American Journal of Pathology, Vol. 143, No. 1, July 1993

Immunohistochemical Study of Intimal Microvessels in Coronary Atherosclerosis

Yixia Zhang,* Walter J. Cliff,* Gutta I. Schoefl,* and Grace Higgins†

From the Clinical Science Division, John Curtin School of Medical Research, The Australian National University, Canberra, and the School of Pathology,† University of New South Wales, Sydney, Australia*

Two hundred ninety-nine human coronary artery paraffin-embedded tissue blocks were examined for intimal microvessel invasion by probing for factor VIII-associated antigen with indirect immunofluorescence and high resolution confocal microscopy. The results obtained confirm that intimal microvessels originate in the adventitia and show that the richness of intimal microvessels is strongly positively correlated with intimal thickness and negatively correlated with relative lumen size. A number of plasma constituents were examined in serial sections. Comparison of immunofluorescence distribution patterns of these components with intimal microvessel distribution patterns reveals that intimal microvessels leak plasma albumin into artery walls, exude fibrinogen, and are associated with the build-up of plasma cells within atherosclerotic lesions. Therefore, intimal microvessels are demonstrated to play important roles in the development of atherosclerosis. (Am J Pathol 1993, 143:164–172)

Coronary arteries, as is the rule for most muscular arteries,[1] normally have no vessels in their intima or media. Intimal vascularization of human arteries was first noticed and linked with atherosclerosis, and intimal thickening in general, by Koester[2] in 1876. This observation has been confirmed by a large number of observers[3–6] and has been beautifully demonstrated by perfusion of India ink[7] and most recently of silicone polymer into the walls of cleared coronary arteries with accompanying cinematography.[8]

The microvessel invasion of plaques has been emphasized as being crucial in the pathogenesis of atherosclerosis.[9–11] Osborn,[5] after the analysis of over 1000 hearts, concluded that nobody with abnormal vascularization of the coronary artery wall was free from the risk of death from coronary artery disease. However, some workers consider that vessels invading the coronary intima are of no significance[12,13] and are a function of intimal thickness and not of atherosclerosis.[14]

Those who emphasize the importance of intimal vascularization propose that the microvessels may play two roles: one, of providing blood components to "nourish" the growing plaques,[2,10,15] and the other, of causing intimal hemorrhages by rupturing. Repeated intimal hemorrhages may eventually lead to coronary artery thrombosis.[11] As to the first hypothesis, no direct evidence of plasma components leaking from intimal microvessels to nourish atherosclerotic plaques has yet been obtained but newly formed vessels are known to be highly permeable.[16] In addition, newly formed small vessels are deficient in supporting connective tissue and basement membrane and are fragile.[16,17] The hemorrhage hypothesis generally postulates that the ruptured microvessels arise directly from the arterial lumen.[11] This is not in agreement with Barger's observations[18] that intimal capillaries originate more frequently from the adventitia than the lumen. The origin of intimal capillaries and their role in intimal hemorrhage are, therefore, still uncertain.

The endothelial cells of these thin-walled microvessels contain factor VIII-associated antigen (VIII.a.a.).[19] Applying anti-factor VIII.a.a. antibody to coronary arteries will, therefore, reveal any microvessels present in their walls. The present work examines the intramural microvascular distribution patterns using the sensitive indirect immunofluorescence technique with anti-factor VIII.a.a. as the first antibody in conjunction with high resolution confocal microscopy. Through analysis of the microvascular patterns, the origin of intimal microvessels and their relation to the development of atherosclerosis can be examined. Comparison of microvessel distributions with those of

Accepted for publication January 14, 1993.

Address reprint requests to Dr. W. J. Cliff, John Curtin School of Medical Research, The Australian National University, Canberra, ACT 2601, Australia.

plasma components such as albumin, fibrinogen, immunoglobulin γ (IgG), α (IgA), and μ (IgM) in serial sections, will establish whether blood components are entering the atherosclerotic plaques from the intimal microvessels. Furthermore, the significance of intimal neovascularization in the development of coronary atherosclerosis will be analysed through relating its presence and richness to intimal thickness and arterial stenosis.

Materials and Methods

Tissue Procurement, Fixation, and Embedding

Hearts were obtained at autopsy from the Pathology Department of Royal Canberra Hospital and from the School of Pathology, University of New South Wales, Sydney. The coronary arteries were cannulated and perfused at 100 mm Hg pressure with warm heparinized saline followed by 10% neutral buffered formalin (NBF) and then barium gelatin.[20] The coronary arteries were clamped and fixation continued by immersing the heart in NBF for at least 24 hours. They were then dissected free from the surface of the heart and made transparent by prolonged soaking in two or three changes of glycerine. The arteries were examined minutely under the dissecting microscope and numerous blocks were taken for histology of normal and diseased segments. Sixty-eight cases were selected from subjects dying of acute myocardial infarction, other cardiovascular diseases, infections, neoplasms, alcoholism, and suicide-accident.

