Identification and Distribution of Fibrinogen, Fibrin, and Fibrinogen) Degradation Products in Atherosclerosis

Use of Monoclonal Antibodies

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Samples of normal and atherosclerotic vessels obtained from vascular and cardiothoracic surgery were examined for the distribution of fibrinogen/fibrin I, fibrin II, and fibrinogen (ogen) degradation products (Fragment D/DD) by using recently characterized monoclonal antibodies that recognize and distinguish the three molecular forms (MAbs IBC8, T2G1, and GC2, respectively) with the ABC-immunoperoxidase technique. In normal aortas, little fibrinogen/fibrin I or fibrin II was present and no fibrinogen (ogen) degradation products could be detected. In early lesions and in fibrous plaques, fibrinogen/fibrin I and fibrin II were distributed in long threads and surrounding vessel wall cells and macrophages. Fibrinogen (ogen) degradation products were not seen in early lesions. In fibrous and advanced plaques, fibrinogen/fibrin I, fibrin II, and fibrinogen (ogen) degradation products were detected in areas of loose connective tissue, in thrombus, and around cholesterol crystals. The results of this study suggest that increased fibrin formation and degradation may be associated with progression of atherosclerotic disease. The observed distribution of the different molecular forms of fibrinogen also suggests the possibility that the cells present in the lesions actively participate in the fibrinogen-to-fibrin transition within the vessel wall. (Arteriosclerosis 9:109–121, January/February 1989)

The presence of fibrinogen and fibrin in atherosclerotic plaques has already been documented by several morphologic or immunochemical studies. However, with the reagents and methods available at the time of those studies, it was not possible to distinguish fibrinogen from fibrin or their degradation products. Polyclonal antisera to fibrinogen could not provide detailed information on its molecular form in the lesion and the use of immunofluorescence in tissues did not allow fine morphological detail to be determined. Moreover, the characteristic periodicity of fibrin seen with the electron microscope is typical only of fibrin that has polymerized under certain conditions and has not undergone any degradation. In two more recent studies, the fragments derived from the amino terminus of fibrinogen-related material found in human atherosclerotic plaques and thrombi were characterized immunohistochemically. Those studies showed that thrombi consisted mainly of fibrin II. In the vessels studied, intact fibrinogen was the predominant molecular form found in normal aortas; fibrinogen, fibrin I, and fibrin II were all present in different proportions in fibrous and fatty atherosclerotic plaques; fibrin II was the main component in complicated plaques. Thus, the proportion of fibrin II in atherosclerotic lesions was shown to increase with progression of the lesions.

As already mentioned, direct information on the fibrinogen (ogen) degradation products present in atherosclerotic lesions or on the distribution of the fibrinogen and fibrinogen-derived antigens in the lesions was not possible from those previous studies. Among the various questions were: 1) Are fibrinogen/fibrin I and fibrin II present only on the lumen of the vessels studied? 2) In addition to the endothelium and the intima, are deeper layers of the vessel wall and vascular wall cells involved in the disease process? and 3) Are fibrinogen (ogen) degradation products present and, if so, are they detected in the same areas as fibrinogen/fibrin I and fibrin II? These questions were investigated in the present study.

The avidin-biotin complex immunoperoxidase technique with recently characterized monoclonal antibodies was used to study the distribution of fibrinogen-related antigen in human atherosclerotic lesions and thrombi. The following monoclonal antibodies (MAb) were used: 1) MAb IBC8, which recognizes Bβ 1-42 peptide as well as intact fibrinogen and fibrin I and does...
Fibrin I is formed from fibrinogen by thrombin cleavage of the bond Aα 16–17 and release of fibrinopeptide A only. Fibrin II is formed from fibrinogen by thrombin cleavage of both Aα 16–17 and Bβ 14–15 and release of both fibrinopeptides A and B. Fragment D consists of a heterogeneous population (MW range 85 to 100 kd) of early and late plasmin digestion products of fibrinogen and non-crosslinked fibrin. Fragment DD consists of a heterogeneous population (MW range 180 to 200 kd) of plasmin digestion products from crosslinked fibrin II.

not crossreact with fibrin II:11 2) MAb T2G1, which reacts with Bβ 15–42 peptide as well as fibrin II and cross-linked fibrin II but does not react with Bβ 1–42, fibrinogen, or fibrin I;12 and 3) MAB GC4, which recognizes the Fragment D moiety in early and late plasmin degradation products of both fibrinogen and fibrin but not in intact fibrinogen.13 In previous studies,6 it was shown that free Bβ 1–42 and Bβ 15–42 constituted less than 3% of the total fibrinogen immunoreactivity. Therefore, it can be assumed that the immunoreactivity with these monoclonal antibodies in tissues would be due to recognition of fibrinogen/fibrin I (I8C6) or of fibrin II (T2G1).

