Adaptive Remodeling of Internal Elastic Lamina and Endothelial Lining During Flow-Induced Arterial Enlargement

Hirotake Masuda, Yong-Jie Zhuang, Tej M. Singh, Koichi Kawamura, Masayo Murakami, Christopher K. Zarins, Seymour Glagov

Abstract—Gaps in the internal elastic lamina (IEL) have been observed in arteries exposed to high blood flow. To characterize the nature and consequences of this change, blood flow was increased in the carotid arteries of 56 adult, male, Japanese white rabbits by creating an arteriovenous fistula between the common carotid artery and the external jugular vein. The common carotid artery proximal to the arteriovenous fistula was studied at intervals from 1 hour to 8 weeks after exposure to high flow. In the controls, the IEL showed only the usual, small, physiological holes, 2 to 10 μm in diameter. At 3 days, some of the holes in the IEL had become enlarged, but they could not be detected by scanning electron microscopy, despite manifest endothelial cell proliferation. At 4 days, gaps in the IEL appeared as small, luminal surface depressions, 15 to 50 μm wide. At 7 days, the gaps in the IEL had enlarged and formed circumferential, luminal depressions occupying 15±5% of the lumen surface. Endothelial cell proliferation persisted in the gaps while proliferative activity decreased where the IEL remained intact. At 4 weeks, as the artery became elongated and dilated, the gaps in the IEL widened as intercommunicating circumferential and longitudinal luminal depressions occupying 64±5% of the lumen surface. At 8 weeks, the rate of elongation and dilatation of the artery slowed and the widening of the gaps in the IEL diminished. Endothelial cells covered the gaps throughout. We conclude that flow-induced arterial dilatation is accompanied by an adaptive remodeling of the intima. The gaps in the IEL permit an increase in lumen surface area while endothelial cell proliferation assures a continuous cell lining throughout. (Arterioscler Thromb Vasc Biol. 1999;19:2298-2307.)

Key Words: endothelial cells □ internal elastic lamina □ blood flow □ internal elastic lamina gap □ arterial remodeling

Arteries enlarge in response to a chronic increase in blood flow.1-9 This enlargement requires structural alteration (remodeling) of the arterial wall. Missing portions of the internal elastic lamina (IEL), which are known as tears,10-12 gaps,4 or fragmentation7 of the IEL and which are much larger than the usual fenestrae of the IEL,13 are known to appear in dilated arteries afferent to an arteriovenous fistula (AVF) in rabbits. Within 4 weeks, the arteries dilate to nearly double their original size.4,6 Therefore, gaps in the IEL appear to be an important morphological event during flow-induced arterial remodeling. To characterize the level and progress of changes in the IEL and the associated changes in the endothelial lining in arteries suddenly exposed to markedly increased blood flow and wall shear stress, we used a well-established model to induce high flow and arterial enlargement: creation of a carotid artery-to-jugular vein AVF.2,5-9,12 We studied the ultrastructural features of the transformation of the IEL while monitoring luminal surface integrity and endothelial cell proliferation. We report that the formation of gaps in the IEL and endothelial cell proliferation are crucial features in the preservation of an adequate, intact, lumen surface during flow-induced arterial dilatation.

Methods

Animals
We used adult, male, Japanese white rabbits (n=68) (Oriental Bioservice Co, Japan) weighing 3 to 4 kg.

Operation
Animals were sedated with xylazine (4 mg/kg IM) and ketamine (25 mg/kg IM) and anesthetized by inhalation anesthesia of Sevoflurane (1% to 1.5% in O2/N2O, 2/1, vol/vol). With the use of sterile techniques, a midline cervical incision was made, and both common carotid arteries and the left jugular vein were exposed. The thyroid artery branch was used to identify and establish a consistent reference to a standard anatomic segment of carotid artery in every animal. After clamping of the left common carotid artery and left

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From the Second Department of Pathology (H.M., Y.-J.Z., K.K., M.M.), Akita University School of Medicine, Akita, Japan; the Department of Surgery (T.M.S., C.K.Z.), Division of Vascular Surgery, Stanford University, Stanford, Calif; and the Department of Pathology (S.G.), University of Chicago, Chicago, Ill.
Correspondence to Hirotake Masuda, MD, Second Department of Pathology, Akita University School of Medicine, 1-1-1 Hondo, Akita 010-8543, Japan. E-mail masuda@med.akita-u.ac.jp
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with a calibrated scale during the surgical procedure (Figure 1).

