American Journal of Pathology, Vol. 137, No. 5, November 1990
Copyright © American Association of Pathologists

Human Abdominal Aortic Aneurysms

Immunophenotypic Analysis Suggesting an Immune-mediated Response

Alisa E. Koch,* G. Kenneth Haines,† Robert J. Rizzo,‡ James A. Radosevich,* Richard M. Pope,* Philip G. Robinson,† and William H. Pearce‡

From the Departments of Medicine, Pathology,† and Vascular Surgery,‡ Northwestern University Medical School and Veterans Administration Lakeside Medical Center, Chicago, Illinois*

Cellular immunity may play a role in the pathogenesis of atherosclerosis. In this report the potential role of these cells in the formation of abdominal aortic aneurysms by immunohistochemistry was investigated. Aortic tissues from 32 patients were examined: 4 normal aortas, 6 aortas with occlusive atherosclerotic disease, 17 abdominal aortic aneurysms, and 5 inflammatory abdominal aneurysms. Using monoclonal anti-CD3 (T cells), anti-CD19 (B cells), anti-CD11c (macrophages), anti-CD4 (T helper cells), and anti-CD8 (T suppressor cells), several distinctions among these groups were found. The amount of inflammatory cell infiltrate was as follows: inflammatory aneurysms more than abdominal aortic aneurysms more than occlusive aortas more than normal aortas. CD3-positive T lymphocytes rarely were found in the adventitia of normal or occlusive aortas. In contrast, abdominal aortic aneurysms and inflammatory aneurysms exhibited most of the CD3-positive infiltrates in the adventitia. CD19-positive B lymphocytes were present mainly in the adventitia of all pathologic tissues. The CD4-positive:CD8-positive ratio was greater in abdominal aortic aneurysms and inflammatory aneurysms than in the other groups, both in the adventitia and in the media of the aortas. CD11c-positive macrophages were present throughout the diseased tissues, often surrounded by lymphoid aggregates; the greatest numbers of macrophages were found in the inflammatory aneurysm group. Our data suggests that the aneurysmal disease may progress from occlusive disease and is accompanied by an increase in chronic inflammatory cells as well as a redistribution of these cell types. Therefore it is suggested that aneurysmal disease may represent an immune-mediated event. (Am J Pathol 1990, 137:1199–1213)

The aortic atherosclerotic plaque is characterized by intimal cell proliferation, lipid accumulation, and an increase in connective tissue.[1] Clinical symptoms often are caused by ulceration of an atherosclerotic plaque, by arterial occlusion, or by perforation of a dilated aneurysm.

Smooth muscle cell proliferation is a key event in the development and progression of atherosclerosis, but the role of other cell types, such as inflammatory cells, is less well defined.[2] Mononuclear cells, including lymphocytes and monocytes, are among the first participants in the early phases of experimental and spontaneous atherogenesis.[2] Joris et al[3] and Emeson et al[2] demonstrated that there are mononuclear infiltrates in human coronary arteries of children and adults dying of acute trauma. Indeed cellular infiltrates have been found in early human fatty streaks, diffuse intimal thickening, as well as in complicated atherosclerotic plaques.[4–6] To date, much attention has been paid to the histologic changes found in the intimas of diseased arteries, with very little attention paid to the adventitial and medial areas of the aortas.

Because inflammatory cells appear to be important in the immunopathogenesis of atherosclerosis, they may also be involved in the formation of aneurysms. Atherosclerosis is the most common cause of abdominal aortic aneurysms in Western societies, although mycotic aneurysms, secondarily infected atherosclerotic aneurysms, aneurysms associated with connective tissue disorders such as Marfan's syndrome, aneurysms due to Takayasu's arteritis,

Supported by NIH Multipurpose Arthritis Center Grant AM30692 (to Alisa Koch and Richard Pope), funds from the V.A. Research Service (to Alisa Koch, Richard Pope, James Radosevich, and William Pearce), Arthritis Foundation Fellowship (to Alisa Koch), and Arthritis Foundation Grants (to Alisa Koch and Richard Pope).

Accepted for publication June 25, 1990.

Address reprint requests to Dr. Alisa Erika Koch, Department of Medicine, Section of Arthritis and Connective Tissue Disorders, Ward Building 2-250, 303 E. Chicago Ave., Chicago, IL 60611.

and inflammatory aneurysms occur.[7-12] Whether 'inflammatory aneurysms' are a variant of atherosclerotic aneurysms or represent a separate periaortic inflammatory process characterized by adventitial inflammation and fibrosis is not clear.[13]

Human aortic aneurysmal tissues have not been analyzed using inflammatory cell-specific monoclonal antibodies to determine whether cells of the immune system play a role in aneurysm formation. To this end, monoclonal antibodies directed against surface antigens of B cells, macrophages, and T cells and their subsets were used to compare the frequency and distribution of these cell types in normal aortas, abdominal aortic aneurysms, inflammatory abdominal aortic aneurysms, and occlusive atherosclerotic aortic diseases. We present evidence suggesting involvement of inflammatory cells in the development of not only inflammatory abdominal aortic aneurysms but also noninflammatory abdominal aortic aneurysms, as well as occlusive aortas.

Materials and Methods

Tissue Samples

Tissue samples of infrarenal abdominal aortas were obtained from 32 patients. These tissues included 4 normal aortas obtained at autopsy. These specimens were compared with abdominal aortic samples obtained during surgery for occlusive aortic disease (6 patients), and abdominal aortic aneurysmal disease (23 patients). Occlusive aortas were atherosclerotic aortas without an enlarged diameter. Among the 23 patients with abdominal aortic aneurysmal disease, 5 were classified as inflammatory aneurysms, while 17 were classified as abdominal aortic aneurysms. Inflammatory abdominal aortic aneurysms were clinically defined by the white glistening appearance of the adventitia at the time of surgery. In addition, they adhered to surrounding structures such as the duodenum, inferior vena cava, left renal vein, or ureters, and often were associated with retroperitoneal fibrosis. Each of the surgical patients gave informed signed consent before donating tissue.

In addition, to confirm the reactivity patterns of the monoclonal antibodies studied, normal lymph node and splenic tissues were obtained from patients at autopsy within 4 hours of death.

Monoclonal Antibodies

For most cases, five monoclonal antibodies were used. All antibodies were obtained from Becton-Dickinson (Mountain View, CA). Leu 4 (anti-CD3) recognizes all peripheral blood T lymphocytes. B4 (anti-CD 19) defines a bimolecular structure of 40 and 80 kd found on all normal B lymphocytes. Leu 3a (anti-CD4) defines the helper/inducer subset of T lymphocytes. Leu 2a (anti-CD8) defines the suppressor/inducer subset of T lymphocytes. Leu M5 (anti-CD11c) detects monocytes and macrophages. In addition, to confirm macrophage reactivity, in select cases monoclonal HAM56, which reacts with tissue macrophages but not smooth muscle, was used (Enzo Biochemical, Inc, New York, NY).

Immunohistochemistry

Sections cut from frozen tissues embedded in optimal cutting temperature compound (OCT) (Miles Scientific, Naperville, IL) were stained using the avidin-biotin complex (ABC; Vector Laboratories, Burlingame, CA), as we and others have previously described.[14-17] Serial tissue sections were air dried for 5 to 7 minutes and fixed in cold acetone for 20 minutes. All antibody incubations were performed for 15 minutes at 37°C in a moist chamber. The tissue sections were pretreated with normal horse serum containing 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS) and then with either one of the monoclonal antibodies or an isotype-specific irrelevant control monoclonal antibody. Slides were washed twice with PBS. The tissue sections then were incubated with a 1:400 dilution of anti-mouse biotinylated antibody in PBS-BSA, washed twice with PBS, and then incubated with ABC. After washing the slides in PBS twice, they were treated with a solution of 5 mg of diaminobenzidine tetrahydrochloride (DAB; Sigma, St. Louis, MO) for 7 minutes. The sections were rinsed in tap water for 2 minutes and counterstained with hematoxylin.

Microscopic Evaluation

Hematoxylin and eosin staining of the frozen tissues or formalin-fixed, paraffin-embedded tissues was performed to confirm the identity of the sampled specimens. All of the tissue specimens were assessed histologically and demonstrated well-preserved cellular detail. Both the adventitial and medial areas of the blood vessels were examined for inflammatory infiltrates. Approximately 10 sections of each tissue were examined.

