The Endothelium of Advanced Arteriosclerotic Plaques in Humans

Karl-Friedrich Bürrig

The functional morphology of the endothelial cells (ECs) covering advanced but uncomplicated sclerotic plaques in humans was studied in carotid endarterectomy specimens and in coronary arteries from hearts explanted because of advanced ischemic heart disease. The endothelial layer was nearly always intact, and the endothelial patterns reflected the anticipated local flow patterns along the narrowed arteries, with the majority of flow irregularities downstream from the stenosis. Large (giant) ECs (defined as ECs with a surface area of ≥800 μm²) were frequently found on the plaque surface, probably indicating accelerated EC senescence attributable to sustained nondenuding injury in the region of disturbed flow. Ultrastructurally, activation of ECs with hyperplasia of organelles was frequent. In addition, as a sign of immunological activation, about 5% of ECs express class II antigens (HLA-DR and rarely focal HLA-DQ), as demonstrated by double immunofluorescence with von Willebrand factor to identify the ECs. EC activation may be responsible for adherence to the intact luminal surface by activated platelets and monocytes, which were always present (in contrast with nonsclerotic artery segments). Furthermore, an increase in myo-endothelial contacts to subendothelial modified smooth muscle cells was a regular feature of the sclerotic lesions; this feature represents an unknown process of EC and smooth muscle cell interaction in the sclerotic lesion and may be a compensatory process for EC control of smooth muscle cell proliferation. In advanced plaques the ECs are altered without denudation but with changed properties, which may contribute to plaque growth and which are consistent with the postulated EC dysfunction in the pathogenesis of arteriosclerotic lesions. (Arteriosclerosis and Thrombosis 1991;11:1678–1689)

Human arteriosclerosis is a long-standing chronic disease, and the process that leads to the initiation of the disease may likewise be responsible for the progression of plaques, which are known to grow over quite a lengthy period, as seen in longitudinal Doppler sonographic studies of the human carotid bifurcation. Endothelial injury or dysfunction is considered to be a key event in the evolution of arteriosclerotic plaques, and a body of literature has accumulated concerning endothelial alterations during the pathogenesis of arteriosclerosis (for a review see References 2 and 3). However, the morphological features of endothelial cells (ECs) during the development of sclerotic lesions have been studied almost exclusively in animal models, whereas little attention has been paid to the structural details of the endothelium covering human sclerotic plaques. The study of such human tissue, however, may provide evidence for or against hypotheses concerning the pathogenesis of human arteriosclerosis that are drawn from experimental studies.

Methods

Procurement of Tissue

Advanced arteriosclerotic plaques (fibrolipid plaques or atheromatous plaques) lacking complications (such as extensive calcification or thrombosis) from 28 carotid endarterectomies and 15 coronary artery plaques were investigated by electron microscopy (Table 1). The coronary arteries were taken from explanted hearts of eight patients who underwent cardiac transplantation because of advanced ischemic heart disease. The percentage of stenosis in coronary artery lesions was estimated by planimetric measurement of photographs of arterial cross sections (Figures 2 and 3). Nonlesional areas of these specimens were likewise studied.

Glutaraldehyde (2.5%) buffered in 0.2 M cacodylate (pH 7.2, 320 mosm/l) was used as a standard...
The endarterectomy specimens were pinned on cork plates and fixed by immersion. The explanted hearts were perfused with the fixative via the cannulated coronary arteries (about 30 minutes' delay from explantation to the start of the perfusion) at a constant pressure of 120 mm Hg (measured in the perfusion system) for 15 minutes. (The smooth contour of the internal elastic lamina of nonlesional areas of the coronaries was used as a control of the quality of the perfusion.) The coronary arteries were then dissected from the epicardial fat tissue and cut stepwise in cross sections. Segments with plaques were removed and pinned on cork plates for further fixation by immersion. Flow direction was marked by an incision at the proximal site.

For immunoscanning electron microscopy, seven carotid endarterectomy specimens were treated by a modified technique with a mixture of 4% formalin and 0.5% glutaraldehyde as the fixative.