Coronary Artery Sections

Two hundred ninety-nine paraffin-embedded blocks of decalcified human coronary arteries from 68 patients were selected according to histological appearances without knowledge of cause of death, sex, or age. The blocks were chosen to include a range from normals through the different aspects of coronary artery disease to provide examples of major pathological features such as fibrosis, inflammatory cell infiltration, intimal vascularization, plaque hemorrhage, and lipid deposition. Ten 4-μ-thick serial sections were cut from each block for the investigation of various components present in the coronary arteries and for comparison of their distribution patterns. The sections were mounted on poly-L-lysine coated slides and air-dried at 60 C for 1 hour.[21]

Control Tissue Sections

Human appendix (Woden Valley Hospital, Canberra), tonsil (Woden Valley Hospital, Canberra), and selected human coronary sections were chosen as positive controls for IgG, IgA, IgM, CRP, fibrinogen, albumin, and factor VIII.a.a.

Antibodies

Rabbit anti-human polyclonal antibodies were used as the primary antibodies in the indirect immunofluorescence methods. Antibody specificity was proved by absorption of the antibody with the corresponding pure antigen (Sigma Chemical Co, St. Louis, MO) at 4 C for 24 hours. After such absorption, the antibodies showed no immunoreactivity on human coronary arteries. The second antibody used was sheep anti-rabbit polyclonal antiserum conjugated with fluorescein isothiocyanate (FITC) (Silenus, Melbourne, Australia) diluted 1:100 with phosphate-buffered saline (PBS) before use. Normal rabbit serum (Dakopatts, Glostrup, Denmark) at the same dilution as that of the tested antibodies was used as the negative control in the indirect immunofluorescence method. Normal sheep serum was used to prevent nonspecific binding of the secondary antibody and was separated by centrifugation from the blood of normal sheep maintained at the John Curtin School of Medical Research, Australian National University.

Immunohistochemical Techniques

Paraffin sections were deparaffinized, rehydrated, washed, and digested with 0.1% trypsin (Sigma) solution in Tris buffer (pH 7.8) for 2 to 3 hours at 37 C in a humidified chamber. Sections were then rinsed three times with distilled water and twice with PBS for 5 minutes each. The final wash with PBS contained 0.5% Tween 20 (TPBS) to reduce nonspecific staining. Sections were incubated for 20 minutes at room temperature with 10% normal sheep serum to block nonspecific binding. The serum was drawn off and the sections were then incubated with the desired rabbit anti-human antibodies. Adjacent sections were incubated with normal rabbit serum for 30 minutes at room temperature in the humidified chamber to serve as controls. After three 5-minute washes with TPBS, sections were incubated with the FITC-conjugated second antibody for 30 minutes at room temperature in the humid chamber. Sections were then rinsed three times with TPBS and mounted in PBS and glycerol (1:9) at pH

8.6 under glass coverslips. In general, the sections were examined immediately but when this could not be done, they were stored briefly in light-proof packets at 4 C until used.

Fluorescent microscopy was performed using an MRC-500 Confocal Image System (Bio-Rad Microscience Division, Hemel Hempstead, England) with an argon ion laser operating at 488 nm wavelength. The thinner parts of the artery walls were assembled into a single frame buffer, but the wider parts of the walls were stored separately as three or more frames and then connected on the screen. Twenty scans were averaged for each image to reduce noise. Experimental images matched by their corresponding control fields were recorded on floppy disks.

Morphometric Techniques

A coronary section from each block was measured with the MD 1 Microscope Digitizer system (Minnesota Datametrics, St. Paul, MN) and the following areas were derived.

Total cross-sectional area: To allow for distortion during processing the total cross-sectional area (T) was assumed to be a circle calculated from the measured perimeter (P)[22]: $T = P^2/4\pi$. Media area (M): scanned and calculated by computer. Intima area (I): scanned and calculated by computer. Wall area (W): media area + intimal area. $W = I + M$. Adjusted lumen area (L): Total cross-sectional area (T) minus wall area. $L = T - W$. I/W: the ratio of intima to wall area and reflects the size of plaques. L/T: the ratio of lumen size to total cross-sectional area and is a measure of stenosis.

To help assess their roles in the pathogenesis of atherosclerosis, intimal neovascularization and plasma components were graded on scales of 0 to 3 in terms of their specific immunofluorescence intensities and distributions in the wall.

Statistical Methods

Correlation coefficients between factor VIII.a.a immunoreactivity grades and I/W or L/T were calculated with Macintosh computer software (Cricket Graph). The frequency plot was analyzed for the 299 data points and showed a statistically normal distribution pattern (Figure 1). The significance of differences between average ratios for each grade are calculated by two-tailed Student's t-test, and $P \leq 0.05$ is recognized as significant difference occurring between two groups. Rank correlation was used to identify the correlation coefficients of immunoreactivity grades between pairs of different intramural components.