Each tissue was assigned an inflammatory score of 0 to 4 for both the lymphocytes and macrophages present. A score of 0 represented no inflammation, while a score of 4 represented severe inflammation. For each tissue, the entire frozen section was examined and the inflammatory scores were averaged over all areas of each tissue section. The same scale was applied to both cell types. The entire analysis of immunohistochemical staining was

Table 1. *Immunohistochemical Analysis of Occlusive Atherosclerotic Aortas*

Case	Inflammatory score	Percentage CD3+ T lymphocytes	CD4:CD8+ T-lymphocyte ratio	Percentage CD19+ B lymphocytes	CD11c+ macrophage score
1	1	90	2.0	0	1.0
2	2	70	5.0	5	1.0
3	1	90	5.0	5	0
4	3	80	5.0	5	1.0
5	1	90	5.0	0	1.0
6	1	60	4.0	10	1.0
Group mean ± SE	1.5 ± 0.3	80 ± 5	4.3 ± 0.5	4 ± 1	0.8 ± 0.5

performed by a pathologist who was blinded to the disease category to which the samples belonged.

For each tissue, the percentage of cells consistent with the morphology of lymphocytes that were CD3 positive was determined. Depending on the degree of inflammatory infiltrate, approximately 400 cells per tissue were counted. In those areas that were CD3 positive, serial 5-μ-thick frozen sections of tissue were examined to determine the percentage of cells that was also positively stained with anti-CD4 or anti-CD8. B cells were identified by anti-CD19. The tissues were assigned a CD11c-positive score based on the number of CD11c positive cells morphologically consistent with macrophages in the tissues. There was some cross-reactivity noted with smooth muscle cells using anti-CD11c. These cells were excluded based on their spindle-shaped appearance. Ham56 was used to confirm macrophage reactivity in select tissues.

Statistical Analysis

Differences between the mean values of groups was determined using analysis of variance.[18] P values less than 0.05 were considered significant.

Results

General Histology and Analysis of Abdominal Aortic Tissues

Overall most of the abdominal aortic aneurysms and inflammatory aneurysms examined had intact medial and adventitial layers. However the intimal layer was generally replaced by atherosclerosis. The intimal changes may represent the end stage of the aortic atherosclerotic process. The aortic tissues examined in this study did not show evidence of medial attenuation. Because the medial and adventitial areas of the aorta are integrally involved in aneurysm formation, we confined the analysis of the tissues to these areas.

The normal aortic tissues had little or no inflammatory cells present. In contrast, all three pathologic groups studied had significantly greater inflammatory scores than the normal tissues ($P < 0.05$; Tables 1 to 3). The pathologic inflammatory score corresponded to the surgical diagnosis of inflammatory aneurysm, with these tissues displaying the greatest amount of inflammatory infiltrate (mean inflammatory score of 3.0 ± 0.49, SE).

Table 2. *Immunohistochemical Analysis of Abdominal Aortic Aneurysms*

Case	Inflammatory score	Percentage CD3+ T lymphocytes	CD4+:CD8+ T-lymphocyte ratio	Percentage CD19+ B lymphocytes	CD11c+ macrophage score
1	3	80	6.5	30	2
2	1	20	15	0	2
3	2	60	5.5	30	2
4	3	55	10	60	1
5	1	80	20	20	2
6	2	50	5	60	3
7	3	80	3	20	2
8	2	85	*	25	2
9	2	85	4.3	15	1
10	3	80	4	10	0
11	2	80	4.3	25	0
12	1	20	8	80	3
13	1	70	13	10	2
14	1	80	3	0	0
15	3	75	4.3	30	0
16	2	70	7	25	0
17	3	80	9	30	0
Mean ± SE	2.0 ± 0.19	67 ± 5	7.6 ± 1.1	25 ± 5	1.3 ± 0.2

* Represents a score of 80:1, which was not included in the analysis.

Table 3. *Immunohistochemical Analysis of Inflammatory Abdominal Aortic Aneurysms*

Case	Inflammatory score	Percentage CD3+ T lymphocytes	CD4+:CD8+ T-lymphocyte ratio	Percentage CD19+ B lymphocytes	CD11c+ macrophage score
1	3	60	11	40	2
2	4	80	9	20	2
3	1	80	7	20	1
4	3	80	5.3	20	3
5	4	80	5.7	20	4
Mean ± SE	3.0 ± 0.49	76 ± 3	7.6 ± 0.95	24 ± 3	2.4 ± 0.46

Normal Abdominal Aortic Tissues

Four tissues were examined from autopsy samples of patients who died of nonvascular causes. Among the four tissues studied, only one had any inflammatory cells, and this infiltrate was minimal. The inflammatory cells in this tissue were located mainly in the adventitia. Eighty percent of these cells were CD3-positive T lymphocytes, less than 10% were CD19-positive B lymphocytes, and a small percentage were CD11c-positive macrophages. Because only one of the tissues displayed any degree of inflammation, these tissues were not further classified as to their content of CD4-positive helper or CD8-positive suppressor T lymphocytes.

Occlusive Atherosclerotic Abdominal Aortic Tissues

Six tissues were examined from stenotic atherosclerotic abdominal aortas (Table 1). Generally these tissues had low inflammatory scores compared to the aneurysmal aortas. The most predominant inflammatory cell type was the CD3-positive lymphocyte, representing a mean of 80% ± 5% of the lymphocytes. The bulk of these cells were located in the media (75% ± 13%) rather than in the adventitia (25% ± 13%) (Figure 1). The CD4-positive:CD8-positive T-lymphocyte ratio in these tissues was approximately 4:1. This ratio did not vary greatly between adventitia and media (Figure 2, Table 1). CD 19-positive B

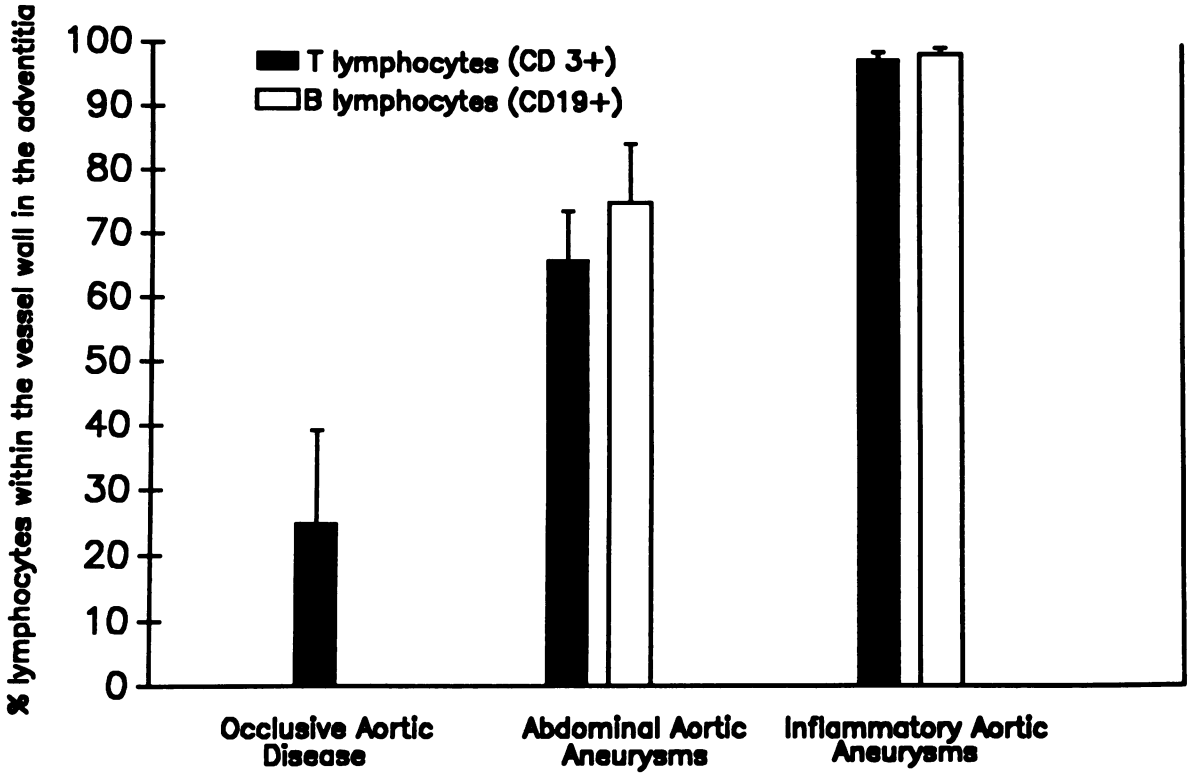

Figure 1. *The percentage of CD3+ T lymphocytes located in the adventitia (as compared to the media) is shown (solid bar). The percentage of CD19+ B lymphocytes in the adventitia (as compared to the media) is also shown (open bar). Because the number of CD19+ B cells within the occlusive aortas was so small, no percentage of cells located in the adventitia is shown for this group.*