This new approach using three different monoclonal antibodies has allowed us to identify and distinguish the distribution of fibrinogen/fibrin I from fibrin II and from fibrinogen degradation products in normal and atherosclerotic vessels.

Methods

Tissue Specimens

The samples were collected under a protocol approved by the Institutional Review Board of the Columbia Presbyterian Medical Center. Upon excision, the vascular surgery specimens were placed immediately into sterile 50 ml tubes filled with 0.1 M NaCl, 0.05 M Tris (pH 7.4) additionally containing 0.1 M NaCl and 100 KIU/ml aprotinin (TrasyloL Mobay Chemical Corporation, New York, NY) and 1 mM benzamidine (benzamidine hydrochloride hydrate, Alrich Chemical Company, Milwaukee, WI) to inhibit plasmin and thrombin activity. Within 2 hours of collection, the samples were rinsed in three changes of the same buffer. Aortas and right and left coronary arteries from heart transplant recipients were dissected immediately after removal of the heart and carefully rinsed as above. The samples were then divided into grossly homogeneous parts, and areas of atherosclerotic aortas were collected with paired normal adjacent areas. Each sample was fixed in Bouin's and was embedded in paraffin. As indicated in Tables 2 to 4 by the number on the left, a total of 51 samples from 29 patients were examined. The samples were 12 areas of normal aortas from three heart transplant recipients; 21 samples of atherosclerotic plaques from 16 patients, and 18 areas of thrombi from 13 patients undergoing cardiothoracic or vascular surgery. In the heart transplant recipients (55 through 58), different lesions from the same patient were examined. In Table 4 the results obtained in different portions of some of the thrombi (38, 43, 44) are indicated.

Antibodies

A summary of the antibodies used and their specificity is illustrated in Table 1. In brief, the identity, specificity, isotype, and mode of purification of all the antibody probes used in this study are as follows:

MAb I8C6 is an IgG2a kappa that recognizes the peptide Bβ1–42 but also crossreacts with intact fibrinogen and fibrin I. In the present study, this antibody also reacted with crosslinked fibrinogen14,15 if it was present in the vessel wall.16 This antibody, as found in culture fluid, was used without further purification.

Table 1. Monoclonal Antibodies and Polyclonal Antisera Used in Immunocytochemical Analysis

| Designation | Specificity | Fragment/molecule identified | Reference source | Concentration* (μg/ml) |
|-------------|-------------|-----------------------------|------------------|-----------------------|
| I8C6        | Bβ1-42      | Bβ1-42, fibrinogen           | B. Kudryk        | 5†                    |
|             |             | fibrin I                    | NY Blood Center  |                       |
| T2G1        | Bβ15-42     | Bβ15-42, fibrin II           | B. Kudryk        | 5‡                    |
|             |             | fibrin II                   | NY Blood Center  |                       |
| GC4         | Fragments   | Fragments                   | B. Kudryk        | 20‡                   |
|             | D and D dimer| D and D dimer               | NY Blood Center  |                       |
| Anti-Fg     | Fibrinogen  | Fibrinogen                  | Cappel Labs      | 45§                   |
| Anti-Alb    | Albumin     | Albumin                     | Dako Labs        | 20§                   |
| Anti-IgG    | IgG heavy chain | IgG                         | Cappel Labs      | 60                    |
| Anti-IgG    | IgG Fc fragment | IgG                         | Cappel Labs      | 79                    |

†Confluent growth medium. Concentration determined by titration on ELISA using a purified MAb I8C6 preparation as standard.
‡HPLC purified.
§As polyclonal antiserum supplied by manufacturer.
||IgG fraction.

The concentration for monoclonal antibodies is referred to antibody concentration. For polyclonal antisera, it is the total protein concentration used.
IMMUNOHISTOCHEMISTRY OF ATHEROSCLEROSIS Bini et al. 111

MAb T2G1 is an IgG1 kappa that is specific for the peptide Bj15–42, which also reacts with fibrin II of both human and dog origin. This antibody was purified from ascites by high performance liquid chromatography (HPLC) using a DEAE column.