Results

Factor VIII.a.a. specific antibody reacted with the endothelium lining the artery lumen, the vasa vasorum in the adventitia and the thin-walled microvessels in the intima and related media when present (Figure 2). Compared with H&E staining, indirect immunofluorescence combined with high resolution confocal fluorescence microscopy was considerably more sensitive in detecting microvessels in the walls of human coronary arteries. There were no microvessels detected in the intima or media of normal coronary arteries. Microvessels first appeared in intimas that were uniformly thickened around the entire coronary circumferences and

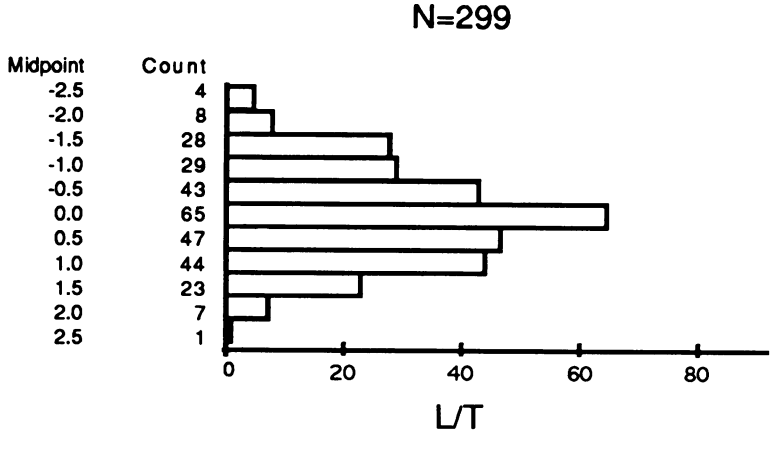

Figure 1. *Frequency plot for the 299 data points for L/T ratios used in Figure 10.*

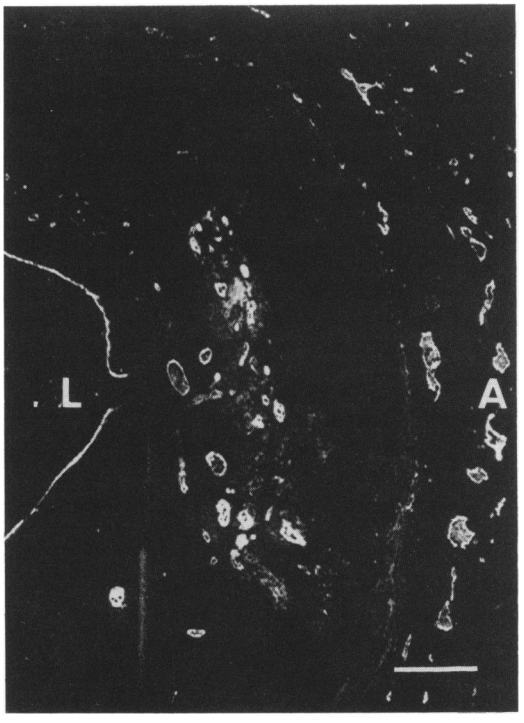

Figure 2. *Coronary section shows many fluorescing microvessels in the intima. The endothelium lining the artery lumen* (L) *and the vasa vasorum in the adventitia* (A) *also show strong immunoreactivity. First antibody: rabbit anti-human factor VIII.a.a. Second antibody: FITC-conjugated sheep anti-rabbit Ig. Scale bar = 100 μ. Confocal microscopy.*

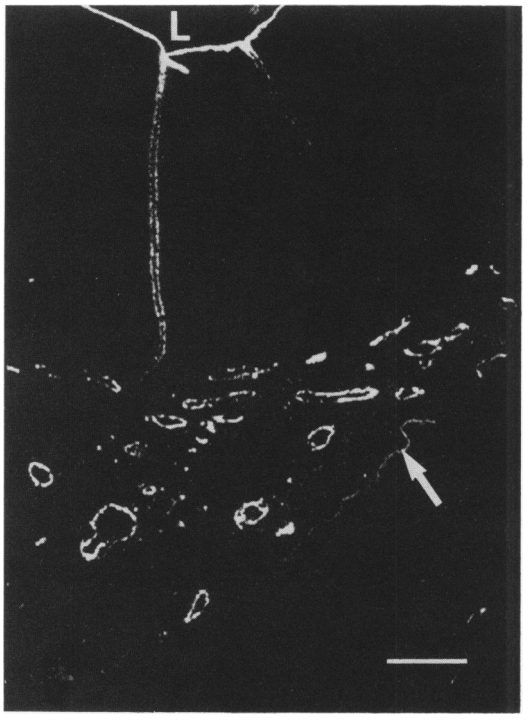

Figure 3. *Intimal microvessels present in a plaque shoulder region. Note: wavy autofluorescent IEL (*arrow*).* L: *artery lumen. First antibody: rabbit anti-human factor VIII.a.a. Second antibody: FITC-conjugated sheep anti-rabbit Ig. Scale bar = 100 μ. Confocal microscopy.*

which had no intra or extracellular lipid deposits detectable. In these instances the microvessels were always very small and sparse.