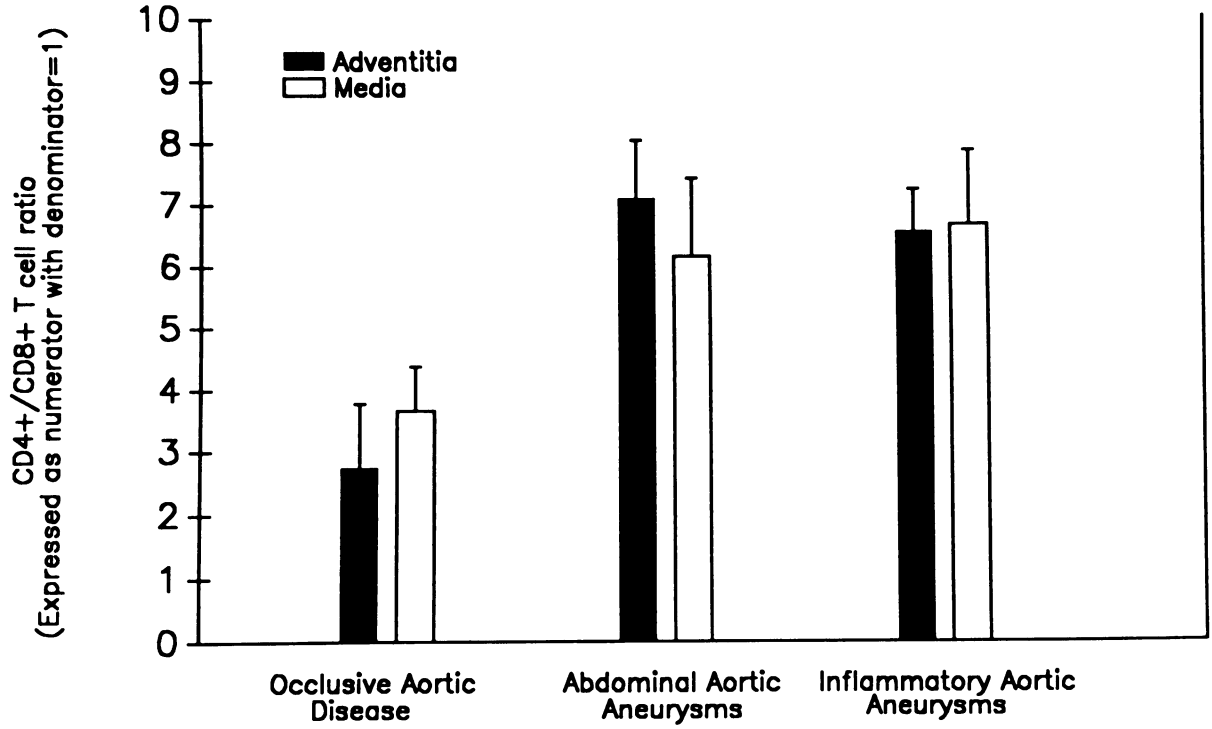

Figure 2. *The CD4+:CD8+ T-cell ratio for aortic tissues is shown. The numbers on the abscissa represent the numerator of the ratio, with the denominator equal to one.*

lymphocytes represented less than 10% of lymphocytes. There were no CD19-positive B cells in two of the six tissues studied. Among the other four tissues, 100% of the CD19-positive cells were located in the adventitia. The overall macrophage score assigned to these tissues ranged form 0 to 1, with the macrophages distributed throughout the adventitia and media (Figure 3, Table 1). Macrophages were seen near the atheromatous plaques and scattered among lymphocytes. Figure 4 shows an example of an occlusive aorta staining with anti-CD3. Greater numbers of CD3-positive T lymphocytes were located in the media as compared to the adventitia of the aortas.

Abdominal Aortic Aneurysms

Seventeen tissues were studied in this group (Table 2). The percentage of CD3-positive cells was less than the percentage of CD3-positive cells found in the occlusive aortas (mean of 67% *versus* 80%). Unlike the occlusive aortas, there was a significantly ($P < 0.05$) greater percentage of CD3-positive cells present in the adventitia (66%) compared to the media 34% (Figure 1). The CD4-positive:CD8-positive T-lymphocyte ratio for the abdominal aortic aneurysms ranged from 3:1 to 20:1. One tissue displayed a ratio of 80:1. This tissue was omitted from the analysis. Overall the mean CD4-positive:CD8-positive ratio was 7.6:1, which was slightly greater than the 4.3:1 ratio found in the occlusive aortas (Figure 1). The percentage of B lymphocytes was significantly greater in the abdominal aortic aneurysms, ranging from 0% to 30%, with a mean of 25% ± 5% *versus* 0% to 10% (mean, 4% ± 1%) compared to the occlusive aortas ($P < 0.05$; Tables 1 and 2). In both the abdominal aortic aneurysms and occlusive aortas, most CD19-positive B lymphocytes were located in the adventitia ($P < 0.05$; Figure 2). The CD11c-positive macrophage score ranged from 0 to 3, with a mean of 1.3 ± 0.2 (Table 2). This score was somewhat greater than the mean score of 0.8 for macrophages in the occlusive aortas. There was essentially no significant difference in the number of CD11c-positive macrophages located in the adventitia *versus* the media of the abdominal aortic aneurysms. Staining with HAM56 confirmed the observations made with anti-CD11c. Several histologic patterns were seen in the abdominal aortic aneurysms as well as in the inflammatory aneurysms. CD19-positive B lymphocytes often were surrounded by CD3-positive T lymphocytes. The lymphocytes often were located around the vasa vasorum, particularly in the adventitial areas of aorta. CD11c-positive macrophages were found scattered within lymphoid aggregates. While well-developed follicles with germinal centers were not present, many lymphoid aggregates were seen. The distribution of CD3-positive

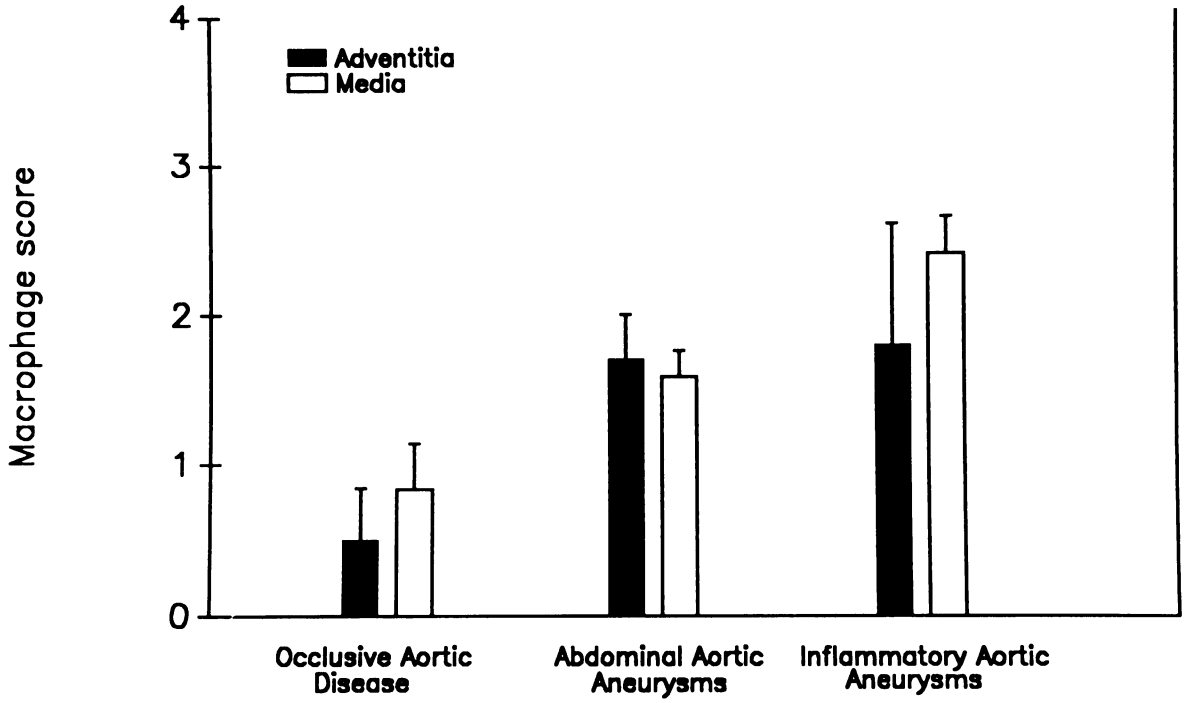

Figure 3. *The CD11c+ macrophage score for the media and adventitia of aortic tissues is shown for occlusive disease, abdominal aortic aneurysms, and inflammatory abdominal aneurysms.*

T lymphocytes in both the media and the adventitia of blood vessels is depicted in Figure 5. Compared to the occlusive aortas, many more CD19-positive B lymphocytes were present. There were more CD11c-positive macrophages present than in the occlusive aortas.

