-14] and both types of cell migrate more slowly in vitro when incubated with serum from blood that has clotted after platelet removal.[14]

Synthesis of fibrofatty plaque matrix: Both in vivo and in vitro studies have shown that smooth muscle cells synthesize the connective tissue elements that are identified in atherosclerotic plaques.[7] The plaque cells secrete large amounts of connective tissue material, some of which appears to be either qualitatively or quantitatively different from the connective tissue elements synthesized and secreted by medial smooth muscle cells in noninjured vessel walls. In fact, data on elastin show that there is an increase in elastin-containing polar amino acids in atherosclerotic plaques that may permit more binding of calcium and then of lipids.[15] Changes in the total content and in the relative amounts of individual glycosaminoglycans have been observed during both induction and regression of atherosclerosis in nonhuman primates,[16] as well as in atherosclerosis in humans.[17] In addition, glycosaminoglycans have been shown to bind to certain lipoproteins.[17] Thus, the complex interactions occurring in the vessel wall could promote fibrofatty plaque development through changes in the types of substances synthesized and secreted by smooth muscle cells in the injured aortic or arterial wall.

Proliferation of smooth muscle cells: Investigators have attempted to identify the agents that regulate the proliferation of smooth muscle cells associated with the pathogenesis of atherosclerotic plaques. Platelets and lipoproteins were studied since they are likely to be present at the site of arterial injury and have been associated with the pathogenesis of the fibrofatty plaque.

Ross and associates[18] characterized a factor that was mitogenic for smooth muscle cells in culture: PDGF is a low-molecular-weight polypeptide that is localized in platelet α-granules and released when platelets aggregate. The Ross group and several others have shown that this factor can promote endocytosis, increase cholesterol synthesis and stimulate protein synthesis as part of a general increase in cellular protein synthesis in smooth muscle cells in vitro.[18]

Smooth muscle cells, like other mammalian cells, take up LDL-cholesterol through the LDL-receptor pathway,[9] which itself can be regulated by the availability of cholesterol to the cell and by the needs of the cell for this substance. LDL is bound to the cell by a specific cell-surface receptor and is subsequently taken up by the cell. This leads to stimulation of the enzymatic esterification of cholesterol in the cell and to a suppression of cholesterol synthesis in the cell. In this manner cholesterol metabolism is finely regulated. Since hypercholesterolemia is a definite risk factor for the development of atherosclerosis it is important to study the capacity of these lipids to make smooth muscle cells proliferate. Hyperlipemic serum, as well as the LDL fraction of normolipemic serum, stimulates the growth of smooth muscle cells.[19] Hyperlipemic LDL is mitogenic for smooth muscle cells, independent of any effect of PDGF,[11] which suggests that these two mitogens may act on different intracellular mitogenic pathways.

Because of the relation of diabetes to atherosclerosis, studies were carried out on the effect of diabetic serum[20] and insulin on smooth muscle cell proliferation. The proliferative effect of the former may be related to increased serum levels of growth hormone, while insulin appears to be at least a cofactor in growth.[21]

Recently it has been shown that endothelial cells secrete a protein growth factor that is different from PDGF but also stimulates the growth of smooth muscle cells.[22] Macrophages have also been shown to secrete a factor that promotes the growth of smooth muscle cells.[23] Endothelial cells secrete a heparin-like molecule that inhibits the growth of smooth muscle cells.[24] Thus, the regulation of the proliferative activity of smooth muscle cells appears to be complex, involving several important factors. The proliferative activity in the developing fibrofatty plaque is self-limiting; that is, the proliferation stops at a time that coincides, generally, with the re-endothelialization of the injured area. If no further damage occurs after a single mild initial injury the proliferative and synthetic activities of smooth muscle cells return to normal and the lesion regresses.[25]

Endothelial cells

The endothelium has been known to function both as a thromboresistant surface and as a regulating barrier for the transfer of macromolecules into the vessel wall. In the last few years it has become apparent that the importance of endothelial cells, both in health and in disease, is due largely to their metabolic activity and to their ability to synthesize biologic substances that interact with both the vessel wall and the constituents of the blood.[26] Injury to the endothelium, characterized

either by functional abnormalities or by actual cell loss, will promote the initiation and growth of fibrofatty plaque.

