


Characterization of the inflammatory and apoptotic cells in the aortas of patients with ascending thoracic aortic aneurysms and dissections

Rumin He, MD,^{a*} Dong-Chuan Guo, PhD,^{a*} Anthony L. Estrera, MD,^b Hazim J. Safi, MD,^b Tam T. Huynh, MD,^b Zhengnan Yin, MD, PhD,^a Shi-Nian Cao, MD,^c Jing Lin, MD,^a Thomas Kurian,^a L. Maximillian Buja, MD,^c Yong-Jian Geng, MD, PhD,^a and Dianna M. Milewicz, MD, PhD^a

 Supplemental material is available online.

Objective: The histopathologic abnormality underlying ascending aortic aneurysm and dissection is medial degeneration, a lesion that is described as the noninflammatory loss of smooth muscle cells and elastic fibers. This study sought to determine whether inflammatory cells are present in medial degeneration and assess any possible contribution of these cells to apoptosis of smooth muscle cells.

Methods: Aortic specimens were obtained from patients undergoing prophylactic surgical repair of an ascending aortic aneurysm (n = 9) and type A dissection (n = 7), along with control patients dying of causes unrelated to aortic disease (n = 5). Immunohistochemical staining was performed to evaluate the presence of lymphocytes and macrophages, and markers of apoptosis were assessed in the aortas of patients with ascending aortic aneurysm and dissection.

Results: Immunohistochemical study indicated significantly more CD3⁺ cells in the aortas of patients with aneurysms or dissections than in control aortas ($P = .020$ and $P = .0022$, respectively). In addition, aortas of patients with aneurysms or dissections had more CD68⁺ cells ($P = .01$ and $P = .005$, respectively). CD3⁺ cells were localized in the media and surrounding the vasa vasorum in the adventitia. Cells yielding a positive result on in situ terminal transferase-mediated deoxyuridine triphosphate nick end-labeling were found in increased numbers in the aortas of patients with aneurysms or dissections relative to control aortas ($P = .005$ and $P = .002$, respectively). Furthermore, Fas and FasL were increased in the aortic samples from patients with aneurysms and dissections relative to control aortas.

Conclusion: The coexistence of inflammatory cells with markers of apoptotic vascular cell death in the media of ascending aortas with aneurysms and type A dissections raises the possibility that activated T cells and macrophages may contribute to the elimination of smooth muscle cells and degradation of the matrix associated with thoracic aortic aneurysms and dissections.

From the Departments of Internal Medicine,^a Cardiovascular Surgery,^b and Pathology,^c The University of Texas Medical School at Houston, Houston, Tex.

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Address for reprints: Dianna M. Milewicz, MD, PhD, The University of Texas Medical School at Houston, 6431 Fannin St, MSB 4.202, Houston, TX 77030 (E-mail: Dianna.M.Milewicz@uth.tmc.edu).

*The first two authors contributed equally to this work.

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The major disease processes affecting the aorta are aortic aneurysms and dissections, and these diseases account for nearly 16,000 deaths annually. The most common aortic aneurysms are infrarenal abdominal aortic aneurysms (AAAs), followed by ascending thoracic aortic aneurysms (TAAs). TAAs and type A aortic dissections (TADs) are related conditions, as evidenced by the fact that progressive enlargement of a TAA can lead to a TAD in the absence of prophylactic surgical repair. The pathology associated with AAAs is atherosclerosis. In contrast, the most common pathology associated with TAAs and TADs is Erdheim cystic medial necrosis, which is described as a noninflammatory lesion characterized by a triad of smooth muscle cell (SMC) loss, fragmentation of elastic fibers, and accumulation of basophilic ground substance within cell depleted areas