Microvessels were observed with a high degree of consistency in arteries with early plaques, which were identified by the appearance of asymmetrical encroachment upon the lumen. New vessels invading such lesions were usually located in the shoulder regions (Figure 3) where transition from plaque to normal intima occurs. With increasing plaque size, the microvessels extended from the plaque shoulders toward the plaque centers. As more lipid appeared in the lesions, more new vessels were observed. As the microvessels disappeared from the degenerate lipid-rich centers they increased in numbers in the surrounding areas of the plaques (Figure 4). The bases of plaques were, in general, the most richly vascularized areas of all. Often vasa vasorum could be traced extending from the adventitia into the plaques via the media (Figure 5).

Some new vessels with endothelial multilayered "onion skin" pattern were seen occasionally in intimal lesions (Figure 6). Although some new vessels were present very close to the arterial endothelium, they were not actually identified arising directly from the artery lumen in these experiments.

Intimal Microvessels and Distribution of Plasma Components

Albumin distribution followed very closely the intimal microvessel distribution. It was the best marker of plasma leakage of the antigens tested in this study. The microvessels themselves showed strong albumin immunoreactivity (Figure 7). Surrounding the microvessel walls were zones of albumin immunofluorescence decreasing in intensity with distance and with the level of fluorescence being related to the number of microvessels present. Rank correlation analysis between albumin and factor VIII.a.a. distributions within intima and plaques produced a coefficient of $r = 0.99$ ($P < 0.001$).

Fibrinogen, when present within atherosclerotic regions, was always related to the presence of microvessels. In some neovascularized areas, however, there was no fibrinogen immunoreactivity present, while in other regions levels of fibrinogen immunoreactivity increased with the number of intimal microvessels present. Fibrinogen was distrib-

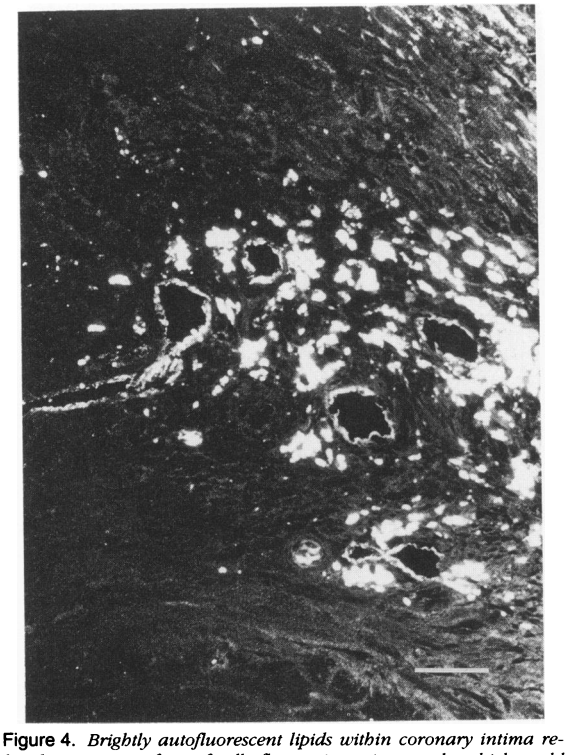

Figure 4. *Brightly autofluorescent lipids within coronary intima related to a group of specifically fluorescing microvessels, which could be readily distinguished by their different color. First antibody: rabbit anti-human factor VIII.a.a. Second antibody: FITC-conjugated sheep anti-rabbit Ig. Scale bar = 50 μ. Confocal microscopy.*

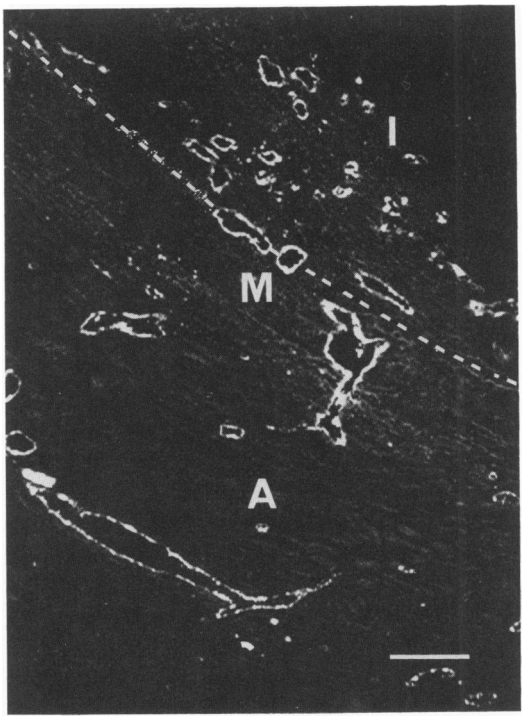

Figure 5. *Microvessels extend from coronary adventitia* (A) *through the media* (M) *and ramify within the intima* (I). *The region of the intimal-medial border is indicated by a* dotted line. *First antibody: rabbit anti-human factor VIII.a.a. Second antibody: FITC-conjugated sheep anti-rabbit Ig. Scale bar = 100 μ. Confocal microscopy.*

uted in two forms, either as small discrete spots or else spread diffusely over entire areas. The small spots were often actually within individual intimal microvessels and the smaller diffuse areas were localized around groups of new vessels present in the intima (Figure 8). In such instances, there was always associated inflammation with cellular infiltration. The strongest diffuse fibrinogen staining was in the necrotic lipid gruel in the centres of plaques where, in addition to rich surrounding neovascularization, associated heavy cellular infiltration was always identified.