MAb GC4 is also an IgG1 kappa that reacts with the Fg-D moiety from both fibrinogen and fibrin II but does not react with either parent molecule. It would be expected to recognize plasmin degradation products from cross-linked fibrinogen.14 This antibody was purified from ascites by chromatography on DEAE A-50 (Pharmacia Fine Chemicals Incorporated, Piscataway, NJ) as well as by HPLC using an H-PHT column (Bio-Rad Laboratories, Richmond, CA).

Rabbit antihuman fibrinogen antiserum (lot 20830) was from Cappel, Malvern, Pennsylvania, and it had previously been shown to be noncrossreactive with fibronectin.17

Goat antihuman IgG (heavy chain-specific, lot 9292) and IgG Fc fragment-specific (lot 26590) were also purchased from Cappel. The immunoreactivity of these antisera was absorbed with human gammaglobulin (fraction II, lot 40) Miles Laboratories, Elkhart, IN.

Rabbit antihuman albumin (IgG fraction 20 mg/ml) was from Accurate, Westbury, New York. The immunoreactivity of this antiserum was completely absorbed with crystallized albumin (Human Albumin, A-9511, lot 126C-8-70, Sigma Chemical Company, St. Louis, MO).

Immunohistochemistry

Twenty serial sections (5 μm) were cut from each block after embedding and used for histochemistry and immunocytochemistry. The ABC immunoperoxidase technique18 was performed essentially as previously described.19,20 Controls for each antigen included sections of surgical thrombus as a positive control and adjacent sections from each specimen incubated with diluent in place of the primary antibody as a negative or method control. Diaminobenzidine (brown) was used as the chromogen and methylene blue, as the counterstain.19 The polyclonal antiserum to fibrinogen was used to compare its distribution in the intima with the three monoclonals. Antiserum to albumin and IgG were used as controls and as permeability markers of the vessel wall in the development of atherosclerotic disease.21–25 The avidin-biotin complex reagents were obtained from Vector Labs Incorporated, Burlingame, California. 3,3‘-diaminobenzidine tetrahydrochloride was from Eastman Kodak Company, Rochester, New York.

Routine Histochemical Staining

Hematoxylin-eosin (H&E) and hematoxylin-phloxin-saffran (HPS) staining were performed exactly as described.26

Results

The tissues were classified histologically as follows:

1. Early atherosclerotic plaque: lesion affecting only the intimal layer and consisting of areas of loose connective tissue and foam cells without calcium or cholesterol.

2. Fibrous plaque: lesion deep in the inner one-third of the media consisting of areas of dense connective tissue and areas of loose connective tissue and foam cells. May have acute or organized focci of thrombus on the surface.

3. Advanced plaque: lesion affecting the intima and the inner two-thirds or full thickness of the media, with calcium and cholesterol, and with or without surface thrombus.

4. Acute thrombus: fibrin clot with discernible lines of Zahn.

5. Organized thrombus: evidence of granulation tissue in fibrin clot.

6. Old thrombus:acelular accumulation of eosinophilic material with extensive vacuolization.

Normal Aortas

The results from normal aortas are shown in Table 2. Fibrinogen/fibrin I (MAb GC6-reactive) was detected in only two of 12 specimens. Staining was focal in the intima (Figure 1A) and diffuse in the adventitia in one specimen, while in the other, it was diffuse throughout the intima, media, and adventitia, with traces of fibrin II (MAb T2G1-reactive) only in the media and the adventitia. Fragment D/D (MAb GC4-reactive) was not observed in any sample of normal aorta. Albumin was present mainly in the intima (Figure 1B) and in the adventitia, often in the same areas where fibrinogen was present. IgG was not detected in most samples of normal aortas, but when present it was mainly localized to the adventitia.

Atherosclerotic Plaques

The results obtained with atherosclerotic plaques are shown in Table 3.