Serial frozen sections with a thickness of about 5 μm were obtained from another 10 endarterectomy specimens, mounted on poly-L-lysine-coated coverslips, fixed in acetone for 15 seconds, and stored at −80°C until used.

Sections of paraffin-embedded tissue (hematoxylin and eosin, resorcin-fuchsin) were available in all cases. A total of 53 plaques were obtained from 46 patients, who ranged in age from 26 to 76 years (median, 60 years; 21 women, 25 men).

Electron Microscopy

For transmission electron microscopy, small blocks were taken from the surface of the fixed plaques, washed in buffer, postfixed in 1% OsO₄, dehydrated in graded ethanol and propylene oxide, and embedded in Epon 812. Five to 10 blocks were studied in each case, and semithin sections were stained with toluidine blue. Ultrathin sections were doubly contrasted with 0.5% aqueous uranyl acetate and 30% lead citrate and viewed with a Zeiss 109 transmission electron microscope.

For scanning electron microscopy, samples with a maximum length of 10 mm were critical-point dried (Polaron E 3100) in CO₂ after dehydration in graded ethanol and acetone; they were then mounted on aluminum stubs, coated with palladium–gold (Cool Sputter Coater E5100, Polaron), and examined with a Philips 515 scanning electron microscope. In addition, some of these samples were reembedded with a mixture of propylene oxide and Epon 812 at a 1:1 ratio in Epon 812 for transmission electron microscopy.

Immunoscanning Electron Microscopy

After the samples were washed in phosphate-buffered saline (PBS), they were incubated with normal porcine serum at ambient temperature for 30 minutes. They were then transferred to small flasks, and the primary monoclonal mouse anti-human antibody (leukocytic common antigen; see Table 2) was applied to the entire mount for 45 minutes at room temperature. After intervening washes with PBS, the samples were then incubated with Dynabeads M-450 (Dynal, Oslo, Norway) for 45 minutes at a dilution of 1:30. During incubation, the flasks with the samples were shaken gently to prevent sedimentation of the beads, which were used as the detection system. These beads were conjugated with goat anti-mouse immunoglobulin (Ig G and had a diameter of 4.5 μm (Figure 9). After postfixation with 1% OsO₄, the scanning electron microscopy technique described was used.

Immunohistochemistry

Immunohistochemistry was performed on the frozen acetone-fixed sections. For the indirect immunoperoxidase technique, the sections were transferred from 96% ethanol to a mixture of 3% H₂O₂ and methanol at a 1:4 ratio for 10 minutes. After washes in PBS, the sections were incubated with normal porcine serum at ambient temperature for 20 minutes. The primary antibody was applied overnight at 4°C (see Table 2). After the sections were washed in PBS, they were incubated for 30 minutes with the secondary peroxidase-conjugated anti-mouse or antirabbit antibody. The peroxidase reaction was developed with 0.1 M 3,3′-diaminobenzidine (Sigma Chemical Co., St. Louis, Mo.) in PBS (5 minutes). The sections were counterstained with hemalaun and dehydrated in graded ethanol, and then mounting media and coverslips were applied.

For the double-immunofluorescence technique, the frozen sections were warmed to ambient temperature, fixed in acetone for 10 minutes, and air dried. They

<table>
<thead>
<tr>
<th>Method</th>
<th>Carotid artery</th>
<th>Coronary artery</th>
<th>No. of sections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transmission EM</td>
<td>14</td>
<td>15</td>
<td>~450</td>
</tr>
<tr>
<td>Scanning EM</td>
<td>17</td>
<td>15</td>
<td>~700</td>
</tr>
<tr>
<td>Immunoscanning EM</td>
<td>7</td>
<td>ND</td>
<td>~100</td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td>10</td>
<td>ND</td>
<td>180</td>
</tr>
<tr>
<td>Histology</td>
<td>31</td>
<td>22</td>
<td>106</td>
</tr>
</tbody>
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EM, electron microscope; ND, not done.