Abbreviations and Acronyms

AAA	= abdominal aortic aneurysm
SMC	= smooth muscle cell
TAA	= ascending thoracic aortic aneurysm
TAD	= type A aortic dissection
TUNEL	= terminal transferase-mediated deoxyuridine triphosphate nick-end labeling

of the medial layer of the aorta.¹ Because the lesion is neither necrotic nor cystic, more recently the term *medial degeneration* has been adopted to describe this pathology. Medial degeneration is not just a pathologic condition but is commonly seen in aging aorta and in patients with hypertension.^{2,3} It has been shown that these changes are both qualitatively and quantitatively much greater in patients with TADs.⁴ The lesion is also observed in genetic conditions that predispose individuals toward TAA and TAD, such as Marfan syndrome and familial TAD.^{5,6}

Inflammation has been documented in only a small number of causes of human TAAs, such as giant cell arteritis, syphilitic aortitis, and very rare cases of inflammatory aneurysms.⁷⁻⁹ In this study, we tested the hypothesis that immune infiltrates are present in the aortas of patients with medial degeneration and that the presence of these inflammatory cells contributes to the local expression of death-promoting mediators in the diseased aortas. We observed that T lymphocytes and macrophages were present in the aortic media in both TAAs and TADs but occurred in greater numbers in the aortas of patients who had dissections. We observed that the aortas from patients with aneurysms and dissections had higher levels of Fas, FasL, and caspase-3 than did normal aortas, indicating that these pathways are activated in both aortic diseases. With a combination of immunostaining and in situ terminal transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL), we detected numerous TUNEL-positive cells that exhibited either SMC markers or CD3 staining, evidence that both α -actin-positive and CD3⁺ cells are undergoing apoptosis. Thus this study indicates that inflammation is a component of medial degeneration and may contribute to apoptosis and degeneration of the aortic wall during the development of aneurysms and dissections.

Materials and Methods**Aortic Tissue**

After appropriate informed consent was obtained, full-thickness aortic wall specimens were collected above the aortic valve from patients undergoing elective repair of TAA (n = 9, 3 male and 6 female, average age 62.9 years) and emergency repair of acute TAD (n = 7, 4 male and 3 female, average age 59.9 years). All patients had tricuspid aortic valves. None of the patients had a known genetic syndrome causing the aortic disease (eg, Marfan or Turner syndrome) or a family history of

TAA or TAD. Control ascending aortas were obtained from 5 individuals found at autopsy to have died of causes unrelated to aortic disease (2 male and 3 female, average age 57.2 years). Medical histories pertinent to aortic disease were obtained from the patients' medical records (Table E1). Specimens were immediately transferred to the laboratory in a container with cold Waymouth MB 752/1 medium (Invitrogen Corporation, Carlsbad, Calif). Each sample was divided into two parts. The first part was used for histopathologic studies, and the second was used for protein and RNA extraction and for explanting SMCs. The study was approved by the institutional review committee at the University of Texas Health Science Center at Houston and Memorial Hermann Hospital.

SMC Culture

Aortic layers were separated, minced, and digested for 16 hours in an enzyme mixture containing elastase type III (15 U/mL), collagenase type I (165 U/mL), and soybean trypsin inhibitor (0.375 mg/mL). Digestion was stopped with SmGm2 medium (BioWhittaker Molecular Applications, Rockland, Maine) supplemented with 20% fetal bovine serum. The released cells were washed, pelleted, and seeded into tissue culture flasks.

RNA Preparation and Quantitative Reverse Transcriptase-Polymerase Chain Reaction

Total RNA was purified from aortic tissue and SMC cultures with TRIzol Reagents (Invitrogen, Carlsbad, Calif.). First-strand complementary DNA was synthesized with the Superscript II Reverse Transcriptase Kit (Invitrogen) and CD3 α -specific reverse primers. The primers were 5'-CCCCAGAGGAAGCAAACCA-3' (forward) and 5'-TGGCCACCGACATCACATC-3' (reverse). Real-time polymerase chain reaction was performed with the QuantiTectTM SYBR Green PCR kit (QIAGEN Inc, Valencia, Calif) on ABI 7700 Real-Time PCR system (Applied Biosystems, Foster City, Calif.). β -Actin was used as the endogenous standard.