Early Atherosclerotic Plaques

In seven early atherosclerotic plaques (Figure 2 and Figure 3), fibrinogen/fibrin I was detected on the luminal surface and in the intima (Figure 2A) and around foam cells and in areas of loose connective tissue (Figure 3A). Intimal staining was often seen in the absence of luminal staining. In two lesions, fibrinogen/fibrin I was distributed in the intima as short threads and around vessel wall cells that appeared to be smooth muscle cells and macrophages (Figure 3A). In other specimens, fibrinogen/fibrin I threads were more closely intertwined and parallel to the lumen. Fibrin II was present on the endothelium of one coronary artery (Figure 2B) and in two lesions in small flecks around what appeared to be smooth muscle cells and macrophages. Fragment D/D was rarely observed in early plaques (Figure 2C is negative for Fragment D/D, while this Fragment is present in Figure 3C). Albumin was present in six of seven lesions and IgG, in four of five. Albumin was more abundant than IgG in areas where fibrinogen/fibrin I and fibrin II were detected (Figure 2E and Figure 3E) and was diffusely present throughout the vessel layers, while some IgG was distributed in the same areas where fibrinogen and albumin were present, and some was observed with the connective fibers in both the intima (Figure 2F) and the adventitia.
Table 2. Vascular and Cardiovascular Samples:
Normal Aortas

| Case   | I8C6 | T2G1 | GC4 | Fg  | Alb | IgG |
|--------|------|------|-----|-----|-----|-----|
| 55/13  | -    | -    | -   | -   | +   | ND  |
| 55/15  | -    | -    | -   | -   | -   | ND  |
| 56/2B  | -    | -    | -   | ±   | +   | -   |
| 57/3   | -    | -    | -   | ±   | +   | +   |
| 57/4   | -    | -    | -   | ±   | +   | +   |
| 55/17  | -    | -    | -   | +   | ++  | +   |
| 55/18  | -    | -    | -   | +   | ++  | +   |
| 57/2a  | -    | -    | -   | ±   | +   | -   |
| 57/2b  | -    | -    | -   | ±   | +   | +   |
| 56/1a  | -    | -    | -   | ±   | +   | +   |
| 55/10  | -    | -    | -   | +   | -   | ND  |

The samples were all taken from the ascending aorta.
ND = not determined, - = negative, ± = weak, + = positive, ++ = strongly positive.
Fg = fibrinogen, Alb = albumin.

Fibrous Plaques

In six fibrous plaques, fibrinogen and fibrin I were present either within the tissue or on the luminal surface in organizing thrombi. Fibrinogen/fibrin I was distributed in short threads and bundles or around smooth muscle cells and macrophages with a patchy stellate appearance (Figure 4A and Figure 4B). Fibrin II was detected in luminal thrombus in four samples and around cells or in long threads and bundles in the intima and into the media (Figure 4C). Fragment D/DD was present within large areas of fibrin deposition (Figure 4D) but not often surrounding vessel wall cells (Figure 4D). The polyclonal antiserum to fibrinogen recognized most of the material stained by the monoclonal antibodies (Figure 4E). Albumin (Figure 4F) and IgG were present in the areas where fibrinogen and fibrin were distributed and also in other areas.

Advanced Plaques

In eight advanced lesions, the areas staining for fibrinogen-related material were more extensive than in the earlier lesions. Fibrinogen and fibrin I were distributed in large foci in areas of loose connective tissue, around macrophages and smooth muscle cells in the typically observed flecks, and in small tightly packed bundles. Staining was also seen surrounding cholesterol crystals and calcium deposits (Figure 5A). Fibrin II was observed around foam cells and in small flecks and bundles both in the intima and into the media, essentially as seen for fibrinogen/fibrin I. Long threads were observed parallel to the luminal surface in some lesions, suggestive of mural thrombus incorporation. Where cholesterol crystals and calcium deposits were present, fibrin II was usually seen around them (Figure 5B). Fragment D/DD was present in all but one advanced plaque samples and was distributed in the same areas where fibrin II was detected (Figure 5C). The distribution of albumin (Figure 5D) followed the distribution of fibrinogen-related antigens, while IgG was more localized. As previously noted, the polyclonal antiserum to fibrinogen reacted more diffusely than did the monoclonals (not shown).