**TABLE 1. Location and Number of Sclerotic Plaques (N=53) Studied With Different Morphological Methods**

<table>
<thead>
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<td>Histology</td>
<td>31</td>
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<td>106</td>
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</table>

**TABLE 2. Antibodies Applied to Antigens Listed**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Dilution</th>
<th>Source</th>
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</thead>
<tbody>
<tr>
<td>von Willebrand factor</td>
<td>1:300</td>
<td>DAKO*</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>1:10</td>
<td>Becton Dickinson†</td>
</tr>
<tr>
<td>L243 (IgA)</td>
<td>1:10</td>
<td>ATCC†</td>
</tr>
<tr>
<td>HLA-DQ</td>
<td>1:10</td>
<td>Becton Dickinson†</td>
</tr>
<tr>
<td>Leukocytic common antigen</td>
<td>1:40</td>
<td>DAKO†</td>
</tr>
<tr>
<td>Leu M3</td>
<td>1:1</td>
<td>Becton Dickinson†</td>
</tr>
<tr>
<td>Leu M5</td>
<td>1:1</td>
<td>Becton Dickinson†</td>
</tr>
</tbody>
</table>

DAKO, Dakopatts, Glostrup, Denmark; Becton Dickinson, Becton Dickinson Immunocytometry Systems, Mountain View, Calif.; ATCC, American Type Culture Collection, Gaithersburg, Md.

*Rabbit, polyclonal; †mouse, monoclonal.
were then transferred to PBS and incubated with the rabbit anti-human von Willebrand factor for 30 minutes at room temperature. The sections were then rinsed in PBS, and the secondary rhodamine-conjugated porcine anti-rabbit Ig (dilution, 1:20; Dakopatts, Glostrup, Denmark) was applied for 30 minutes. After several washes in PBS, the procedure was repeated for mouse anti-human HLA-DR, L243, or HLA-DQ, with a fluorescein-conjugated rabbit anti-mouse Ig (dilution, 1:20; Dakopatts). After a final wash in PBS, the slides were viewed on a Leitz fluorescence microscope equipped with appropriate barrier filters. In controls both primary antibodies were omitted, or the fluorescence was blocked by staining with primary antibody, followed by unlabeled and then labeled secondary antibody. Alternatively, one of the primary antibodies was omitted or a nonimmune serum was used as a first or second layer, and the sections were then handled as described above.

**Results**

The endothelial morphology of carotid plaques and coronary plaques was identical; thus, the following text comprises the findings relating to both of these different locations.

With the scanning electron microscope, only a few areas with endothelial defects were detected that were totally covered by networks of fibrin and aggregations of thrombocytes and red blood cells. Semithin sections of these defects cut from reembedded blocks clearly showed the lack of ECs on the luminal surface.

**Endothelium Outside of Plaque Areas**

The endothelial pattern in nonsclerotic areas of the coronary arteries of explanted hearts was regular, with endothelial nuclei uniformly aligned in parallel and with a spindle-shaped cytoplasm. Their small cytoplasmic rims contained only a small number of mitochondria, lamellae of rough endoplasmic reticulum, and Weibel-Palade bodies. Sometimes, nonmembrane-bound cytoplasmic inclusions, which were always located downstream from the nucleus, were found at perinuclear locations. Adherent leukocytes and platelets were very rare.

**Endothelial Patterns on Plaques**

The endothelial pattern was somewhat irregular in most places on the surface of the lesions compared with the pattern seen on uninolved segments of the coronary arteries. EC morphology depends on the grade of the stenosis (Figures 1, 2, and 3). As a rule, most of the endothelial irregularities present at the outlet of the stenosis had a cobblestone (polygonal) appearance (Figure 4). In low-grade stenosis (lesion with a residual lumen of 40% or more; seven plaques) the changes were fairly mild, whereas in high-grade stenosis (lesion with a residual lumen of 20% or less; six plaques) areas with a whirl-like arrangement were also detected (Figure 4). On the other hand, the EC morphology was rather less disturbed in the region of the upstream shoulder of the plaque, where the endothelial layer consisted of spindle-shaped cells with nuclei arranged in parallel. In the throat of high-grade stenoses, extremely elongated ECs were present in parallel alignment (Figure 3). In contrast, flat plaques had a polygonal EC layer, even on the surface of the lesion, this layer being continuous with the cobblestone layer of the downstream shoulder of the plaques.