Inflammatory Aortic Aneurysms

Overall these tissues demonstrated a higher inflammatory score than any of the other patient groups studied (Table 3). As a group, these tissues also displayed a much narrower range of CD3-positive T cells than did either the occlusive or abdominal aortic aneurysm groups. Almost all of the CD3-positive T cells were located in the adventitia *versus* the media ($P < 0.05$), differing from the situation in the occlusive aortas (Figures 1 and 6). The CD4-positive: CD8-positive ratios in this group were approximately 7:1. This ratio was greater than that seen in the occlusive group ($P < 0.05$) and the same as that found in the abdominal aortic aneurysm group (Figure 6). CD19-positive B lymphocytes constituted from 20% to 40% (mean, 24% ± 3%) of the lymphocytes found in the tissues, again differing from the paucity of CD19-positive B cells found in the occlusive aortas. Ninety-nine percent of the CD19-positive B cells were located in the adventitia as compared to a mean of 75% lymphocytes present in the adventitia of abdominal aortic aneurysms ($P < 0.05$; Figure 1). The macrophage scores in this were higher than both the occlusive aorta group ($P < 0.05$), as well as the abdominal aortic aneurysm group. The predominance of CD11c-positive macrophages is shown in Figure 6. There was essentially no appreciable difference between the location of the macrophages within the adventitia and the media of these tissues.

Discussion

This study aimed to define the interrelationships between CD3-positive T lymphocytes, CD19-positive B lymphocytes, and macrophages in the vessel wall of various groups of diseased abdominal aortas. Representative sections from each tissue were examined, making it unlikely that sampling bias occurred. We found several differences between the groups of tissues examined. A schematic diagram of the main findings, comparing the groups, is shown in Figure 7.

Normal aortic tissues contain few, if any, inflammatory cells, in sharp contrast to the diseased tissues that appear to display a spectrum of responses. The degree of reactivity ranges from mild inflammatory changes found in the occlusive aortas, to more severe changes found in the abdominal aortic aneurysms, to the even more severe changes found in the inflammatory aneurysms. The amount of inflammation present in these tissues is re-

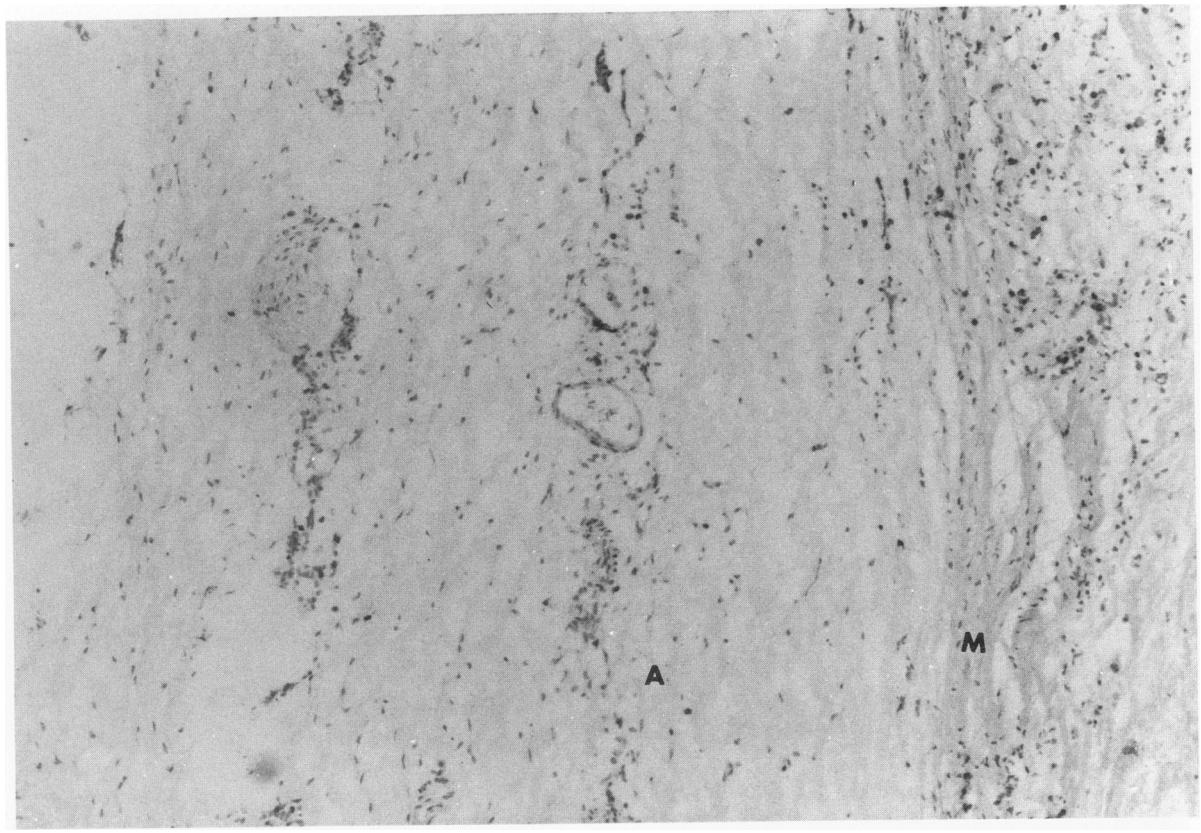

Figure 4. *Frozen section of an occlusive aorta. Immunoperoxidase staining with anti-CD3. The adventitia is indicated by A, while the media is indicated by M. The predominance of CD3+ T lymphocytes located in the media of the aortas is shown (100×).*

flected by the overall inflammatory score assigned to each group, with the normal tissues having a mean inflammatory score of 0.25 and the inflammatory aneurysms having a mean score of 3.0. Because occlusive aortic disease lesions progress to aneurysmal degeneration, most inflammatory cells become located in the adventitia rather than in the media of the aortas. These findings suggest that abdominal aortic aneurysms and inflammatory aneurysms may be considered variants within the spectrum of aneurysmal disease rather than distinct pathologic entities.

In this study we found that in all of the disease groups, the bulk of the inflammatory cells present in the media and adventitia of the aortas were CD3-positive T lymphocytes, ranging from a mean of 67% to 80%. CD3-positive T lymphocytes varied in location, exhibiting a difference between the groups of tissues studied. In the occlusive aortas, only one fourth of CD3-positive T lymphocytes were located in the adventitia, while most were present in the media. In contrast, in the abdominal aortic aneurysms and in the inflammatory aneurysms, the CD3-positive T lymphocytes were found predominantly in the adventitia (66% and 99%, respectively). It is unlikely that these findings represent changes due to attenuation of the media because, using ocular micrometer measurements, medial thickness was not attenuated in the aortic or inflammatory aneurysms in comparison to the normal or occlusive aneurysms. In all of the tissues, the lymphocytes generally were found in aggregates around the vasa vasorum.

The finding of many CD3-positive lymphocytes in the adventitia of inflammatory and abdominal aortic aneurysms is not surprising. While much attention has been paid to the histologic changes found in the intimas of atherosclerotic vessels, little has been reported about the inflammatory cells present in the media and adventitia of the same vessels. Swartz et al[19,20] used histochemistry of formalin-fixed, paraffin-embedded tissues to study more than 700 atherosclerotic arterial blocks of aortic, coronary, cervical, and iliac arteries, but did not study aneurysms. They found that adventitial cellular infiltrates, particularly lymphocytic, was related to the atheroma, and that the degree of inflammation showed a constant relationship to the degree of plaque formation. They did not study the type of cells involved by immunohistochemistry. The inflammatory cells in the nonintimal areas of blood vessels may play a role in the perpetuation of aortic disease.

To better understand the function of these CD3-positive T lymphocytes, we determined the CD4-positive:CD8-