No diffuse zones of IgG, IgA, or IgM were observed surrounding coronary intimal microvessels. IgG, IgA, and IgM containing plasma cells were, however, found related to microvessels and the numbers of such cells increased with the richness of neovascularization.

Diffuse immunoglobulin staining regions were present in advanced plaques which were richly vascularized, these immunoglobulins appeared to have been produced locally by plasma cells or to have been extravasated in hemorrhages from ruptured microvessels.

Intimal Microvessels Related to Intimal Thickness

All the artery sites are plotted for intimal factor VIII.a.a. immunoreactivity grades versus their I/W (Figure 9). The avascular artery walls showed the widest range of relative intimal thickness (I/W), but almost all factor VIII.a.a. positive ones had I/W ratios over 0.54 (of 163 positive sections only 2 had I/W less than 0.5). As factor VIII-a.a. immunoreactivity grades increased, there was a proportionate increase in relative intimal thickness (I/W) (Figure 9). The correlation coefficient is positive and strong, $r = 0.663$. The increase of I/W ratios for microvessel grades from 0 to 1, 1 to 2, and 2 to 3 are all significant ($P < 0.001$, Student's t-test).

Intimal Microvessels Related to Lumen Size

In Figure 10 all the arterial sites are plotted for factor VIII.a.a. immunoreactivity grades versus their (L/T) ratios. As factor VIII.a.a. grades increase, there is a definite decrease in L/T ratios. The correlation coefficient r is 0.721. The decreases in L/T ra-

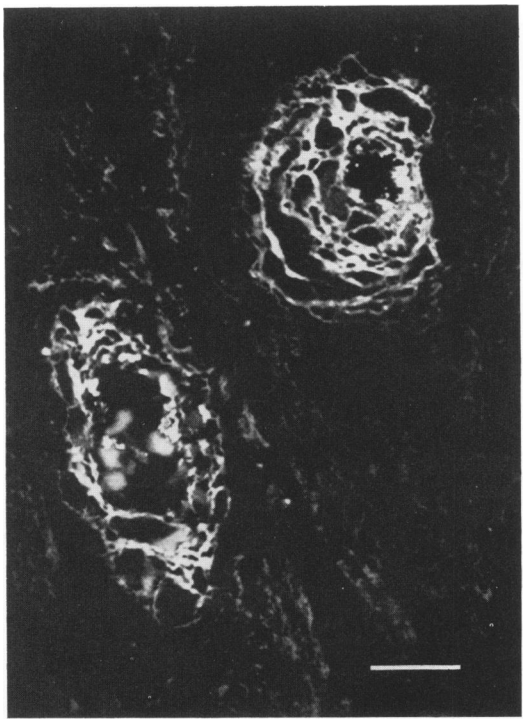

Figure 6. *Microvessels with marked endothelial reduplication in a coronary plaque. First antibody: rabbit anti-human factor VIII.a.a. Second antibody: FITC-conjugated sheep anti-rabbit Ig. Scale bar = 25 μ. Confocal microscopy.*

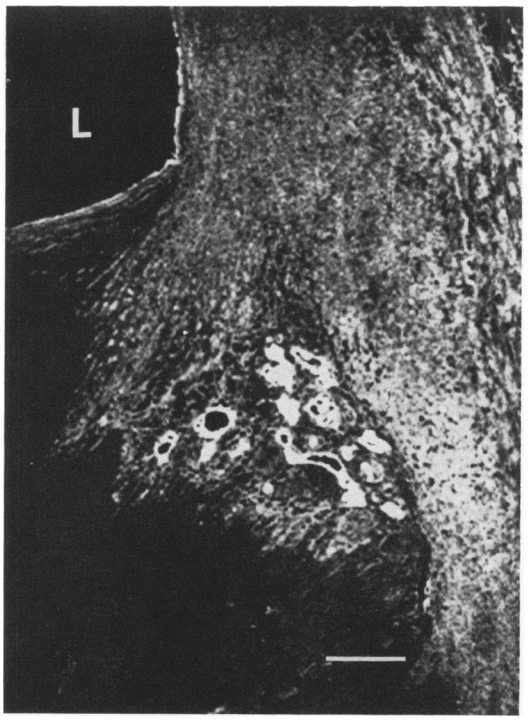

Figure 7. *Group of microvessels present in plaque shoulder region of coronary artery. Strong albumin-specific immunofluorescence surrounds their walls and diffuse albumin staining is related to the neovascularized area (particularly to right of figure).* L: *lumen. First antibody: rabbit anti-human albumin. Second antibody: FITC-conjugated sheep anti-rabbit Ig. Scale bar = 100 μ. Confocal microscopy.*

tios from 0–1, 1–2, 2–3 are all significant ($P < 0.005$, student's *t*-test) and indicate progressive stenosis.