In situ outer diameter (OD) of the common carotid artery, 0.5 to 1 cm proximal to the thyroid branch, was obtained from photographs taken during the surgical procedure. A round mesh grid is used as a scale of 3-mm diameter. Carotid artery at 8 weeks after AVF operation was slightly winding, whereas carotid artery of the sham control (left) is straight. Lower photographs, Cross section of sixth segment of the left common carotid arteries. Enlargement and elongation at 8 weeks after AVF operation were apparent (Figure 1). Segments 1 and 2 were considered as the proximal carotid, 3 and 4 as the middle carotid, and 5 and 6 as the distal carotid arteries. Specimens for light microscopy (3 mm long), scanning electron microscopy (SEM; 3 mm long), and transmission electron microscopy (TEM; 1 mm long) were obtained from each segment. Specimens for histology were stained with hematoxylin-eosin and elastic Masson’s trichrome stains. Specimens for SEM were dehydrated through a series of alcohols, critical point–dried, coated with evaporated gold-platinum, and observed in a JSM-5200 (JEOL Co). Specimens for TEM were dehydrated through a series of alcohols, critical point–dried, coated with evaporated gold-platinum, and observed in a JSM-5200 (JEOL Co). Ultrathin sections were obtained from each segment. Specimens for TEM were dehydrated through a series of alcohols, critical point–dried, coated with evaporated gold-platinum, and observed in a JSM-5200 (JEOL Co). Ultrathin sections were observed in an LEM2000 (Topcon Co).

Histometric Measurements
Histomorphometry was performed on elastica–Masson’s trichrome–stained histological sections. With a profile projector (Nikon V-16, Nikon Co), the contours of the luminal margin and the outer margin of the media were traced. Because no distinct intimal thickening was detected in the experimental animals, the lumen contour usually coincided with the measured contour of the IEL. Contours were then digitized, and perimeters of the lumen (circumference) and cross-sectional area of media (CSA) were obtained by using image analysis software (Cosmozone-1, Nikon Co), the contours of the luminal margin and the outer margin of the media were traced. Because no distinct intimal thickening was detected in the experimental animals, the lumen contour usually coincided with the measured contour of the IEL. Contours were then digitized, and perimeters of the lumen (circumference) and cross-sectional area of media (CSA) were obtained by using image analysis software (Cosmozone-1, Nikon Co). From these values, calculations were made for corrected lumen radius (r = 1.25 × CSA/circumference) and corrected medial thickness (t = 1.25 × CSA/circumference) of all segments and for the luminal surface area of the sixth segment (SA = 2πrL/6). Calculations assumed that the artery was tubular; a correction factor of 1.25 was used to account for the shrinkage due to the fixation process. 1 2 3 4 5 6 7 8 9 10

Calculation of Hemodynamic Parameters
Mean blood flow velocity (U, cm/s) was calculated using the formula U = BFR/60πr², where blood flow rate (BFR) is expressed in milliliters per minute and r is expressed in centimeters. The Reynolds’ number (Re) was calculated using the equation Re = 1.054 × 2r × U/0.03, in which 0.03 poise was used for blood flow.
viscosity and 1.054 g/mL for blood specific gravity. Wall shear stress (WSS) was calculated using arterial pressure (AP, in mm Hg) as follows:

\[ \text{WSS} = \frac{\text{AP} \times 0.03}{r/t}, \]

where 0.03 is viscosity in poise.