Large cells and giant cells (respectively defined by Repin et al7 as cells of the endothelial layer with an area of 800 μm² or more and 1,200 μm² or more) were found to be regular constituents of the endothelium on the plaque surface (Figure 4).

**Cytosplasmic Filaments and Inclusions**

The ECs covering the plaque were rather rich in intermediate filaments, which were fairly randomly distributed throughout the cytoplasm (Figure 5). On the other hand, in places where ECs overlapped each other extensively, the abluminal ECs displayed a light cytoplasm containing only very few intermediate filaments (Figure 5).

Cytosplasmic microfilaments were well developed in the ECs. As with many other cells, they occurred as peripheral networks adjacent to interendothelial clefts, stress fibers, or subplasmalemmal anchoring filaments. Stress fibers were present on the luminal aspects as well as on the abluminal side of the ECs, where they were quite often connected to the anchoring filaments. Clusters of intracellular lipid drops were present in some ECs (Figure 6c). Furthermore,
FIGURE 2. Panel a: Scanning electron photomicrograph of low-grade stenosis with cobblestone endothelium. Arrow indicates flow direction. ×40. Inset shows cross section of coronary artery with a residual lumen of 43%. Panel b: Ultrathin section of re-embedded tissue from plaque surface from the region indicated by the crosswires in panel a. Endothelial cell (ec) is covered by a small rim of palladium–gold and has a plump cuboidal appearance. This cell probably represents an activated EC. In the subsurface area, foam cells (fc) are present. ×15,360.

FIGURE 3. Scanning electron photomicrograph of high-grade coronary stenosis with extremely elongated endothelial cells. Arrow indicates flow direction. ×310. Inset shows cross section of the coronary artery with a residual lumen of 10%.
in some areas single non-membrane-bound rather than electron-dense inclusions were found.

**Endothelial Contacts to Subendothelial Intimal Cells**

Cellular contacts of surface ECs and subendothelial intimal cells, which are usually modified smooth muscle cells, were present in large numbers in sclerotic plaques. Two principal types of myo-endothelial contact were observed: 1) Single or multiple delicate finger-like processes from the EC to the adjacent subendothelial smooth muscle cell were common, whereas projections originating from the smooth muscle cells were rare (Figure 7a). The small cytoplasmic projections sometimes engaged with a furcate cytoplasmic projection of the corresponding cell. These contact zones were always simple appositions; tight junctions or gap junctions (nexus) were not observed. 2) On the other hand, there were broad attachment sites between ECs and modified smooth muscle cells, which sporadically included primitive junction-like densities on both sides of the attached cytoplasmic membranes (Figure 7b).

**Signs of Activation in Endothelial Cells**

In semithin sections small clusters of plump cuboidal cells were found within the endothelial monolayer (Figure 8b). By transmission electron microscopy, these cells showed signs of hypertrophy, with hyperplasia of organelles such as mitochondria or Weibel-Palade bodies (Figure 6a), but not all hypertrophic ECs contained Weibel-Palade bodies. Furthermore, the cytoplasm of these hypertrophic ECs was well endowed with a rough endoplasmic reticulum, which often displayed dilatation and vesiculation and had an electron-dense granular content (Figure 6b). Reembedding of areas with polygonal endothelium likewise showed cells with hyperplasia of cytoplasmic organelles (Figure 2b).

With the indirect immunoperoxidase technique, about 5% of the luminal surface cells displayed...
positive sigs for HLA-DR (expressed as a percentage of the stained surface line from the total surface line of the sectioned tissue, as measured with a Kontron MOP-Videoplan) (Figure 8). As shown by the number of nuclei in stained surface areas, either single cells or even small clusters of cells were found to be positive. In addition, in some areas subendothelial spindle-shaped intimal cells expressed HLA-DR, regardless of the HLA-DR-positive or -negative character of the corresponding surface-lining cells (Figure 8e). With the double immunofluorescence technique, the luminal surface was found to be almost entirely positive for von Willebrand factor, and small areas expressing HLA-DR were detected in the same section (Figures 8c and 8d). ECs that were positive for HLA-DQ were rarely found.