The left and right coronary arteries from a heart transplant recipient are shown in Figure 6. The left coronary artery (Figure 6B) was more severely affected and showed areas in which cholesterol crystals were present, areas rich in foam cells and macrophages, and also an area with an organizing thrombus. The right coronary artery (Figure 6A) showed early, fatty lesions in the vessel wall with large numbers of monocytes/macrophages in the subendothelium, very close to the lumen, and deeper into the intima. Foam cells were generally found in the intima. Cholesterol crystals were present in a very small area of this vessel, deeper into the intima under a large group of foam cells. As shown in the figure, staining with MAb ICS showed that fibrinogen/fibrin I was present in both these vessels in the intima, neointima, and subintima, and also deeper into the media in the left coronary artery (Figure 6B), and some staining was present in the adventitia in both vessels. Fibrinogen/fibrin I was detected in small areas on the luminal surface in the right and left coronary arteries. Fibrinogen/fibrin I was also seen at the margin of the thrombosed area in the sample from the left coronary (Figure 6B). In the right coronary artery, some fibrin II was focally present in one area surrounding macrophages and foam cells. Fragment D/DD was seen in loose connective

Figure 1. A section of the wall of a normal ascending aorta from a heart transplant recipient. A. A small amount of fibrinogen and fibrin I are distributed in the intima and subintima layers only. No other fibrinogen-related antigens tested were observed. × 129. B. Albumin is present in the deeper layers. × 129.
tissue and around foam cells. In the left coronary artery, fibrin II was detected mainly in the thrombus area and in the intima and neointima in long threads, as well as in areas of loose connective tissue where Fragment D/DD was also detected. The latter was also seen surrounding cholesterol crystals in the same lesion.

**Thrombi**

The immunohistochemical results obtained in thrombi are shown in Table 4. All thrombi stained positively for all the antigens tested.

**Acute Thrombi**

In eight acute thrombi, the distribution of staining varied from more intensely stained central areas to more lightly stained regions toward the edge of the tissue sections. In most thrombi, at the edge of the section, a thin dark ring of staining positive for all the antigens tested and surrounding a larger light ring of nonreactive material was seen. Fibrinogen/fibrin I, fibrin II, albumin, and IgG were detected around leukocytes and platelets. In some samples, a diffuse network-like pattern of tissue staining for all the antibodies with no preferential distribution was observed, while in others, the distribution of fibrinogen-related antigens looked as if the fibrin had formed in parallel, wave-like layers in the direction of blood flow.

**Organized Thrombi**

In four organized thrombi, all the fibrinogen-related antigens were seen in a fibrin mesh pattern, either wavy or more uniform. There was often a light, nonstaining band at the margin of the thrombus. Staining for fibrinogen/fibrin I was weak (Figure 7B), while staining for fibrin II (Figure 7C), and particularly for Fragment D/DD (Figure 7D), was much more intense. In many samples, fibrinogen/fibrin I and fibrin II staining appeared to be surrounding leukocytes and platelets, but not all leukocytes. Staining for Fragment D/DD was found to be concentrated in more central areas of the thrombi. The distribution of fibrinogen-related antigen detected with the polyclonal antiserum to fibrinogen usually included most of the areas seen with the monoclonal antibodies combined (Figure 7E). Staining for albumin (Figure 7F) and IgG was always present in these specimens in the fibrin mesh and it was also observed surrounding leukocytes. Albumin was detected around clusters of cholesterol crystals present in one of the thrombi.

**Old Thrombi**

In the six old thrombi, the same wavy or mesh-like fibrin observed for organized thrombi was seen. Staining for fibrinogen/fibrin I was very light in all the samples studied.

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**Table 3. Vascular and Cardiovascular Samples: Atherosclerotic Plaques**

| Type of lesion and case | Tissue site | TCS | TCS | GC | Fg | Alb | IgG |
|-------------------------|------------|-----|-----|----|----|-----|-----|
| Early plaque            |            |     |     |    |    |     |     |
| 58/1                    | coronary a | +   | -   | +  | +  | ++  | +   |
| 58/2                    | coronary a | +   | +   | -  | +  | ++  | ++  |
| 57/1b                   | as aorta   | -   | -   | -  | -  | -   | -   |
| 55/1b                   | as aorta   | +   | -   | -  | +  | +   | +   |
| 55/2                    | as aorta   | +   | -   | -  | +  | +   | +   |
| 58/4                    | as aorta   | +   | -   | -  | +  | +   | +   |
| 56/1b                   | coronary a | +   | -   | -  | +  | +   | +   |
| Fibrous plaques         |            |     |     |    |    |     |     |
| 24                      | pop-fem an | +   | +   | ±  | ±  | ++  | +   |
| 25                      | pop e      | +   | +   | +  | +  | ++  | ND  |
| 27                      | fem-pop a  | +   | +   | +  | +  | ++  | +   |
| 33                      | fem an     | +   | +   | +  | +  | ++  | +   |
| 47/1                    | fem-pop a  | -   | -   | -  | -  | -   | -   |
| 55/5                    | as aorta   | ±   | -   | ±  | +  | +   | ND  |
| Advanced plaques        |            |     |     |    |    |     |     |
| 23                      | pop-fem a  | +   | +   | -  | +  | +   | +   |
| 26                      | a-bifem a  | +   | +   | +  | +  | +   | +   |
| 31                      | fem-pop a  | +   | +   | +  | +  | +   | +   |
| 42                      | fem-pop a  | +   | +   | +  | +  | ++  | ND  |
| 49                      | fem-pop a  | +   | +   | +  | +  | +   | +   |
| 50                      | fem-pop a  | -   | -   | -  | -  | +   | +   |
| 58/1b                   | coronary a | +   | +   | +  | +  | ++  | +   |
| 36                      | AAA        | +   | +   | +  | +  | +   | +   |