Endothelial injury in fibrofatty plaque formation

The importance of maintaining the integrity of the aortic endothelium has been a prime consideration in atherosclerosis research. It has been known for several years that areas of the aorta that are more prone to atherosclerosis have a higher rate of endothelial cell turnover.[27] It was felt that this indicated recurrent episodes of desquamation in these areas due to hemodynamically induced injury that were resulting in the progressive growth of the fibrofatty plaque. Increased cell turnover has also been reported in experimentally induced hypertension[28] and hyperlipidemia,[29] conditions associated with atherosclerosis. However, the true nature of the endothelial injury in human atherosclerosis is still not known.

Role of endothelial cells in preventing platelet and fibrin deposition during the genesis of fibrofatty plaques

Since both platelet adherence to injured subendothelium and formation of mural thrombi on atherosclerotic plaques have been shown to be important in the initiation and growth of fibrofatty plaques (Fig. 4),[30] studies have focused on identifying the factors that normally make the endothelium thromboresistant.

Thromboresistance had initially been attributed to the glycocalyx, the carbohydrate-rich cell coat that is present on the luminal surface of vascular endothelium.[31] It was thought that negative charges both on platelets and on endothelial cells resulted in the repulsion of platelets. As well, since α_2-macroglobulin was located on the luminal side of endothelial cells, and since it inhibits a number of proteases associated with coagulation and fibrinolysis, it was suggested that it may be associated with thromboresistance.[32] Furthermore, type V (AB_2) collagen appears on the surface of endothelial cells. Since it does not promote platelet aggregation it was suggested that this type of surface collagen may indeed be associated with thromboresistance.[33] These three suggestions, although interesting, have not received confirmation.

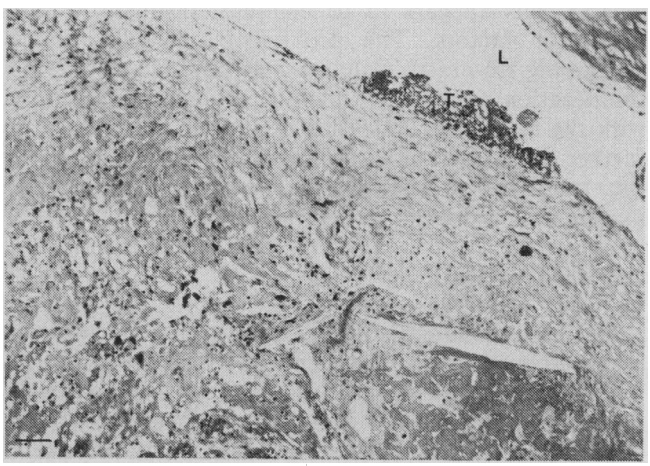

FIG. 4—Part of an atherosclerotic plaque with small mural thrombus (T) on luminal surface (L = lumen) becoming incorporated into plaque (hematoxylin–phloxine–saffron × 143). Bar is 0.069 mm long.

Exciting work on prostacyclin (PGI_2)[34] has shown that this prostaglandin is a potent inhibitor of platelet aggregation and that it promotes relaxation of blood vessels. It is synthesized in relatively large amounts in the intimal portion of various vessels, including the human aorta. Could this prostaglandin synthesized by endothelial cells be the elusive "thromboresistance factor"? It is likely that PGI_2 plays a role in thromboresistance, but how important that role is, especially as it pertains to the development of atherosclerosis, remains to be seen.

Recent studies have shown how the coagulation system also interacts with the functions of endothelial cells that relate to thromboresistance. Thrombin, the important coagulation factor, is a very potent aggregator of platelets that causes the release of biologic agents from platelets. Endothelial cells also have receptors for thrombin, and it appears that thrombin stimulates PGI_2 release from vascular endothelium.[35] To make matters more complex, endothelial cells are also associated with the coagulation system since they provide procoagulant substances.[36] Recently it has also been shown that the surface of vascular endothelial cells provides a cofactor that accelerates the thrombin-catalyzed activation of protein C, itself a potent and specific inhibitor of coagulation.[37]

Another important function in maintaining thromboresistance is the production by endothelial cells of a plasminogen activator that promotes fibrinolysis. Tissue culture studies have been most useful in investigating the properties of this important biologic function since the blood and the vessel wall have fibrinolytic activators and inhibitors that make definitive in vivo studies difficult at present.[38] Thus, it appears that vascular endothelial cells are likely to be very important in regulating the thromboresistant state by a variety of mechanisms that act directly on both platelets and the coagulation system. When the thromboresistance mechanisms are interfered with in those cells, platelet adherence and thrombus formation are likely to occur and to play their role in the initiation and growth of atherosclerotic plaques.