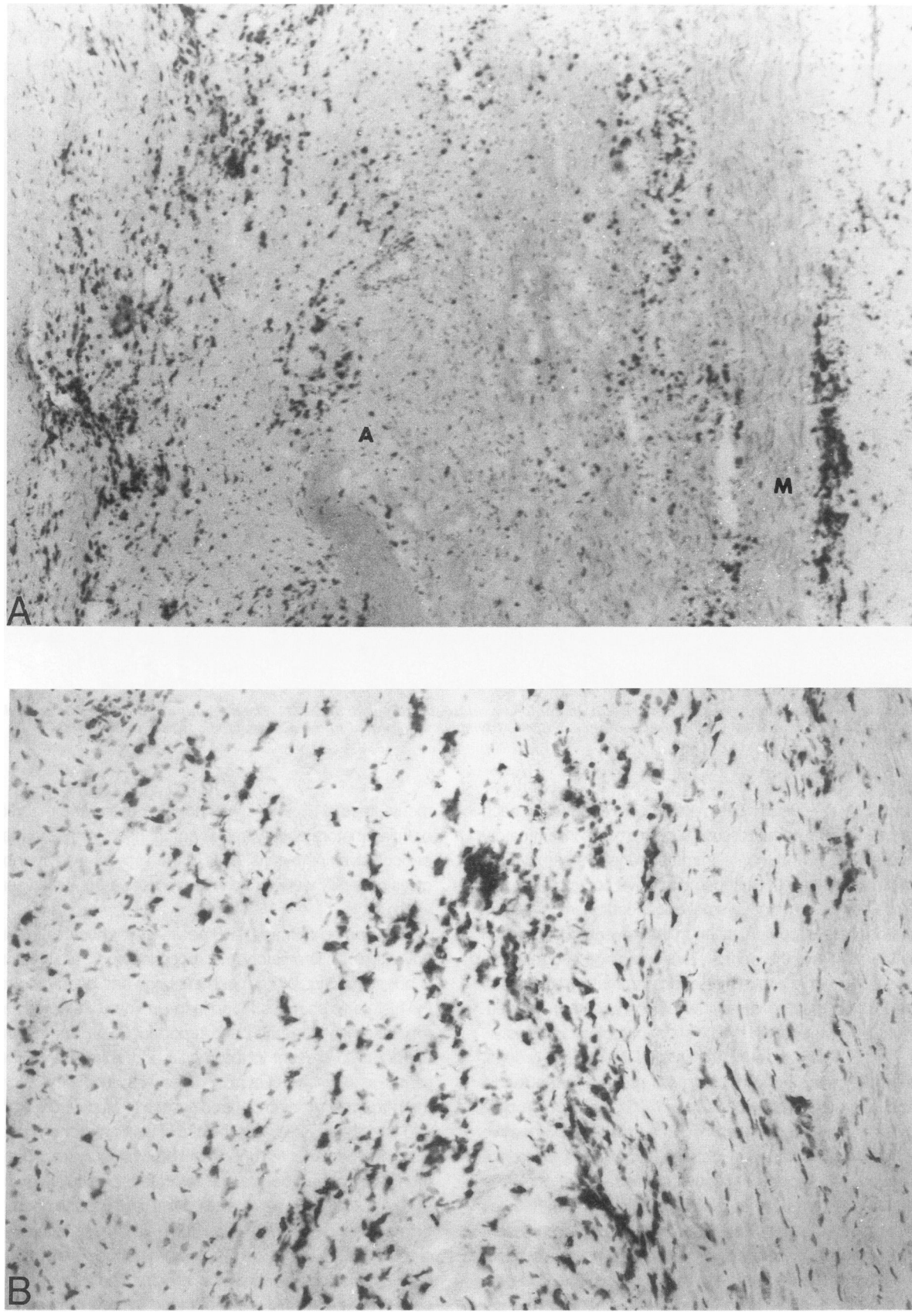

Figure 5. *Immunoperoxidase staining of frozen sections of an abdominal aortic aneurysm.* **A:** *The similar distribution of CD3+ T lymphocytes in the adventitia (A) and media (M) of the aneurysm (80×).* **B:** *High-power view of anti-CD3+ T-lymphocyte staining in the media of the aorta (200×).* **C:** *Anti-CD19+ staining of a large number of B lymphocytes in the media (M) and adventitia (A) of the aorta (200×).* **D:** *Anti-CD11c staining of macrophages scattered among lymphocytes (arrow) (510×).*

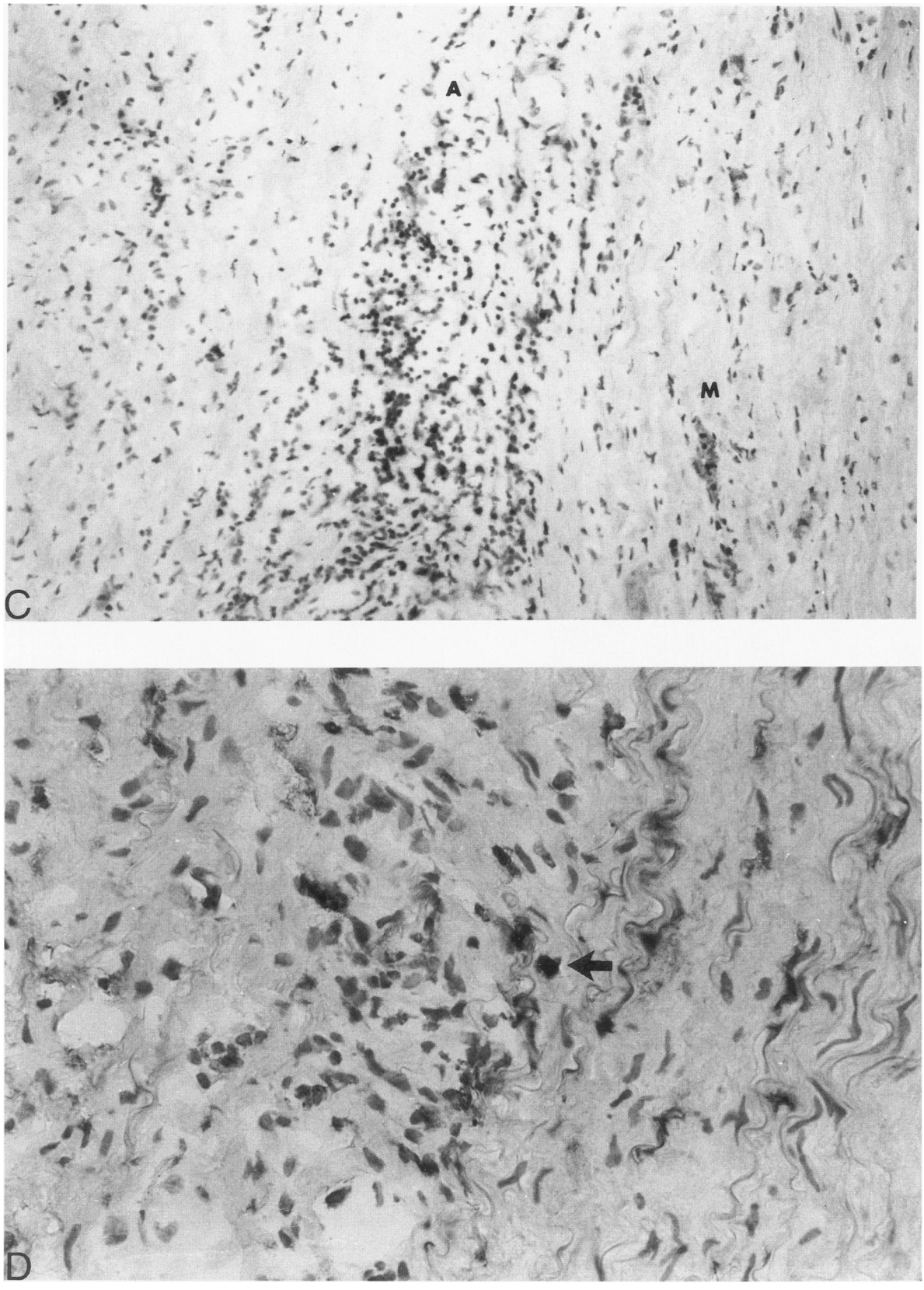

Figure 5 (*continued*)