a = artery, as = ascending, an = aneurysm, AAA = abdominal aortic aneurysm, pop = popliteal, fem = femoral, a-bifem = aorto-bifemoral, Fg = fibrinogen, Alb = albumin.

For reactivity score explanation, see legend to Table 2.
Staining for fibrin II was also light in most samples, whereas staining for Fragment D/DD was occasionally more intense and was detected in wider areas. Albumin and IgG were diffusely distributed.

A summary of the results of the immunohistochemical staining of thrombi and atherosclerotic plaques is shown in Table 5. Fibrinogen/fibrin I was found in two of the normal aortas and fibrin II was present in only one normal specimen. Fragment D and D dimer were absent. In contrast, in the atherosclerotic plaques, fibrinogen and fibrin I were detected in six of seven early plaques, in five of six fibrous plaques, and in seven of eight advanced plaques. Fibrin II deposition was present in three of seven early plaques and in four of six fibrous plaques, but the frequency increased to seven of eight in advanced lesions. Fragment D/DD was seen in only one of seven samples in early lesions but was detected in four of six and in six of eight fibrous and advanced plaques, respectively. All the antigens were present in the fibrin mesh of thrombi and frequently surrounded leukocytes and platelets, although intensity and extent of staining varied.
**Discussion**

The distribution of fibrin(ogen)-related antigens in atherosclerotic plaques observed in this study suggests that the presence of these antigens in these lesions is due to multiple causes. Previous studies have attributed the presence of fibrin(ogen)-related material in atherosclerosis to thrombus formation or to increased permeability of the endothelium, with passage of plasma proteins through the vessel wall, especially in the early stages of atherosclerosis. 1-6 Some pioneering studies have linked the presence of fibrin(ogen)-related material in atherosclerosis to lipoproteins and have shown a correlation between lipid-rich lesions and fibrin(ogen) content. These studies also indicated that both soluble fibrinogen and insoluble fibrin were present in the lesions, suggesting that fibrin(ogen)-related antigen in the vessel wall was not only thrombus related. 27-30 The adsorption of LDL onto fibrinogen was also suggested. 31 The distribution of fibrin(ogen)-related antigen described in previous work 33-6 and in the present study is similar.

The improved fixation (Bouin's vs. frozen), together with a highly sensitive ABC immunoperoxidase technique and well characterized monoclonal antibodies, has led to better morphology with a higher degree of resolution and specificity. This has allowed the detection of fibrinogen/fibrin I, fibrin II, and Fragment D/DD directly in the tissue, with morphologic detail and information that was not previously available. In this, as in the previously men-
tioned studies, thrombi were observed on the luminal surface in a number of lesions, and the incorporation of mural thrombi was strongly suggested when large threads of fibrinogen-related antigen detected with the monoclonal antibodies were distributed in the intima, subintima, and media parallel to the endothelial surface.

Little fibrin II was seen along the endothelial surface in the aorta samples, while larger deposits were detected in the coronary artery samples, not only in areas of thrombus. This might be related to the size and rate of blood flow in such vessels. Previous studies have hypothesized the existence of a fibrin lining of blood capillaries as an important factor in controlling vascular permeability.31,32

The present study has examined only larger vessels in which this fibrin layer has not been identified with the method used. However, in capillaries, such as glomerular
Figure 5. An area of cholesterol crystals in an advanced lesion. A. Fibrinogen/fibrin I is present in the central area. B. Fibrin II is more widespread. C. Fragment D/DO is localized in the same area. D. Staining for albumin in the same area is very intense. × 130.