TUNEL Testing

TUNEL reaction was performed with an in situ cell detection kit (Promega Corporation, Madison, Wis) according to a standard protocol. Counterstaining for total nuclei was performed by mounting the sections with 4',6-diamidino-2'-phenylindole-containing glycerol solution. Total and TUNEL-positive nuclei were counted in 10 contiguous high-power fields under an Olympus fluorescent microscope (Olympus America Inc, Melville, NY) then converted to TUNEL-positive nuclei per square millimeter by two independent observers (R.H. and D.-C.G.).

Immunohistochemical Testing

The antibodies and supplier used are listed in Table E2, and biotin-conjugated antibodies (Vector Laboratories, Inc, Burlingame, Calif) were used as second antibody. An avidin-alkaline phosphatase-fast red reagent or the peroxidase-DAB system (Vectastain ABC kit; Vector Laboratories) was used to visualize the antibody stains. Normal mouse immunoglobulin G (Sigma, St Louis, Mo) served as the control for the immunostains. Infiltrated inflammatory cells were counted in 10 contiguous high-power

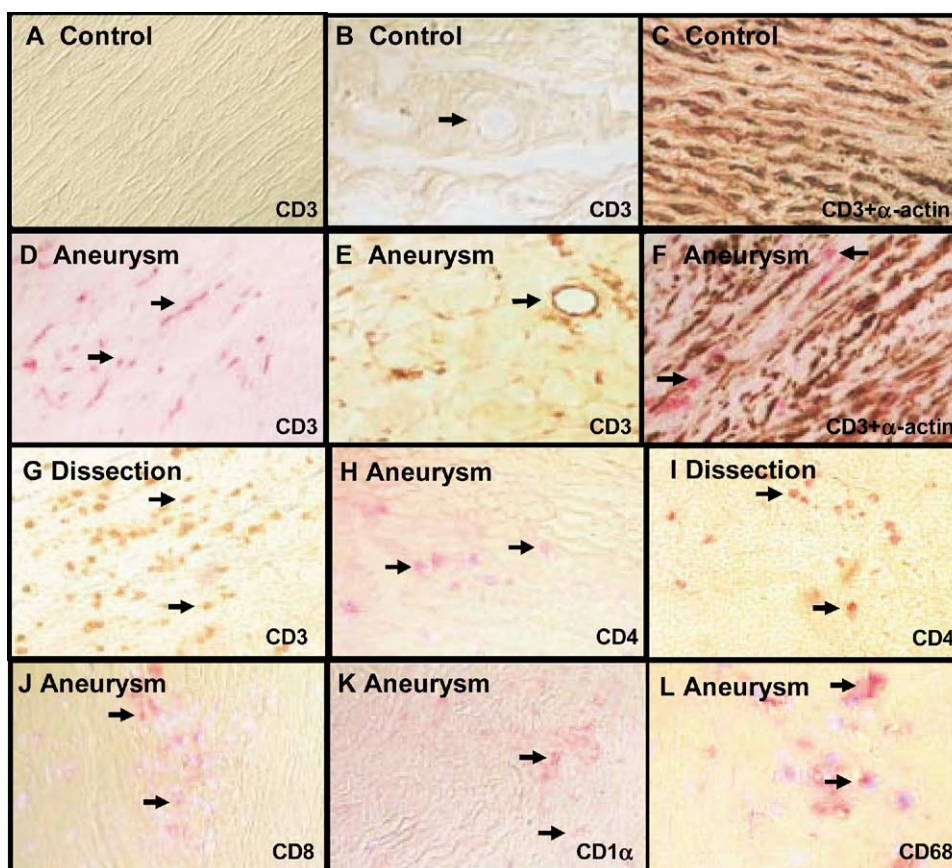


Figure 1. T-lymphocyte infiltrates in TAA and TAD. For orientation, lumen of aorta is at *top* with adventitia at *bottom*. A through C, Immunohistochemical staining demonstrates that CD3⁺ cells (*arrow*) are rarely present in control aorta. D, F, and G, Media from patients with TAA and TAD demonstrate CD3⁺ cells (*arrows*) throughout media of aorta. E, Adventitia from patient with TAA demonstrates CD3⁺ cells throughout adventitia. H through J, CD4⁺ and CD8⁺ cells are present in aortic media from patients with TAA and TAD. K and L, Media from patient with TAA demonstrates that CD1 α ⁺ and CD68⁺ cells are present in aneurysmal aortic media. Monoclonal anti-CD3 antibody used in D was obtained from DAKO Corporation, Carpinteria, Calif; antibodies in panels A through C and E through F were obtained from Zymed Laboratories Inc, South San Francisco, Calif, for comparison. Original magnification 400 \times .

fields under an Olympus fluorescent microscope by two independent observers.

For double staining with TUNEL and immunostaining, the paraffin sections were stained by DeadEnd Fluorometric or Colorimetric TUNEL system (Promega Corporation, Madison, Wis), followed by immunohistochemical assay with an avidin-alkaline phosphatase–fast red reagent or the peroxidase-DAB system. The TUNEL-positive nuclei emitted green fluorescence when the DeadEnd Fluorometric TUNEL system was used or stained brown when the DeadEnd Colorimetric TUNEL System was used. Immunostaining for cell type–specific antigens showed pink color, which was clearly visible under a bright field microscope.