Discussion

The current work has unequivocally confirmed the existence of microvessels in thickened intima and atherosclerotic plaques.[2,5,7,8] However, taking advantage of highly specific immunofluorescence techniques and high resolution confocal microscopy examination, the present investigation has found that the presence of intimal microvessels is not limited to atherosclerotic lesions. The earliest invasion by new vessels was in thickened intimas with no atherosclerotic features.

A statistically normal distribution pattern of 299 data plots was established to allow use of classical statistical methods. The earliest stage of intimal neovascularization was closely related to the intimal thickness. Of the sections, 73 of 75 (97%) with I/W ratios <0.54 had no intimal factor VIII.a.a. immunoreactivity and 134/136 of the positive sections (98%) had I/W ratios greater than 0.54. This result supports the conclusion that intimal vascularization is a function of intimal thickness, and that intimal vascularization may be a response to the inadequate nutrition of the thickened intima.[14] Our study showed that only when the intima accounts for 50% or more of the wall thickness will it become vascularized and so is different from Geiringer's[14] absolute value of 0.35 mm. However, even arteries with extremely thick intimas may be free of new vessel ingrowth. This indicates that the fibrovascular reaction in the intima can go through phases of waxing and waning activity.[5,23] The fibrosis and build up of extravascular materials are virtually permanent, whilst microvessels appear and disappear.[9]

When microvessels had developed in the intima, their distribution patterns were associated with similar distribution patterns of plasma albumin and of Ig containing plasma cells. The richness of neovascularization increased with increasing plaque size and decreasing relative lumen size. The hypothesis that intimal microvessels "nourish" the growing plaques[2,10,15] was strongly supported by this study. Visible evidence of albumin diffusing from the microvessels and the strong rank correlation coefficient (0.99) between albumin grading and microvessel grading provide the best evidence. This is perhaps due to albumin being a relatively low

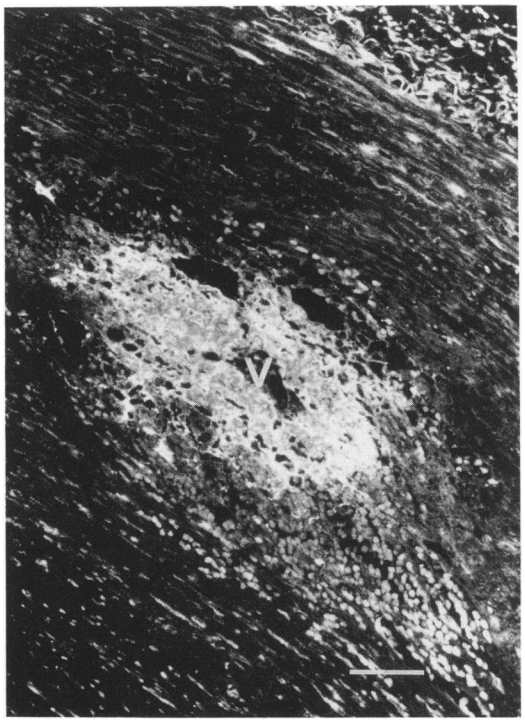

Figure 8. *Strong intimal fibrinogen-specific immunofluorescence related to microvessels (V). First antibody: rabbit anti-human fibrinogen. Second antibody: FITC-conjugated sheep anti-rabbit Ig. Scale bar = 100 μ. Ploem fluorescence.*

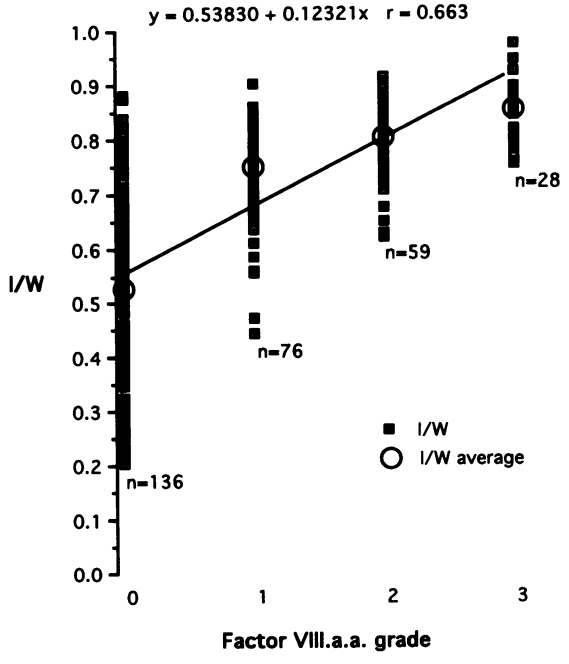

Figure 9. *Factor VIII.a.a. reactivity grade* versus *relative thickness of intima (I/W).*

molecular weight protein, with a high plasma concentration and a stable chemical character. Albumin is unlikely to cause pathological reactions like either