Table 4. Vascular and Cardiovascular Samples: Thrombi

| Type of lesion and case | Tissue site | i8C5 | T2G1 | GC4 | Fg | Alb | IgG |
|-------------------------|------------|------|------|-----|----|-----|-----|
| Acute thrombi           |            |      |      |     |    |     |     |
| 30/2                    | AAA        | +    | ++   | ++  | ++ | +   | ND  |
| 35                      | fem a      | ++   | ++   | +   | ++ | +   | +   |
| 37                      | fem pop a  | ++   | ++   | +   | ++ | +   | +   |
| 38/2                    | AAA        | +    | +    | +   | +  | +   | +   |
| 38/4                    | AAA        | ±    | +    | +   | +  | +   | +   |
| 43/1                    | AAA        | +    | +    | +   | +  | +   | ND  |
| 47/2                    | fem pop a  | +    | +    | +   | +  | +   | +   |
| 48/2                    | embolus    | ±    | +    | +   | ++ | +   | +   |
| Organized thrombi       |            |      |      |     |    |     |     |
| 43/2                    | AAA        | +    | +    | +   | +  | ND  | +   |
| 43/5                    | AAA        | ++   | +    | +   | +  | ND  | +   |
| 44/1                    | fem a an   | +    | +    | +   | +  | ±   | ND  |
| 44/2                    | fem a an   | +    | +    | ND  | +  | +   | ND  |
| Old thrombi             |            |      |      |     |    |     |     |
| 28                      | AAA        | ++   | ++   | ++  | ++ | +   | ND  |
| 32                      | AAA        | –    | +    | ++  | +  | +   | ND  |
| 34/2                    | AAA        | +    | +    | +   | +  | ND  | +   |
| 43/4                    | AAA        | ±    | +    | +   | +  | ND  | +   |
| 45                      | AAA        | ±    | +    | +   | ND | +   | ND  |
| 46                      | AAA        | –    | +    | +   | ND | +   | ND  |

For abbreviations, see legend to Table 3. For explanation of reactivity scores, see legend to Table 2.
capillaries, this is still an open question that is being investigated in a parallel study.\textsuperscript{33} When diffuse staining was observed, insudation of plasma proteins due to altered permeability of the endothelium could be an explanation; although this concept has been challenged.\textsuperscript{34}

In the present study, two patterns of distribution of fibrin(ogen) were observed around vessel wall cells. Flecks of fibrin in an atherosclerotic vessel were previously described;\textsuperscript{3,5} however, the methods used did not allow recognition of an association of such fibrin flecks with vessel wall cells. In a more recent study, an association of foam cells with fibrin was seen in organizing thrombi.\textsuperscript{35} In this work, flecks of fibrinogen/fibrin I and fibrin II with a stellate patchy appearance surrounded cells, which seemed to be smooth muscle cells and macrophages\textsuperscript{36,37} and these flecks were observed in numerous lesions. Moreover, a net-like distribution was clearly observed around lipid-laden foam cells.\textsuperscript{30,39,40}

Monocytes and macrophages have been shown to have specific receptors for fibrin(ogen).\textsuperscript{41-44} These cells have also been shown to possess procoagulant and fibrinolytic properties.\textsuperscript{45-50} By immunofluorescence and immunoelectronmicroscopy, some authors have demonstrated the formation of fibrin strands deposited around both viable and fixed peripheral blood monocytes.\textsuperscript{43,46,51} These fibrin spindles formed around monocytes confer a patchy stellate appearance similar to that observed in the present study. More mature macrophages from lung, thymus, and breast milk did not have this capability, although tonsillar phagocytes did. It has been suggested that fibrin formation was associated with monocyte activation and that macrophages, themselves, might contain fibrinogen stored in granules.\textsuperscript{43} In another study, rabbit peritoneal macrophages were incubated with purified labeled fibrin monomers, and electron micrographs revealed an association with the cell surface and subsequent internalization of the fibrin monomers.\textsuperscript{52} Such studies suggest that macrophages may perform a scavenger function in the clearance of fibrin from the circulation.\textsuperscript{53,54}