Leukocytes and Platelets on the Plaque Surface

Leukocytes were regular residents on the surface; they appeared spread over the ECs, and their positions indicated that they had been active (Figure 9a). These leukocytes were unequivocally identified as such by the use of Dynabeads, indicating the leukocytic common antigen on their surfaces, even on rather flattened cells (Figure 9b).

Most of these leukocytes were fairly flattened and extended numerous lamellipodia or pseudopodia onto the EC membrane. Thus, most of the leukocytes found to be adherent to the EC surfaces, even on rather flattened cells (Figure 9b).

Many sections, however, cells that clearly represented monocytes/macrophages were easily identified by use of Leu-M3 and Leu-M5 antibodies, but they were rare in these sections. In addition, positive mononuclear cells were sometimes found sticking to the endothelial surface in sections incubated with HLA-DR antibodies. Furthermore, as seen with the scanning electron microscope and in cross sections from reembedded probes (Figure 2b), the ECs were distended in some places by subsurface mononuclear macrophages and foam cells.

Scattered platelets were found adhering to the surface of polygonal endothelial cells. They showed signs of activation, with formation of pseudopodia (Figure 10).

**Discussion**

As this study shows, endothelial defects are generally less frequent on the surfaces of uncomplicated human plaques. This corroborates another ultrastructural investigation of human coronary arteries and a postmortem study of segments of aorta and left anterior descending coronary artery obtained from 52 humans aged 15–34 years who had died suddenly: microthrombi composed of a mixture of aggregated platelets and fibrin were observed by scanning electron microscopy in only 6% of these cases.

**Endothelium and Blood Flow Pattern**

It is well established that EC morphology and function (for a review, see Reference 12) respond to hemodynamic stress (shear forces) and that the local blood flow pattern is reflected in the morphology of the ECs. However, in a recent report it has been suggested that EC shape may additionally be influenced by factors that are not yet known. Normal quiescent arterial endothelium consists of a layer of fairly uniformly arranged, flattened spindle-shaped cells orientated in vivo with their long axes in the direction of blood flow. As in normal arteries, the presence of blood flow in regions of artherosclerotic disease is also likely to modify endothelial structure and function. The effects of arterial lesions on flow as indicated by the EC pattern are fairly comparable to the effects of experimental coarctation in animals. As this study indicates, the morphol-
FIGURE 6. Panel a: Transmission electron photomicrograph showing endothelial hypertrophy with hyperplastic organelles, especially Weibel-Palade bodies (wpb). ×25,600. Inset is a high-power magnification view of a wpb of this cell. ×149,600. Panel b: Transmission electron photomicrograph of well-developed rough endoplasmic reticulum in a hypertrophic endothelial cell. ×28,800. Panel c: Scanning electron photomicrograph of endothelial cells with clusters of lipid inclusions (asterisks) in their cytoplasm. Nu, nucleus. ×1,600.

ogy of the ECs likewise correlates with the anticipated level of shear stress along the stenosed sclerotic arteries and therefore depends to some extent on the degree of narrowing (Figure 1).

Upstream and in the throat of the stenosis, there is increased shear stress. This is indicated by extremely elongated ECs that line the narrow channel of the stenosis. These ECs are apparently protected against denudation by adaption of their cytoskeleton to the altered flow conditions, with hypertrophy of stress fibers, anchoring microfilaments, or intermediate filaments. Such adapted ECs living in a narrowed artery are reported to be resistant to a shear stress load of as much as 25 times the normal level. A similar adaptation of ECs is seen at the tip of the flow divider, which is the point of the highest shear stress at a bifurcation. On the other hand, those ECs that are protected from shear stress load because they are overlain by other ECs, as seen in the coronary artery plaque, contain fewer filamentous networks.