Measurements of Depressed Areas, Endothelial Cell Density, and Endothelial Cell Number
Depressed areas of the luminal surface first appeared at 4 days and appeared significantly enlarged after 7 days. The depressed area as a percentage of total area was measured using 5 SEM photographs (at ×150 magnification) of the sixth segment from each animal in the 7-day, 4-week, and 8-week groups. Endothelial cell density (number of cells per square millimeter) was obtained from 5 SEM photographs (at 1500 magnification) of the sixth segment from each animal. In the 4-day, 5-day, 7-day, 4-week, and 8-week groups, endothelial cell density in the depressed and nondepressed areas was measured separately. Endothelial cell number in the sixth segment was calculated by using the following formula: endothelial cell number = endothelial cell density × SA, where SA of the sixth segment was calculated separately in the depressed and nondepressed areas at 7 days, 4 weeks, and 8 weeks.

Serial Histological Study of the IEL
To understand the 2-dimensional orientation of the gaps in the IEL, 100 consecutive, 1-μm histological cross sections were obtained from the sixth segment of rat-embedded specimens of 3 nonoperated controls, three day 3 animals, one day 7 animal, and one week 4 animal. The contour of the IEL was traced and then digitized. By using the digitized image, 2-dimensional reconstructions of the IEL were made by using the Cosmowze-2 system (Nikon Co.). The mean density, mean area, and percentage area of the small, missing foci of the IEL were analyzed using the reconstructed images of the IEL from control and day 3 animals.

Incorporation of Bromodeoxyuridine
To study endothelial cell proliferative activity, incorporation of bromodeoxyuridine (BrdU) was studied at 3 days, 7 days, and 4 weeks as well as in nonoperated controls. Four animals were used in each group. Similar surgical techniques were used as previously mentioned. One hour before the animals were killed, BrdU (50 mg/kg) in 5% glucose solution was injected into the abdominal cavity. After measurement of blood flow, the animals were killed by injection of an overdose of pentobarbital (100 mg/kg IV). Five minutes after death, the aortic arch and carotid arteries were perfusion-fixed via a catheter introduced into the abdominal aorta with 4% paraformaldehyde solution at 20°C at 100 mm Hg intraluminal pressure for 30 minutes. The left carotid artery was evenly cut in 10 mm proximal from the thyroid artery branch; the outer diameter (OD) was measured ~10 mm proximal from the thyroid artery branch; r is the luminal diameter of the sixth segment; t is the media thickness of the sixth segment; CSA is the cross-sectional area of the sixth segment; SA is the luminal surface area of the sixth segment; \( U \) is mean blood flow velocity at the sixth segment; Re is the Reynolds’ number at the sixth segment; WSS is wall shear stress at the sixth segment; WTS is wall tensile stress at the sixth segment; % Gaps is the percent of depressed areas observed by SEM in the sixth segment; endothelial cell density (ECD) was measured in nondepressed (N) and depressed (D) areas by SEM; total ECs were measured in the sixth segment and in depressed areas in the sixth segment (D).

Statistical significance (P < 0.05): *Greater than control and sham control; †Greater than 3 days; ‡Greater than 1 week; §Smaller than 3 days; ¶Larger than control, sham control, 1-week ECD (N) and ECD (D) 4-week ECD (N) and ECD (D), and 8-weeks ECD (N) and ECD (D); ||Larger than control, sham control, 1-week NG, 4-week ECD (N) and ECD (D) and 8-week ECD (N) and ECD (D) and 8-week ECD (D).
with the total number of endothelial cell nuclei in each section. BrdU incorporation was expressed as a percent of positive cases. At 7 days and 4 weeks, the specific location of BrdU-positive endothelial cells in relation to the missing areas of the IEL was analyzed.

Statistical Evaluation
All data are represented as mean±SD. Statistical analysis was performed by ANOVA followed by Scheffe’s test for multiple comparisons to compare the results for each group and time interval. Differences were determined to be significant when the probability value was <0.05.

Results

MAP and Blood Flow
MAPs showed no significant differences (the Table). Blood flow significantly increased immediately after AVF (68.2±22.8 mL/min), which was 3.97-fold higher than before AVF (17.2±4.2 mL/min; n=44, P<0.0001). Flow increased gradually thereafter, reaching 7.8-fold at 7 days, 15.5-fold at 4 weeks, and 18.0-fold at 8 weeks compared with controls (the Table).