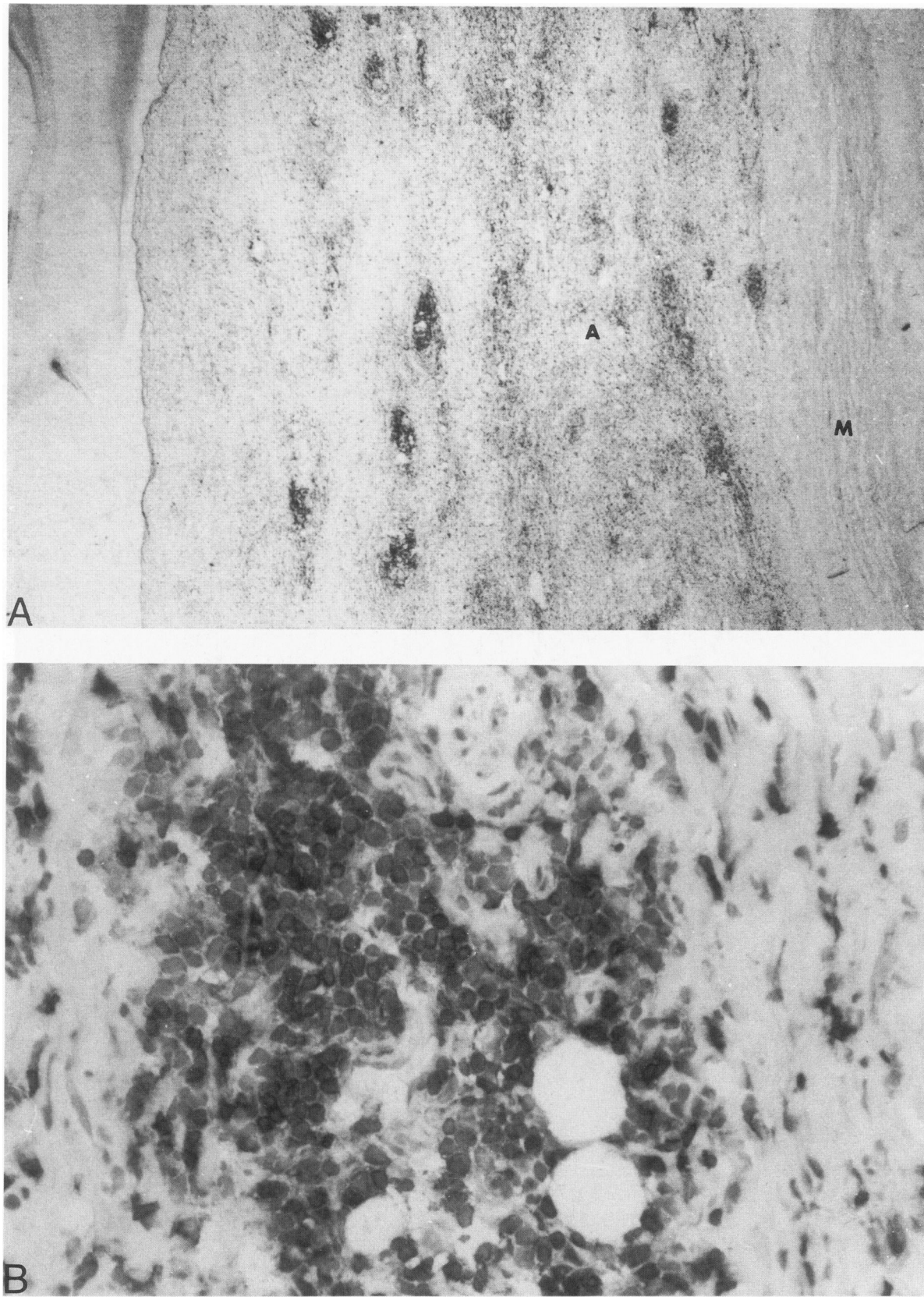

Figure 6. *Immunoperoxidase staining of frozen sections of an inflammatory aneurysm having an inflammatory score of 4.* **A:** *A low-power view of a tissue with CD3+ cells present predominantly in the adventitia (A) as opposed to the media (M) (20×).* **B and C:** *Lymphoid aggregate in the adventitia showing preponderance of anti-CD3+ T-lymphocyte staining* **(B)** *over anti-CD19+ B-cell staining* **(C)** *(500×). CD19+ B-cell staining is indicated by the arrow in* **C. D:** *The distribution of CD4+ lymphocytes in the same lymphoid aggregate (500×).* **E:** *Distribution of CD8+ lymphocytes (arrow). There are fewer CD8+ lymphocytes than CD4+ cells in any given tissue (500×).* **F:** *CD11c+ macrophages scattered throughout the lymphoid aggregate (arrow) (500×).*

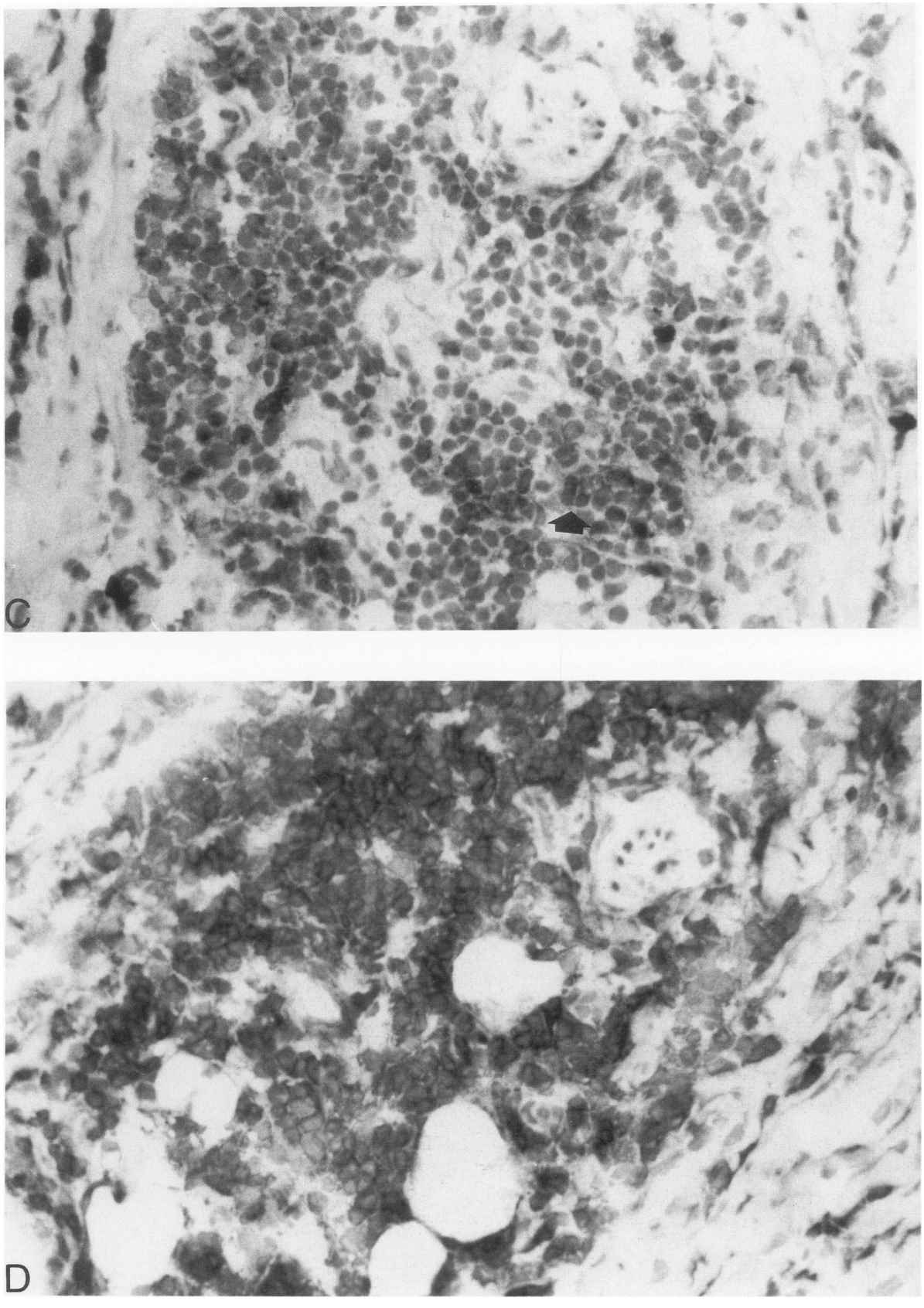

Figure 6 (*continued*)