For assessing the amount of SMC α -actin staining, the IT3 ImageTool (<http://ddsdx.uthscsa.edu/dig/itdesc.html>; Department of Dental Diagnostic Science, The University of Texas Health Science Center, San Antonio, Tex) was used to trace areas of positive

α -actin staining. The pixels of the area with positive α -actin staining were quantified in a 10 \times magnification field for 5 patients with TAA, 6 with TAD, and 4 control patients.

Statistical Analysis

The CD3, TUNEL, and CD68 signals were counted in 10 contiguous high-power fields under an Olympus fluorescent microscope for each individual. The averaged values of these counts for each individual were used for later group comparisons. The 2-tailed Student *t* test was conducted to compare the difference in mean values between study and control groups.

Further, the CD3, TUNEL, and CD68 values were treated as nonparametric data and expressed as median values. Mann-Whitney Wilcoxon rank-sum tests were used to compare groups on nonparametric data from CD3, TUNEL, and CD68. All tests of

significance were 2-sided. All analyses were performed with SAS software (version 8.2; SAS Institute, Inc, Cary, NC).

Results

Loss of SMCs and Elastic Fibers in

Medial Degeneration

The control aortas showed an orderly array of SMCs and elastic fibers in the media (Figure E1, A). In contrast, the aortas of the patients with TAAs and TADs showed medial degeneration affecting primarily the SMCs, leading to bands of cellular loss in the aortic media (Figure E1, B), regions with primarily the elastic fibers associated with increased basophilic ground substance, resulting in the appearance of cystlike structures (Figure E1, C), or both.¹⁰ Consistent with the hematoxylin and eosin staining results, immunostaining with monoclonal antibody against SMC α -actin showed reduced numbers of SMC α -actin-positive cells in medial bands in the diseased aortas, with focal loss of both SMCs and elastic fibers (Figure E1, D through F). The amount of α -actin staining was assessed quantitatively with a digital image analysis system. The amount of staining in histologic sections of control aortas (388,701 pixels) was 5-fold that in aneurysms (73, 417 pixels, $P < .001$) or dissected aortas (75,906 pixels, $P < .001$).