Dimensions of the Left Common Carotid Artery
Dimensions of the left carotid artery are shown in the Table. The left carotid artery had become elongated at 4 and 8 weeks (Figure 1). In situ OD was almost unchanged up to 5 days after AVF and up to 10 weeks in sham controls (Figure 1). At 4 and 8 weeks, OD had increased significantly, to 2-fold greater than in controls (Figure 1). Luminal radii of the controls, sham-operated controls, and the animals at 3 days after AVF were not enlarged. At 7 days, OD was slightly enlarged in the distal carotid (sixth segment). At 4 and 8 weeks, it had enlarged =1.8-fold and 1.8-fold, respectively, relative to controls in the distal carotid, slightly enlarged 1.1-fold and 1.3-fold relative to controls in the middle carotid, and 1.0-fold and 1.1-fold relative to controls in the proximal carotid, respectively. Medial thickness was considerably decreased at 4 and 8 weeks in the sixth segment, but this difference did not reach significance. CSA of the media was significantly increased at 8 weeks in the sixth segment. SA of the sixth segment was 41 to 45 mm² until 3 days. It was slightly enlarged at 7 days, and by 4 and 8 weeks, it was markedly enlarged.

Hemodynamic Parameters of the Distal Segment of the Left Carotid Artery
Hemodynamic parameters of the distal carotid artery (sixth segment) are shown in the Table. At 3 days, U, Re, and WSS were markedly increased =8-fold, 7-fold, and 9-fold, respectively, relative to controls. At 7 days, U and WSS were =7-fold and 6-fold larger than controls, respectively. Re was nearly 500 times higher. At 4 weeks, U and WSS were 7-fold and 4-fold larger than in controls, respectively. Re was nearly 800 times higher. At 8 weeks, U and WSS were 6-fold and 3-fold larger than controls, respectively. Re was nearly 650 times higher. WTS before 1 week was almost as large as in controls, whereas WTS values at 4 and 8 weeks were significantly elevated.

Depressed Areas of the Lumen Surface in the Distal Carotid Artery
Until 2 days after the flow increase, the lumen surface remained flat (Figure 2C). At 3 days, although the lumen surface was still mostly flat (Figure 2, 3d), small, indistinct, round, and shallow dimplelike depressions, ≈15 μm in diameter, appeared occasionally (Figure 2, 3d-h). At 4 days, small, distinct, depressed areas appeared (Figure 2, 4d), which had sharp edges and were 15 to 50 μm wide (Figure 2, 4d-h). They could be differentiated from the shallow dimples appearing at 3 days by their depth, shape, size, and complementary deep indentations at their proximal and distal edges. At 5 days, the depressed areas were increased in number and size. At 7 days, the depressed areas were mostly circumferentially arranged, fissurelike depressions (≈50 μm wide), occupying 15% of the lumen surface (Figure 2, 7d). At 4 weeks, wide, depressed areas consisted of intercommunicating longitudinal and circumferential, fissurelike depressed areas (Figure 2, 4w) occupying 64% of the lumen surface. Nondepressed areas appeared as square “islands” surrounded by depressed areas, and the lumen surface of these nondepressed areas was wavy. At 8 weeks, wide, depressed areas occupied 57% of the lumen surface (Figure 2, 8w), consisting of considerably irregular, intercommunicating, longitudinal and circumferential depressed areas. The nondepressed areas showed a somewhat distorted square shape. The depressed areas were completely covered with endothelial cells as observed from 4 days to 8 weeks.