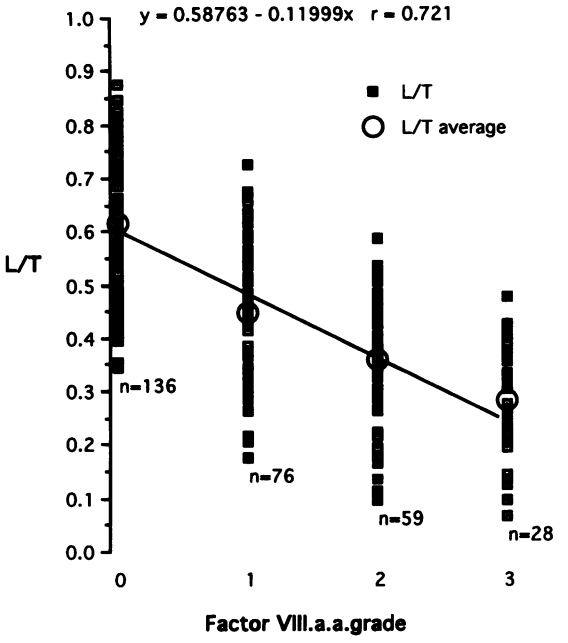

Figure 10. *Factor VIII.a.a. reactivity grade* versus *relative lumen size (L/T).*

fibrinogen[24] or Igs.[25] Its filtration from small vessels will also contribute significantly to plaque growth by causing edema of the artery wall. Other plasma components not tested for are also likely to be diffusing out in the same way as albumin[26] and to be playing roles in atherogenesis.

Fibrinogen leakage can be an expression of the increased permeability of new vessel walls.[16] When the intimal microvessels were uninflamed there was no fibrinogen leakage. When the coronary microvessels were involved in inflammation, with accompanying migration of cells, then fibrinogen started to appear within the intima as focal deposits. In the necrotic areas of advanced plaques, strong diffuse staining for fibrinogen was present. This implies that intimal vessels were directly involved in the production of the surrounding inflammatory infiltrate. Additionally, fibrinogen present in the necrotic plaque cores could also have been derived from microvessels during their terminal inflammatory and necrobiotic stages with increased permeability and hemorrhages adding to the deposits, supporting the concept that hemorrhage derived from intimal microvessels is a significant factor in atherogenesis.[5,7]

Although diffuse Igs were not observed around intimal microvessels, IgG, IgA, and IgM containing plasma cells were identified surrounding microvessels and the numbers of these cells could be related to the degree of neovascularization. It is intended to present these findings more fully and

quantitatively in a subsequent paper. However, to summarize it would appear that intimal microvessels are involved in immune responses during atherogenesis by supplying precursors of plasma cells within the plaques. With regard to the diffuse IgG, IgA, and IgM deposits in the lipid cores, these could possibly be due to bleeds from broken small vessels in the atheromatous plaques[10] as well as to release from local plasma cells. Endothelial cell proliferation has been found in homografts of renal transplants.[27,28] Our study showed similar multilayered endothelial cells in some of the new vessels of coronary atherosclerotic lesions. This could be interpreted as additional evidence of an immune response being involved in atherogenesis. It has been suggested that endothelial cells may proliferate and differentiate to provide tissue cells during the formation of intimal plaques.[29,30]

It can be seen, then, that coronary intimal neovascularization plays a role in the growth of plaques. The microvessels definitely supply plasma components, such as albumin and fibrinogen and thus nourish the thickened wall. They participate in immune reactions by supplying and maintaining inflammatory cells and plasma cells. Hence, they can contribute to the rapid growth and complications of plaques through oedema, inflammation, small vessel rupture, hemorrhage, thrombosis, and necrosis.[5] In contrast to Geiringer's conclusion that intimal vascularization was not a function of atherosclerosis,[14] intimal neovascularization has been demonstrated to be a significant contributor to atherosclerosis.

When the intimal new vessels could be traced to their origins, they always appeared to connect to the adventitial vasa vasorum. The origin of intimal microvessels from the lumen, therefore, must be relatively rare. Nevertheless, intimal vessels still might on occasions arise directly from the artery lumen.[11]

Acknowledgments

The authors thank Dr. M. W. Simpson-Morgan for his help in producing the computer program used for the derivation of the artery wall areas.