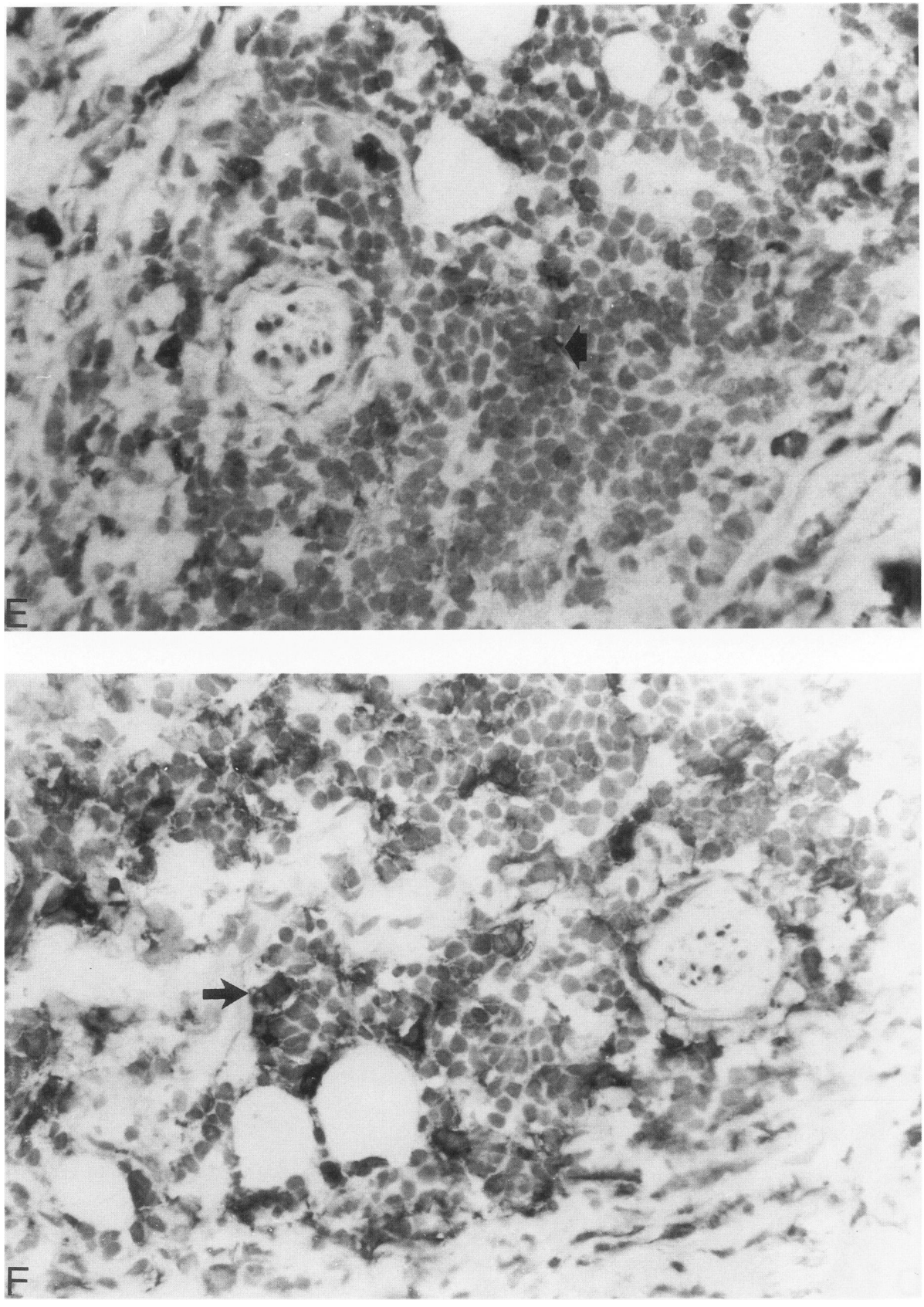

Figure 6 (*continued*)

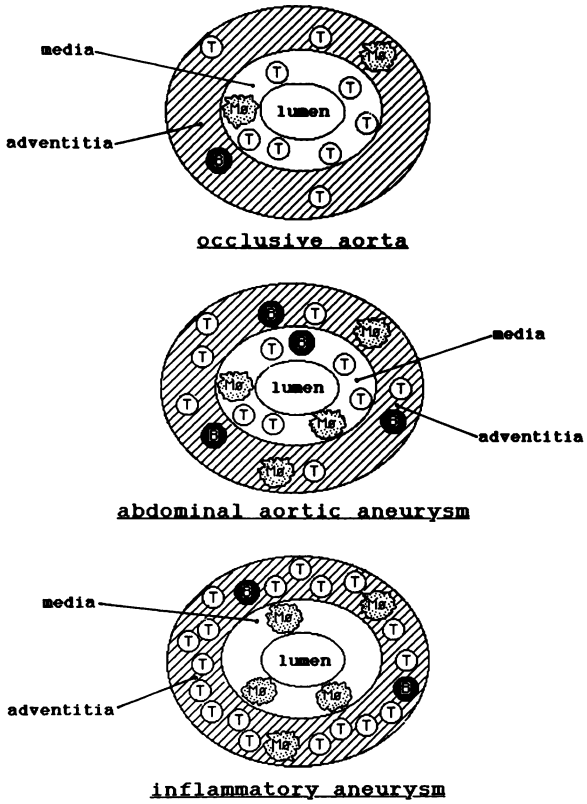

Figure 7. *Diagram of a cross-sectional view of an occlusive aorta, abdominal aortic aneurysm, and inflammatory aneurysm depicting the spectrum of diseases studied. Representations of the relative quantities of inflammatory cells present in the media* versus *the adventitia of blood vessels in the various disease groups is shown.*

positive T-cell helper/surpressor ratio in the tissues. Within each disease category, the CD4-positive helper cell predominated, with the CD4-positive:CD8-positive ratio ranging from 4.3 to 7.6. This ratio appeared to increase as the inflammatory scores increased. These values are considerably increased compared to the ratio in normal peripheral blood, which is approximately 2:1.[21,22] This observation is consistent with that of Hansson et al,[23-25] who found a preponderance of CD4-positive lymphocytes in atherosclerotic plaques.

The function of CD3-positive T lymphocytes within aortic tissues is unclear. However there have been characteristics attributed to CD3-positive lymphocytes that may account for their presence in aneurysmal tissue. CD3-positive lymphocytes, particularly the CD4-positive subset, can release interleukin-2, which causes the activation and proliferation of CD3-positive T and CD19-positive B lymphocytes.[26] CD4-positive T cells can interact with activated macrophages or smooth muscle cells in aortic tissue bearing class II major histocompatibility determinants.[27-30] These macrophages or smooth muscle cells can process foreign or autologous but altered antigen, perhaps perpetuating an aberrant immune response. In aneurysmal tissues, the proportion of CD8-positive T lymphocytes is reduced compared to normal peripheral blood. This might contribute to activation of the immune system, ultimately leading to destruction of the aortic wall.

CD19-positive B lymphocytes were rarely found (mean, 4% of total lymphocytes) in the media and adventitia of the occlusive aortas we studied. This correlates with the findings by others studying the intimal plaque of atherosclerotic carotid arteries.[1,2,22] In sharp contrast, one fourth of lymphocytes in abdominal aortic aneurysms, and in inflammatory aneurysms, were CD19-positive B lymphocytes. These CD19-positive B lymphocytes, if present, were located in the adventitia of occlusive aortas. These cells were present in the adventitia a mean of 75% of the time in abdominal aneurysms, and a mean of 99% of the time in inflammatory aneurysms. Furthermore, in many of the tissues, there were clusters of lymphocytes containing CD19-positive B lymphocytes surrounded by CD3-positive T lymphocytes. CD3-positive T lymphocytes can exhibit a variety of mechanisms for recruiting CD19-positive B lymphocytes to sites of inflammatory reaction. For instance, interleukins-4, -5, and -6 may be important in stimulating the growth and differentiation of CD19-positive B lymphocytes.[31] CD19-positive B cells can serve as antigen-presenting cells, much as smooth muscle cells and macrophages do.[32-34] The increase in CD19-positive B lymphocytes in aneurysmal tissue could result in increased immunoglobulin deposition. Indeed atherosclerotic plaques have been shown to contain deposits of immunoglobulins that are not found in nonatherosclerotic tissue.[35,36]

CD11c-positive macrophages were noted in each type of aortic tissue studied. Their frequency tended to parallel the overall inflammatory scores. These cells did not appear to be preferentially located in the adventitia *versus* the media within any category. Macrophages often were scattered within the lymphoid aggregates. Macrophages may be involved in disease pathogenesis in many ways. They may present antigen to CD4-positive T lymphocytes and thus perpetuate the immune response.[1] It may be that mediators such as interleukin-1, after release by a macrophage, act as a chemotactic agent for both CD19-positive B cells and CD3-positive T cells, accounting for their presence in aneurysmal tissue.[37] Macrophages, as well as vascular smooth muscle cells, can produce tumor necrosis factor-alpha, which can induce reorganization of monolayers of endothelial cells as well as control lipolytic activity.[1,38] Lipoprotein lipase is synthesized by parenchymal cells of tissues, including the arterial wall.[1,36] Tumor necrosis factor-alpha and interleukin-1 can suppress lipoprotein lipase activity and thus contribute to the catabolic activity of these cytokines.[1,39-43] In addition, macrophages can secrete lipids, including prostaglandins and

leukotreines, and thus influence additional leukocyte recruitment.[19] Macrophages also may contribute to the pathogenesis of aneurysmal disease by secreting collagenase and elastase, the enzymes that are likely to be responsible for the connective tissue degradation observed within aneurysmal aortas.[5,44]

While it is likely that aortic aneurysmal disease represents an immune-mediated response, it is also possible that the inflammation seen in these tissues is an epiphenomenon. The antigen that may drive the immune-mediated mechanisms seen in aortic aneurysms is not clear at this time. However analogies can be drawn between the findings reported in this study and the findings found in biopsies of patients with temporal arteritis.[45] Most of the interaction seen in these temporal arteritis vasculitic lesions appears to be between CD4-positive lymphocytes and macrophages in response to persistent stimulation by antigenic substances that are not easily eliminated by inflammatory mechanisms. Cid et al[45] and O'Brian[46] postulated that the putative antigenic substance might be an inert substance such as the elastic fibers of the blood vessels. The elastic fibers could potentially degenerate and alter their structure by the aging process. On the other hand, lipoproteins that are extracellularly modified may be recognized as antigenic by the immune system.[1] Antibodies to phospholipids have been observed in the sera of patients with myocardial infarction and other atherosclerotic diseases.[1] Patients with anti-cardiolipin antibodies may be at increased risk for recurrent cardiovascular disease.[42] Further studies will be needed to establish the relationship between the inflammatory cells found in the aneurysmal lesions and the progression of atherosclerotic disease. The role of cytokines and other factors released by the inflammatory cells within aneurysmal aortas is under active investigation in our laboratory and should lead to further correlations between the immunohistochemical localization of these cells and their functional significance.