Missing Portions of the IEL in the Distal Carotid Artery
In the controls, the IEL was regular (Figure 3C) and usually exhibited small spaces, 2 to 5 μm in diameter. Occasionally the spaces were as large as 10 μm in diameter. The reconstructed IEL (n=3) revealed small holes (Figure 3, C-iel). Their density was 965±126/mm², their mean area was 29.7±4.1 μm², and their percent area relative to the whole IEL area was 6.9±0.9%. Until 2 day after flow increase, holes in the IEL were mostly similar to those in controls. At 3 days, some holes in the IEL were large, nearly 15 μm in diameter (Figure 3, 3d). The reconstructed IEL (n=3, Figure 3, 3d-iel) revealed that their density was 1522±712/mm², their mean area was 41.2±19.7 μm² (significantly larger than controls; P<0.05), and their percent area relative to the whole IEL area was 14.7±7.2%. At 4 days, distinct missing portions of the IEL (gaps in the IEL; Figure 3, 4d), which were wider (mostly 15 to 30 μm) than the holes in the IEL, occasionally appeared. The lumen surface was sharply depressed, with frequently observed edges of the IEL that appeared curled downward. At 5 days, the gaps in the IEL were more frequent and larger than those at 4 days. At 7 days, the gaps were frequently observed and enlarged (Figure 3, 7d). They were usually >100 μm wide in cross section. The IEL was slightly wavy and sometimes curled at its edges. After histological reconstruction (Figure 3, 7d-iel), they were nearly perpendicular to the vessel axis. At 4 weeks, the gaps in the IEL were frequent, wide (sometimes >200 μm in cross section; Figure 3, 4w), and slightly depressed. The IEL was wavy in many places. Histological reconstruction (Figure 3, 4w-iel) revealed wide gaps in the IEL surrounding undisrupted IEL areas. At 8 weeks, gaps in the IEL (Figure 3, 8w) were frequent, and the lumen surface was not so depressed as at 4 weeks. Throughout the experiments, gaps in the IEL were covered with endothelial cells, and no distinct intimal thick-
ening was observed in these gaps in the IEL as well as in undisrupted areas of the IEL.

Ultrastructural Changes of the IEL in the Distal Carotid Artery

In controls, the IEL was mostly regular, showing rather smooth margins of its luminal side (Figure 4C). At 1 hour to 1 day, the IEL was very similar to that of controls. At 2 days, the luminal side of the IEL showed irregular margins with a deep sawtooth appearance in almost all portions (Figure 4, 2d, 2d-t). In the “valleys” of the sawtooth corrugations where the IEL was absent, endothelial pseudopodia-like projections were frequently found. The basement membrane was irregular and absent in some areas, with a wide and loose subendothelial space. At 3 days, the luminal side of the IEL showed irregular margins (Figure 4, 3d), but the irregularity was not so distinct as at 2 days. Abluminal pseudopodia-like projections of endothelial cells were frequently observed. The basement membrane was thick and irregular, and the subendothelial space was still wide. In some large holes in the IEL (Figure 4, 3d-hole), the lumen surface was slightly indented. At the center of the holes in the IEL, endothelial cells occasionally extended abluminally, and medial smooth muscle cells occasionally protruded and appeared attached to these endothelial cells. At 4 days, gaps in the IEL appeared (Figure 4, 4d), which were completely covered with endothelial cells. The edges of the IEL around its gaps were mostly blunt. In the undisrupted areas of the IEL, it was thin, with irregular margins on its luminal side similar to those noted at 3 days. No intimal thickening was observed. At 5 days, the appearance of gaps in the IEL was similar to those noted at 4 days. At 7 days, gaps in the IEL (Figure 4, 7d) contained endothelial cells that were packed together. The luminal side of the IEL showed rather smooth margins compared with that at 4 days. The subendothelial layer was narrow with a rather regular basement membrane. At 4 and 8 weeks, the gaps in the IEL had become completely covered with endothelial cells (Figure 4, 4w). In the undisrupted areas of the IEL, single smooth muscle cells occasionally appeared in the subendothelial layer.