Monocytes bind little fibrinogen if they are not activated.\textsuperscript{43,44,46,51} However, such cells could be activated on the surface of a biochemically altered endothelium on which thrombin is formed and to which platelets might adhere and release ADP.\textsuperscript{44} In addition, monocytes may be activated by lipoproteins,\textsuperscript{55} by some of their own secretory products of arachidonate metabolism, or by platelet-activating factor.\textsuperscript{56} Activation by any of these mechanisms may confer on such cells the ability to transport fibrinogen through the vessel wall. In addition, monocyte procoagulant activity might be stimulated by the lymphocytes present in the lesion,\textsuperscript{56-58} as it has been reported that activated lymphocytes induce macrophage procoagulant activity.\textsuperscript{47,51-58} What modulates and regulates the function of these cells in the vessel wall is not known. In a recent study in Watanabe and fat-fed rabbits, it has been shown that subendothelial macrophage-foam cells demonstrate thrombi on their surface.\textsuperscript{64,65} The fibrin(ogen)-related antigen association with foam cells in the present study was morphologically distinct from that with macrophages. Generally, foam cells cluster together and are mixed with a fibrin(ogen)-related antigen net-like mesh. No association between smooth muscle cells and fibrin formation is known nor has the procoagulant and fibrinolytic activity of foam cells yet been studied. How the procoagulant and fibrinolytic activity of macrophages, smooth muscle cells, and foam cells might be modulated in atherosclerotic plaques has not been investigated.

Fibrinolytic activity in atherosclerotic vessels has been reported to be decreased. However, it is still a matter of debate whether this is due to a decreased amount of plasminogen activator or to an increase in plasminogen.

**Table 5. Summary of Antibody Reactivity of Human Atherosclerotic Lesions**

| Lesions             | MAB/1BC6 | MAB/T2G1 | MAB/GC4 |
|---------------------|----------|----------|---------|
| Normal wall         | 2/12     | 1/12     | —       |
| Early plaques       | 6/7      | 3/7      | 1/7     |
| Fibrous plaques     | 5/6      | 4/6      | 4/6     |
| Advanced plaques    | 7/8      | 7/8      | 6/8     |
| Acute thrombi       | 8/8      | 8/8      | 8/8     |
| Organized thrombi   | 4/4      | 4/4      | 3/3     |
| Old thrombi         | 4/6      | 6/6      | 6/6     |

Figure 6. Immunoperoxidase with MAB 1BC6 for fibrinogen/fibrin I in sections of the left and right coronary arteries of a heart transplant recipient. A. In the right coronary artery, fibrinogen/fibrin I is distributed in the atherosclerotic lesion involving the intima, mainly in the subendothelium and deeper into the intima as flecks and threads, in areas where macrophages and foam cells were seen (although not visible at this magnification). B. In the left coronary, which was more severely affected, fibrinogen/fibrin I can be seen distributed in a thrombus area along the endothelium and deeper in the media in areas where macrophages and cholesterol crystals were present, although not visible at this magnification. x2.56
Activator inhibitor. The possible influence of fibrinogen and fibrinogen degradation products on the cellular components of plaques is being investigated in parallel studies in which preliminary results have shown an increase in growth factors released by endothelial cells upon incubation with fibrinogen degradation products. No fibrinogen-related antigens were detected in any lesion in the areas of dense fibrous connective tissue.

In all lesions, albumin was detected in the areas where fibrinogen-related antigen was observed and the albumin showed a parallel pattern of distribution, while IgG was not always present. Some recent studies have investigated the interaction between fibrinogen and albumin and have shown an effect of albumin on increased fibrin fiber thickness and on the limitation of growth of the fibrin fibers but not of their density. Such interactions could influence the egress of fluid and blood proteins trapped in the vessel wall, the morphology of the fibrin, and the migration of the macrophages in the atherosclerotic lesions.
The results from the present study indicate that fibrinogen-related antigen in the vessel wall is not related only to thrombus formation or to increased permeability of an injured endothelium. The detection of the different biochemical forms of fibrinogen surrounding macrophages and smooth muscle cells strengthens our hypothesis that such cells may be actively involved in the fibrinogen-to-fibrin transition. The purpose of this study was not to try to determine whether fibrin plays a causative role in the development of atherosclerosis. However, our data show that a higher degree of fibrin polymerization, and presumably its subsequent degradation, is associated with increasing severity of atherosclerotic lesions. We, therefore, believe that fibrin present in atherosclerotic plaques is not merely an inert component of the lesion, but rather it might be formed by and interact with vessel wall cells. More detailed studies on the interaction of fibrinogen with vessel wall cells and with other plasma and/or tissue proteins will be necessary to obtain a more integrated picture of the development of atherosclerotic disease.

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