Endothelial repair following vascular injury

Following endothelial injury endothelial cells migrate and proliferate. When large areas of endothelium are lost, adjacent cells migrate into the denuded area. The proliferation of endothelial cells is less dependent on growth factors than that of smooth muscle cells. In response to small injuries endothelial cells flatten and spread into the adjacent area of denudation, which may be one to two cells wide. In this way small areas of denuded endothelium can be rapidly covered[39] even before cell proliferation occurs. Since it is likely that some human lesions may be initiated at sites where hemodynamic injuries result in the loss of a few endothelial cells, an in vitro model has been used to study the factors that control the spread of endothelial cells.[40] The spread is correlated with changes in the distribution of cytoskeletal fibres within the cell,[41] and the cells spread more slowly in platelet-poor plasma

serum than in whole blood serum, the former being devoid of platelet releasate material.[40] Thus, it is possible that platelets contain a factor that enhances the ability of endothelial cells to spread and rapidly cover small areas of denuded endothelium.

Some very interesting data have been published[42,43] that show that after in vivo injury of the aorta by a balloon catheter the areas of aortic wall that had re-endothelialized contained more lipid than the areas that remained de-endothelialized. This was true in both normocholesterolemic and hypercholesterolemic animals. Work is going on as well to study the amount and types of glycosaminoglycans present in these areas. The important conclusion from these studies is that even in the presence of regenerated endothelium there appear to be changes in the metabolic activity of the vascular wall.

Effect of hemodynamic factors on endothelial cells

It has been known for several years that in areas where laminar flow is disrupted there is an increased likelihood of atherosclerotic plaques.[29] It is not known whether the damage in humans is due to high-shear or, indeed, low-shear stresses on the endothelium. What is known is that endothelial cells respond to flow in many ways.[44] Shear stresses can increase endothelial permeability; acute shear stresses can denude endothelium. Both the orientation and the shape of endothelial cells can be changed by altering blood flow: these cells will realign in the direction of flow after their orientation has been altered. In addition, endothelial cells are oriented with their long axis in the direction of blood flow except in the aortic arch and at branch points. In the former the cells are more rounded and appear to have lost their ellipsoid shape, while in the latter there is no preferred orientation. Thus, hemodynamic forces may indeed play a role in determining the shape of these cells and, perhaps, in some way, in regulating their repair.

Conclusion

Although the precise role of smooth muscle and endothelial cells in the pathogenesis of atherosclerosis is still unknown, several cellular functions are currently being studied that relate to this process. It now appears that there is a very complex interaction between the vessel wall, blood constituents and hemodynamic forces (Fig. 5). The important components of the vessel wall are the endothelial and smooth muscle cells. The former synthesize and secrete numerous substances that affect platelets, coagulation factors and the function of the latter. The smooth muscle cells produce the important elements of the fibrofatty plaque matrix — collagen, elastin and glycosaminoglycans. Lipids in the wall are derived from the blood and synthesized by smooth muscle cells. The blood constituents that appear to be important in fibrofatty plaque development are platelets and their biologically active substances, coagulation factors, lipoproteins and monocytes. Hemodynamic stresses on the wall, especially on endothelial cells, occur when there is a loss of laminar flow, resulting in turbulence or stasis. To date only a few of the findings in experimental systems have been confirmed in humans. It is most likely, however, that the same processes occur in patients with atherosclerosis and that studies focusing on the interactions at the blood vessel–wall interface will allow us to begin to understand the complex function of smooth muscle and endothelial cells in the pathogenesis of the atherosclerotic plaque.

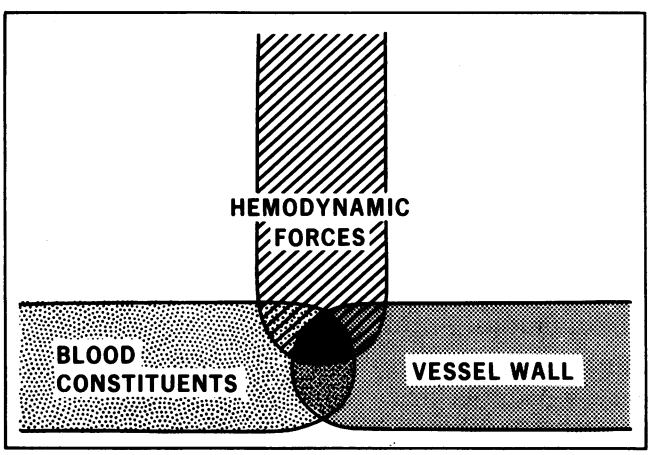

FIG. 5—Interaction of hemodynamic forces, blood constituents and vessel wall in pathogenesis of fibrofatty atherosclerotic plaque.

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