Endothelial Cell Density, Endothelial Cell Number, and BrdU Incorporation in the Distal Carotid Artery

In the controls, endothelial cell density was 3000 cells/mm² (the Table, Figure 5), and BrdU incorporation into these endothelial cells was not detected (Figure 6C). Endothelial cell density increased rapidly from 1 day to 2 to 3 days (the Table, Figure 5): from 4200 to 5100 and to 6900 cells/mm², respectively (Figure 5). At 3 days, endothelial cells showed high but diffuse BrdU incorporation (7.8±1.8% BrdU-positive endothelial cells), and smooth muscle cells also occasionally exhibited BrdU incorporation (Figure 6, 3d). Endothelial cell number in the sixth segment was >2-fold greater than in controls (the Table). At 4 days (Figure 5), endothelial cell density in the depressed areas had increased to 7000 cells/mm², but density in the nondepressed areas decreased slightly, to 5800 cells/mm². At 5 days (Figure 5), endothelial cell density in the depressed areas increased up to ∼9000 cells/mm², while that in the nondepressed areas decreased further to 5200 cells/mm². At 7 days (the Table, Figure 5) in the depressed areas, endothelial cell density was slightly lower, ∼8000 cells/mm², whereas it had decreased even further, to 5000 cells/mm², in the nondepressed areas. Endothelial cell number in the sixth segment increased even further: 23% appeared in the depressed areas and 77% in the nondepressed areas. In the gaps in the IEL, endothelial cells showed high BrdU incorporation (5.2±3.7%) and smooth muscle cells showed frequent BrdU incorporation (Figure 6: 7 days): 85% of BrdU-positive endothelial cells were localized in the gaps in the IEL. At 4 weeks (the Table, Figure 5), endothelial cell density was decreased even in the depressed areas, to 5300 cells/mm², while it was even lower, at ∼3400 cells/mm², in the nondepressed areas. Endothelial cell number in the sixth segment was >3-fold higher than in controls: 73% appeared in the depressed areas and 27% in the nondepressed areas. BrdU-positive endothelial cells amounted to 0.3±0.2%, and almost all (99%) of these were found in the gaps in the IEL (Figure 6, 4w). At 8 weeks (the Table, Figure 5), endothelial cell density was almost the same as that at 4 weeks in the depressed area as well as the nondepressed areas. Endothelial cell number in the sixth segment was ∼4-fold greater than in controls: 66% appeared in the depressed areas and 34% in the nondepressed areas.

Discussion

The rabbit common carotid artery that was suddenly exposed to high blood flow after AVF was significantly elongated and dilated, especially in its distal segment (3 to 4 cm distal to the orifice) at 4 and 8 weeks after flow increase. The middle segment (1.5 to 3 cm distal to the orifice) was slightly dilated while the proximal segment (within 1.5 cm of the orifice) was only minimally dilated. The basis for these axial differences in the degree of dilatation is unclear. We focused our morphological study on the distal segment, where flow-induced arterial dilatation was greatest. During dilatation, depressed areas of the lumen surface and missing portions (gaps) of the IEL were proved to appear simultaneously. Therefore, the former corresponded to the latter, representing underlying gaps in the IEL. Similar lesions have been reported in similar experiments with an AVF and have been called “intimal tears,”10,11 “tears of the endothelium and the IEL,”12 “IEL gaps,”14 or “fragmentation of the IEL.”17 On the contrary, Wong and Langille16 showed that in rabbit common