Flow irregularities such as annular vortexes, flow stagnation, and backflow occur at the outlet of a stenosis, and the altered flow may lead to oscillating shear stress. This has been clearly demonstrated in glass models and in vivo at the carotid bifurcation in humans and monkeys. At the downstream shoulder of the plaque, the altered flow patterns are reflected by a cobblestone (polygonal) endothelial layer. Furthermore, in some places at the outlet of the stenosis, some plaques reveal an EC pattern that closely resembles the pattern of regenerating ECs (for a review, see Reference 3). These features, which are probably healed injuries of the EC, may be attributable to true turbulence, characterized by stochastic motion of fluid elements that may occur in a narrowed vessel as a result of episodes of deviation in blood pressure. In vitro, prolonged exposure of ECs...
to true turbulence leads to cell–cell retraction, cell loss, and increased turnover. However, true turbulence is said not to occur in the undiseased vascular tree. This is supported by the fact that the endothelial layer of undiseased human coronary arteries is monotonous, with nuclei arranged in parallel without any signs of flow irregularity even in the region of bifurcations.

**Giant Cells in the Endothelial Layer**

Constant nondenuding injury and desquamation of ECs may necessitate an increase in the regeneration of the endothelial layer to preserve its integrity; this may lead to accelerated aging. EC senescence, however, may lead to a significant increase of the surface area of the individual cell, as seen from long-term cultures of bovine ECs after 75% of the total life span of the EC clone has been completed. On the other hand, an increase in the cellular surface area correlates with a decline in proliferative capability. However, such giant cells are not usually seen in cultured ECs obtained from rodents, and in contrast to human sclerotic lesions, they have not been described in animal models of arteriosclerosis. In humans the constant finding of such mononuclear or multinuclear (this report and References 7, 26, and 27) giant cells in the layer covering advanced lesions may serve as an indicator of increased regeneration and thus of sustained and intensified nondenuding injury of the endothelial layer. This is supported by the observation of increased EC replication at the shoulders of large raised lesions in hypercholesterolemic rats.

**Leukocytes on the Plaque Surface**

Leukocytic margination is common in small-diameter blood vessels, but in large vessels it is a rare finding. In this study we found monocytes adhering to the EC surface on the top of the plaque, whereas adherent monocytes were very rarely observed in the nonsclerotic segments. Furthermore, there was strong morphological evidence that these monocytes had been activated because they usually appeared spread over the surface of the EC. The mechanisms involved in the phenomenon of intimal blood monocyte recruitment in vivo are only understood to a limited extent (for review, see Reference 29). In advanced plaques, blood flow irregularities may facilitate the collision of blood monocytes with the ECs...
Activated Endothelial Cells

Signs of cellular activation are a constant finding in the endothelial layer covering the advanced plaques. EC activation has recently been defined, by analogy to macrophage activation,\(^3^0\) as a quantitative change in the level of expression of specific gene products (i.e., proteins), which in turn endow ECs with new capabilities that cumulatively allow ECs to perform new functions.\(^3^1\) Nevertheless, although a universally acceptable definition of endothelial activation is unavailable at this time, various signs of endothelial activation are certainly seen in the pathological state of an advanced sclerotic plaque.

By light microscopy, plump or "hypertrophic" ECs were detected on the plaques. Such ECs were first described as activated.\(^3^2\) By electron microscopy, hypertrophy of many ECs on the surface of the plaque was evident. Compared with the more quiescent ECs in undiseased segments of human coronary arteries, there was hyperplasia of organelles with a high content of endoplasmic reticulum in the cytoplasm, indicating a high level of cell biosynthesis that is probably attributable to increased regeneration in the endothelial layer,\(^3^3\) to increased production of von Willebrand factor as seen in injured rat endothelium,\(^3^4\) or to other substances such as growth factors\(^3^5\) or endothelium-derived relaxing factor (nitric oxide\(^3^6\)).