References

1. Vancov V: Structural basis of the microcirculation in the wall of arterial vessels. Bibl Anat 1973, 11:383–388
2. Koester K: Endarteriitis und arteriitis. Klin Wochenschr 1876, 13:454–455
3. Blumenthal HT, Alex M: Arteriosclerosis of the coronary circulation, Cowdry's Arteriosclerosis: A Survey of the Problem, 2nd Ed. Edited by HT Blumenthal. Springfield, Illinois, Charles C. Thomas, 1967, pp 329–62
4. Higginbotham AC, Higginbotham FH, Williams TW: Vascularization of blood vessel walls, Evaluation of the Atherosclerotic Plaque. Edited by RJ Jones. Chicago, University of Chicago Press, 1963, pp 265–77
5. Osborn GR: The Incubation Period of Coronary Thrombosis. London, Butterworths, 1963
6. Paterson JC: Vascularization and hemorrhage of the intima of arteriosclerotic coronary arteries. Arch Pathol 1936, 22:313–324
7. Winternitz MC, Thomas RM, LeCompte PM: The Biology of Arteriosclerosis. Springfield, Illinois, Charles C. Thomas, 1938
8. Barger AC, Beeuwkes R, Lainey LL, Silverman KJ: Hypothesis: vasa vasorum and neovascularization of human coronary arteries: A possible role in the pathophysiology of atherosclerosis. N Engl J Med 1984, 310:175–177
9. Cliff WJ, Schoefl GI: Pathological vascularization of the coronary intima, Development of the Vascular System. Edited by J Nugent, MO Connor. Ciba Found Symp, Pitman, London, 1983, 100:207–221
10. LeCompte PM: Reactions of the vasa vasorum in vascular disease, Cowdry's Arteriosclerosis, A Survey of the Problem, 2nd Ed. Edited by HT Blumenthal. Springfield, Illinois, Charles C. Thomas, 1967, pp 212–24
11. Paterson JC: Capillary rupture with intimal hemorrhage as a causative factor in coronary thrombosis. Arch Pathol 1938, 25:474–487
12. Leary T: Vascularization of atherosclerotic lesions. Am Heart J 1938, 16:549–554
13. Schornagel HE: Intimal thickening in the coronary arteries in infants. Arch Pathol 1956, 62:427–432
14. Geiringer E: Intimal vascularization and atherosclerosis. J Pathol Bacteriol 1951, 63:201–211
15. Groszek E, Grundy SM: The possible role of the arterial microcirculation in the pathogenesis of atherosclerosis. J Chronic Dis 1980, 33:679–684
16. Schoefl GI: Studies on inflammation. III. Growing capillaries: their structure and permeability. Virchows Arch Abt A Pathol Anat 1963, 337:97–141
17. Cliff WJ: Observations on healing tissue: A combined light and electron microscopic investigation. Philos Trans R Soc Lond-Biol Sci 1963, 246:305–325
18. Barger AC, Beeuwkes R: Rupture of coronary vasa vasorum as a trigger of acute myocardial infarction. Am J Cardiol 1990, 66:41G–43G
19. Hoyer LW, Santos RP, Hoyer JR: Antihemophilic factor antigen: localization in endothelial cells by immunofluorescent microscopy. J Clin Invest 1973, 52:2737–2744
20. Schlesinger MJ: An injection plus dissection study of coronary artery occlusions and anastomoses. Am

Heart J 1938, 15:528–568
21. Huang S, Minassian H, More JD: Application of Immunofluorescent staining on paraffin sections improved by trypsin digestion. Lab Invest 1976, 35:383–390
22. Thomas AC, Davies MJ: Post-mortem investigation and quantification of coronary artery disease. Histopathology 1985, 9:959–976
23. Bredt H: Morphology. Atherosclerosis: Pathology, Physiology, Aetiology, Diagnosis and Clinical Management. Edited by FG Schettler, GS Boyd. Amsterdam, Elsevier Publishing Company, 1969, pp 1–48
24. Kadish JL: Fibrin and atherogenesis-a hypothesis. Atherosclerosis 1979, 33:409–413
25. Rosenfeld ME, Khoo JC, Miller E, Parthasarathy S, Palinski W, Witztum JL: Macrophage-derived foam cells freshly isolated from rabbit atherosclerotic lesions degrade modified lipoproteins, promote oxidation of low-density lipoproteins, and contain oxidation-specific lipid-protein adducts. J Clin Invest 1991, 87:90–99
26. Friedman M, Byers SO, George SS: Origin of lipid and cholesterol in experimental thromboatherosclerosis. J Clin Invest 1962, 41:828–841
27. Pedersen NS, Morris B: The role of the lymphatic system in the rejection of homografts: A study of lymph from renal transplants. J Exp Med 1970, 131:936–969
28. Lund B, Jensen OM: Renal transplantation in rabbits. Acta Pathol Microbiol Scand Section A 1970, 78:713–728
29. Reddy GSR, Cliff WJ: Morphologic changes in arterial grafts in rabbit ears. Lab Invest 1979, 40:109–121
30. Altschul R: Histologic analysis of arteriosclerosis. Arch Pathol 1944, 38:305–312