Figure 2. SEM microphotographs of the luminal surface of the distal segment (sixth) of rabbit common carotid arteries. Flow is from left to right. Bars indicate magnification in microns. C, Nonoperated control; 3d, 3 Days after AVF. Endothelial cell density is high. 4d, 4 Days after AVF. Small depressed areas (*) appear. 7d, 7 Days after AVF. There are circumferential fissurelike depressed areas (*). Endothelial cell density in depressed areas is high. 4w, 4 Weeks after AVF. There are intercommunicating longitudinal and circumferential fissurelike depressed areas (*). Nondepressed areas are almost square. 8w, 8 Weeks after AVF. There are intercommunicating longitudinal and circumferential depressed areas (*). The margins of depressed areas are not straight but slightly distorted. 3d-h, High magnification of lumen surface 3 days after AVF. A small, round, dimplelike depression is recognized. Its margin is indicated by arrowheads. 4d-h, High magnification of lumen surface 4 days after AVF. Proximal and distal edges of a small depressed area (*) are sharply indented with straight margins.
Figure 3. Photomicrographs and reconstructed images of the IEL of the distal segment (sixth) of the left common carotid artery. Epon-embedded, 1-μm-thick cross section stained with toluidine blue. Arrow in reconstructed images of the IEL indicates longitudinal vessel axis. Bars indicate magnification in microns. C, Nonoperated control. C-iel, Reconstructed image of the IEL of a control. 3d, 3 Days after AVF. (*) Indicates a small hole of the IEL. 3d-iel, Reconstructed image of the IEL of a day 3 animal. 4d, 4 Days after AVF. There are gaps in the IEL (*). 7d, 7 Days after AVF. There is a wide gap in the IEL (arrowheads), which is slightly wavy. 7d-iel, Reconstructed image of the IEL of a day 7 animal. There are wide gaps in the IEL (*). 4w, 4 Weeks after AVF. There are wide and distinct gaps in the IEL (arrowheads), and the IEL is wavy. 4w-iel, Reconstructed image of the IEL of a week 4 animal. The gap in the IEL (*) is very wide. 8w, 8 Weeks after AVF. The gap in the IEL is wide (arrowheads).
Figure 4. TEM photomicrographs of endothelial cells (E), IEL, and medial smooth muscle cells (SMC) of the distal segment (sixth) of the rabbit left common carotid arteries. All photographs are cross sections. Bars indicate magnification in microns. C, Nonoperated control. 2d, 2 Days after AVF. IEL shows sawtooth appearance of the luminal side. Endothelial cells show frequent pseudopodia-like protrusions (arrows). 2d-t, 2 Days after AVF; section stained with tannic acid, uranyl acetate, and lead citrate. IEL is stained black. Luminal side of the IEL shows an irregular margin with sawtooth appearance. 3d, 3 Days after AVF. Pseudopodia-like projections of endothelial cells are observed. Basement membrane is thick and irregular. 3d-hole, 3 Days after AVF. A large hole in the IEL is observed. A smooth muscle cell protrudes into the subendothelial space (*) through the hole in the IEL and attaches to the endothelial cell (arrowheads). 4d, 4 Days after AVF. A small gap in the IEL with a distinct luminal depression, which is completely covered by endothelial cells. 7d, 7 Days after AVF. Marginal portion of a gap in the IEL (arrowheads). 4w, 4 Weeks after AVF. Marginal portion of the gap in the IEL (arrowheads).
Carotid arteries in which flow increased gradually and physiologically during development or due to contralateral common carotid ligation, flow-dependent enlargement of the fenestrae of the IEL contributed to developmental remodeling of the IEL. They did not observe gaps in the IEL. Therefore, the formation of gaps in the IEL is considered basically related to the sudden and extensive flow increase.

Our experiments revealed that as the artery became enlarged, gaps in the IEL widened. The gaps in the distal sixth segment totaled 8 mm² at 7 days and 62 mm² at 8 weeks. By contrast, the lumen surface over the undisrupted areas of the IEL was almost unchanged for 8 weeks. Thus, widening of the lumen surface corresponded to the widening of the gaps. They first appeared at 4 days in our experiments. Greenhill and Stehbens,10 Stehbens, 11 and Jones et al 12 found gaps in the IEL as early as 2 to 3 days after AVF, which occurred almost 1 day earlier than in our experiments. They suggested that the gaps first appeared as endothelial layer disruptions; however, we never encountered endothelial disruption in the gaps throughout our experiments. From 4 to 7 days, the gaps were mostly circumferential, but after 4 weeks, they had become greatly enlarged and consisted of intercommunicating circumferential and axial components.