Class II antigen expression (e.g., HLA-DR) by ECs is a further example of activation in the cell-biologic sense.\(^3^7\) As shown by immunoperoxidase methods in this study, about 5% of the ECs covering the advanced human sclerotic plaque are positive to HLA-DR antibodies. In contrast, in the endothelial layer of normal human arteries no class II antigens were found.\(^3^8\) However, it is not known whether class II expression by ECs in the advanced plaque is a primary or a secondary event. Regardless of this, the HLA-DR-positive ECs may play a role in perpetuating the atherosclerotic process in the fully developed plaque, as assumed in autoimmune diseases. As
with many cells that do not normally express class II antigens, ECs do so after the application of γ-interferon in cell culture. In sclerotic plaque the expression of class II antigens may be a consequence of lymphocytic infiltration of the plaque and concomitant local γ-interferon production by activated T cells, which represent 5% of the T cells, as seen in the fibrous cap of human atherosclerotic plaques from endarterectomy specimens.

Furthermore, activation may lead to altered procoagulant properties of ECs, as seen in apparently intact endothelium treated with cytokines such as interleukin-1 or tumor necrosis factor. In advanced human plaques, mononuclear macrophages may be a source of both cytokines. From the morphological point of view, the adherent activated platelets that were found on the intact EC layer of human plaques may serve as an indicator of a change in the anticoagulant properties of the ECs. Adhesion of platelets is dependent on the presence of von Willebrand factor and ECs were sometimes crowded with Weibel-Palade bodies, the cytoplasmic store of von Willebrand factor. However, flow disturbance in the region of a plaque may be a further factor leading to platelet adhesion.

**Myo–Endothelial Contacts**

In contrast with humorally mediated interactions, ECs and subendothelial smooth muscle cells, in common with the resident cells of most tissues, communicate via heterocellular junctions. These are well documented for the microcirculation but are less so for larger vessels. In the microcirculation and in the rat carotid artery, the majority of myo-
endothelial junctions are gap junctions that may be involved in the regulation of smooth muscle cell tonus in the vessel wall as a response to signals generated in the EC. In vitro, it has been shown by means of various tracers, dyes, and electrophysiological techniques that myo–endothelial junctions are functional and that substances are transferable from ECs to smooth muscle cells via cellular contacts.48–50 Recently, Davies et al51 have demonstrated gap junction–like contacts in a coronary artery obtained from the heart of an adolescent boy who underwent cardiac transplantation. To date, the role of such myo–endothelial gap junctions in humans is hard to understand because the intima of most human arteries undergoes a diffuse thickening, with accumulation of collagen fibers and smooth muscle cells resulting in a slowly increasing distance between the ECs and the smooth muscle cells of the arterial media. In the human advanced plaque, we found numerous myo–endothelial contacts, but these were always simple adherent junctions that were the widespread version of mesenchymal cell junctions, and gap junctional complexes were never found.

Heterocellular junctions are important for growth control in embryonic development.52 The modification of the normal growth regulatory mechanisms between vascular cells is of some importance for the proliferation of smooth muscle cells in atherogenesis.53,54 The breakdown of gap junctional communication between ECs and smooth muscle cells has been proposed as a potential mechanism for vascular smooth muscle cell proliferation.55 The increase in myo–endothelial contacts in advanced plaque (this report) could thus be a compensatory process for retarding smooth muscle cell proliferation, being unsuccessful because unknown factors impede the building of gap junctional complexes.

FIGURE 10. Scanning electron photomicrograph of activated platelets (p), with pseudopodia adherent to intact endothelium of the surface of advanced sclerotic plaque. ×1,040.

Acknowledgments

We are indebted to W. Ostermeyer and to H.-W. Kniemeyer from the Department of Surgery, Heinrich-Heine University, Düsseldorf, for providing us with endarterectomy specimens and explanted hearts. The skillful technical assistance of U. Geusenhainer, G. Jahn, S. Schneeloch, and M. Schröder is gratefully acknowledged.

References


**KEY WORDS** • endothelium • atherosclerosis • flow patterns • class II antigens • myo-endothelial contacts
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