References

1. Hansson GK, Jonasson S, Seifert PS, Stemme S: Immune mechanisms in atherosclerosis. Arteriosclerosis 1989, 9:567–578
2. Emeson EE, Robertson AL Jr: T lymphocytes in aortic and coronary intimas. Their potential role in atherogenesis. Am J Pathol 1988, 130:369–376
3. Joris I, Billingham ME, Majno G: Human coronary arteries: An ultrastructural search for the early changes of atherosclerosis. Fed Proc 1984, 43:710
4. Gown AM, Tsukada T, Ross R: Human atherosclerosis. II. Immunocytochemical analysis of the cellular composition of human atherosclerotic lesions. Am J Pathol 1986, 125:191–207
5. Munro JM, Van der Walt JD, Munro CS, Chalmers JAC, Cox EL: An immunohistochemical analysis of human aortic fatty streaks. Hum Pathol 1987, 18:375–380
6. Van der Wal AC, Das PK, Bentz van de Berg D, Van der Loos CM, Becker AE: Atherosclerotic lesions in humans. In situ immunophenotypic analysis suggesting an immune mediated response. Lab Invest 1989, 61:166–170
7. Rose AG, Dent DM: Inflammatory variant of abdominal atherosclerotic aneurysm. Arch Pathol Lab Med 1981, 105:409–413
8. Jarrett F, Darling RC, Mundeth ED, Austen WG: Experience with mycotic aneurysms of the abdominal aorta. J Cardivasc Surg 1976, 17:81
9. File TM, Barnishan J, Fass RJ: Campylobacter fetus sepsis with mycotic aneurysm. Arch Pathol Lab Med 1979, 103: 143–145
10. Goldstone J, Malone JM, Moore WS: Inflammatory aneurysms of the abdominal aorta. Surgery 1978, 83:425–430
11. Sommerville RL, Allen EV, Edwards JE: Bland and infected arteriosclerotic abdominal aortic aneurysms: A clinicopathological study. Medicine 1959, 38:207–221
12. Walker DI, Bloor K, Williams G, Jillie I. Inflammatory aneurysms of the abdominal aorta. Br J Surg 1972, 59:609–614
13. Mitchinson MJ. Chronic periaortitis and periarteritis: Histopathology 1984, 8:589–600
14. Koch AE, Burrows JC, Marder RM, Domer P, Leibovich SJ: Reactivity of human tissues with monoclonal antibodies to myeloid activation and differentiation antigens. Pathobiology (In press)
15. Koch AE, Robinson PG, Radosevich JA, Pope RM: Distribution of CD45RA and CD45RO T-lymphocyte subsets in rheumatoid arthritis synovial tissue. J Clin Immunol 1990, 10: 192–199
16. Hsu SM, Raine L, Fanger H: Use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 1981, 29:577–580
17. Robinson PG, Eggena DA, Koch AE, Radosevich JA: Immunochemical staining coupled with a modified prussian blue technique for iron. Lab Medicine 1989, 20:569–571
18. Winer B: Statistical Principals in Experimental Design. New York, McGraw Hill, 1971
19. Swartz CJ, Mitchell RA: Cellular infiltration of human arterial adventitia associated with atheromatous plaques. Circulation 1962, 26:73–78
20. Swartz CJ, Valente AJ, Sprague EA, Kelley JL, Suenram CA, Graves DT, Rozek MM, Edwards EH, Delgado R: Monocyte/macrophage participation in atherogenesis: Inflammatory components of pathogenesis. Seminars in Thrombosis and Hemostasis 1986, 12:79–86
21. Evans RL, Wall DW, Platsoucas CD, Siegal FP, Fikrig SM, Testa CM, Good RA: Thymus-dependent membrane antigens in man: Inhibition of cell-mediated lympholysis by monoclonal antibodies to TH_2 antigen. Proc Natl Acad Sci 1981, 78:544–548
22. Ledbetter JA, Evans RL, Lipinski M, Cunningham-Rundles C, Good RA, Hertzenberg LA: Evolutionary conservation of surface molecules that distinguish T lymphocyte helper/in-

ducer and T cytotoxic suppressor subpopulations in mouse and man. J Exp Med 153:310–323
23. Jonasson L, Holm J, Skalli O, Bondjers G, Hansson GK: Regional accumulations of T cells, macrophages, and smooth muscle cells in the human atherosclerotic plaque. Arteriosclerosis 1986, 6:131–138
24. Hansson GK, Holm J, Jonasson L: Detection of activated T lymphocytes in human atherosclerotic plaque. Am J Pathol 1989, 135:169–175
25. Hansson GK, Jonasson L, Lojsthed B, Stemme S, Kocher O, Gabbiani G: Localization of T lymphocytes and macrophages in fibrous and complicated human atherosclerotic plaques. Atherosclerosis 1988, 72:135–141
26. Smith KA. Interleukin-2. Ann Rev Immunol 1986, 2:319–333
27. Unanue ER, Beller DI, Lu CY, Allen PM: Antigen presentation: Comments on its regulation and mechanism. J Immunol 1984, 132:1–5
28. Unanue ER, Allen PM: The basis for the immunoregulatory role of macrophages and other accessory cells. Science 1987, 236:551–557
29. Hansson GK, Jonasson L, Holm J, Claesson-Welch L: Class II MHC antigen expression in the atherosclerotic plaque: Smooth muscle cells express HLA-DR, HLA-DQ and the invariant gamma chain. Clin Exp Immunol 1986, 6:261–268
30. Jonasson K, Holm J, Scalli O, Gabbiani G, Hansson GK: Expression of class II transplantation antigen on vascular smooth muscle cells in human atherosclerosis. J Clin Invest 1985, 76:125–131
31. Weiss A: T lymphocytes. *In* Kelley WN, Harris ED, Ruddy S, Sledge CB, eds. Textbook of Rheumatology. Philadelphia, WB Saunders, 1989, pp 148–168
32. Chestnut RW, Grey HM. Studies on the capacity of B cells to serve as antigen-presenting cells. J Immunol 1981, 126: 1075–1079
33. Rock KL, Benacerraf B, Abbas AK: Antigen presentation by hapten-specific B lymphocytes. I. Role of surface immunoglobulin receptors. J Exp Med 1984, 160:1102–1113
34. Lanzavecchia A: Antigen-specific interactions between T and B cells. Nature 1985, 314:537–539
35. Hollander W, Colombo MA, Kirkpatrick B, Paddock J: Soluble proteins in the human atherosclerotic plaque. Atherosclerosis 1979, 38:391–405
36. Hansson GK, Holm J, Kral JG: Accumulation of IgG and complement factor C3 in human arterial endothelium and atherosclerotic lesions. Acta Pathol Microbiol Immunol Scand 1984, 92A:429–435
37. Moissec P, Yu C-L, Ziff M: Lymphocyte chemotactic activity of human interleukin-1. J Immunol 1984, 133:2007–2011
38. Stolpen AH, Guinan EC, Fiers W, Pober JS: Recombinant tumor necrosis factor and immune interferon act singly and in combination to reorganize human vascular endothelial cell monolayers. Am J Pathol 1986, 123:16–24
39. Warner SJC, Libby P: Human vascular smooth muscle cells. Target for and source of tumor necrosis factor. J Immunol 1989, 142:100–109
40. Beutler BA, Cerami A: Recombinant interleukin 1 suppresses lipoprotein lipase activity in 3T3-L1 cells. J Immunol 1985, 6:3961–3971
41. Beutler BA, Milsark IW, Cerami A: Cachectin/tumor necrosis factor: Production, distribution, and metabolic fate in vivo. J Immunol 1985, 6:3972–3977
42. Hamsten A, Bjorkholm M, Norberg R, de Faire U, Holm G: Antibodies to cardiolipin in young survivors of myocardial infarction: An association with recurrent cardiovascular events. Lancet 1986, i:113–116
43. Cerami A, Beutler B: The role of cachetin/TNF in endotoxic shock and cachexia. Immunol Today 1988, 9:28–31
44. Rizzo RT, McCarthy WJ, Dixit SN, Lilly MP, Shively VP, Flinn WR, Yao JST: Collagen types and matrix protein content in human abdominal aortic aneurysms. J Vasc Surg 1989, 10: 365–373
45. Cid MC, Campo E, Erilla G, Palacin A, Vilaseca J, Villalta J, Ingelmo M: Immunohistochemical analysis of lymphoid and macrophage cell subsets and their immunologic activation markers in temporal arteritis. Influence of corticosteroid treatment. Arthritis Rheum 1989, 32:884–893
46. O'Brian JP: A concept of diffuse actinic arteritis: The role of actinic damage to elastin in 'age change' and arteritis of the temporal artery and in polymyalgia rheumatica. Br J Dermatol 1978, 98:1–13

Acknowledgments

The authors thank James Burrows and Vera Shively for assistance in the preparation of this manuscript and Dr. James Sinacore for invaluable assistance in statistical analysis.