Before the appearance of gaps in the IEL, there was a marked increase in endothelial cell proliferation, which was widespread up to 3 days. After 4 days, however, when the gaps in the IEL appeared, endothelial cell density increased further in the gaps, peaking at 5 days, while it began decreasing thereafter in the undisrupted areas of the IEL. After 4 weeks, endothelial cell proliferation was markedly decreased even in the gaps of the IEL, while endothelial cells had almost ceased to proliferate in the undisrupted areas of the IEL. Endothelial cell proliferation began before the gaps in the IEL appeared, continued in the gaps during their enlargement, and ceased with the cessation of widening of the gaps. Thus, it is considered that the gaps in the IEL are a newly expanded surface populated by newly proliferated endothelial cells. Jones et al12 suggested that because endothelial cells were considered to regenerate to cover missing portions of the endothelium, endothelial cell density was higher in the gaps in the IEL. However, our present observations suggest another possibility; ie, that the gaps occurred under the preserved layer of already proliferating endothelial cells and that they became enlarged, in parallel with the continued endothelial cell proliferation.

Until 2 days after the flow increase, we noted only small holes in the IEL, similar to those in controls. They were therefore considered to be the usual fenestrae of the IEL.16 At 3 days, shallow, round, dimplelike depressions up to 15 μm in diameter were occasionally recognized. These were considered to be enlarged holes in the IEL. Considering that the gaps in the IEL appeared only at 4 days and that the diameter of the enlarged holes in the IEL at 3 days was similar to the width of the smallest gaps in the IEL, it is probable that the gaps may have originated from the enlarged holes, although we cannot exclude the probability that the gaps also appear independently of the holes.

At 2 days after the flow increase, when the gaps in the IEL were still unchanged, the luminal side of the IEL showed...
irregular margins with a sawtooth appearance. Pseudopodia of endothelial cells were frequently observed between the projections of the IEL. The basement membranes of the endothelial cells also became irregular within a rather wide, loose, subendothelial space. If the sawtooth irregularities imply degeneration of the IEL, we would expect the IEL to be attenuated by this degeneration and eventually disrupted at the enlarged holes in the IEL as well as in the thinner portions of the IEL. It is, however, likely that these changes are part of a biosynthetic and/or degradation process induced by the overlying, proliferating endothelial cells.

At 8 weeks, the distal carotid artery seemed to cease dilation, while conditions might be close to Poiseuille flow, considering that the Re, nearly 650, was less than the critical value of 2000 at which laminar flow may become turbulent. At that time, wall shear stress was still greater than the physiological level. However, the dilatation at 8 weeks was sufficient to reduce the wall shear stress initially present immediately after AVF and up to 3 days after AVF when dilatation did not occur. Therefore, it may be assumed that the arterial dilatation induced by increased flow in this experiment is an adaptive dilatation, which tended to normalize wall shear stress, as Kamiya and Togawa predicted. On the other hand, wall tensile stress is not the initial stimulus for flow-loaded dilatation, for wall tensile stress was unchanged until 7 days.

Despite the evidence showing increased activity of smooth muscle cells, including the protrusions across the gaps in the IEL, to come in contact with endothelial cells and the incorporation of BrdU, there was no intimal thickening except for a few isolated smooth muscle cells in the subendothelial layer. We reported that intimal thickening occurred at low wall shear stress (<0.5 N/m²), whereas intimal thickening did not occur or progress under physiological or high wall shear stress in a similar rabbit model of AVF. It has also been demonstrated that production of some growth factors by endothelial cells, such as endothelin-1 and platelet derived growth factor B, are downregulated by increased shear stress. Kraiss et al showed that proliferation of smooth muscle cells as well as neointimal thickening was lower under conditions of high shear stress. Because in this report we specifically focused on the morphological changes in the IEL rather than on intimal thickening, the relationship between wall shear stress and intimal thickening remains to be investigated.

Disruptions of the IEL can be found in necrotizing arteritis, aneurysm formation, dissection, and atherosclerosis. Although disruptions of the IEL are thought to be caused by flow-induced and other forms of adaptive and disease-related losses of mechanical properties of the arterial wall, there is no clear mechanistic basis for the disruption. Our findings demonstrating a close relationship between endothelial cell proliferation and the formation of gaps in the IEL may provide concepts leading to a further explanation of the mechanisms underlying this aspect of artery wall remodeling and its features during aneurysm formation and atherosclerosis.

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