CD3⁺ and CD68⁺ Cells in the Aortas from Patients with TAA and TAD

Immunohistochemical staining was used to determine whether inflammatory cells, specifically T cells and macrophages, were present in the aortic media of patients with TAAs with medial degeneration. Two different monoclonal anti-CD3 antibodies were used for pan-T lymphocyte detection, along with a monoclonal antibody against leukocyte common antigen (CD45) and a polyclonal antibody specific for macrophages (CD68). Rare CD3⁺ cells and macrophages were observed in the control aortas in the media or adventitia (Figure 1, A through C). In the aortas of patients undergoing prophylactic repair of a TAA, there were significant increases in the number of CD3⁺ cells relative to control aortas ($P = .02$; Figures 1, D through F, and 2). CD3⁺ cells were observed throughout the aortic media, either scattered throughout or localized in foci. Some CD3⁺ cells had the typical round appearance of lymphocytes, but other CD3⁺ cells had a flattened morphology similar to SMCs (Figure 1, D, arrows). The CD3⁺ cells were present in similar abundance when a different monoclonal antibody against CD3 was used for staining. Interestingly, there was a considerable influx of CD3⁺ cells in the walls of the vasa vasorum in the adventitia that was not present in control aortas (Figure 1, B and E). Staining of endothelial cells with an antibody directed against Von Willebrand factor verified that the CD3⁺ cells were associated with the vasa vasorum in the adventitia (data not shown). The presence of CD3⁺ cells was confirmed by immunostaining with an antibody to

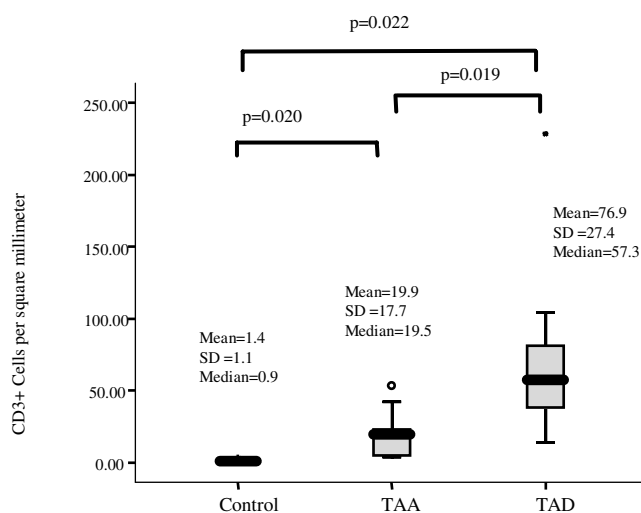


Figure 2. CD3⁺ cells present in media from control, aneurysmal, and dissected aortas. CD3⁺ cells in 1 mm² of aortic media were counted from aortic tissue sections from control subjects, patients with TAAs, and patients with TADs. Total areas examined were 2.2 mm². Box-whisker plots display CD3. Central horizontal bars indicate median, upper and lower ends of boxes indicate 75th and 25th percentiles, and error bars indicate maximum and minimum values of CD3 values. P values were determined by Mann-Whitney Wilcoxon rank-sum tests.

CD45, an antigen present on all lymphocytes. CD45⁺ cells had a similar distribution to that of the CD3⁺ cells in the medial layer.

Focal and diffuse accumulations of CD3⁺ cells were detected in aortas of patients with TADs, and the numbers of these cells were increased relative to control aortas ($P = .022$) and to aneurysmal aortas ($P = .019$; Figures 1, G, and 2). CD3⁺ cells were observed both along the edges of ruptured media of the dissected aortas and distributed throughout the media with a flattened morphology.

To assess the T-cell subsets within aortic lesions, the sections were stained with antibodies that recognized CD4 and CD8 antigens, markers for helper and cytotoxic T cells, respectively. Both CD4⁺ and CD8⁺ T-cell subsets were present in the aneurysmal and dissected aortas but not in control aortas (Figure 1, H through J). Immunostaining with antibodies directed against CD1 α antigen indicated that vascular dendritic cells were frequently observed in the areas containing a large number of T cells but were not found in control aortas (Fig. 1K). Finally, immunostaining with CD68 antibody also indicated that macrophages were frequently present in the aortas from patients with ascending aortic aneurysm and dissection (Fig. 1L).

The flattened appearance of the CD3⁺ cells raised the question as to whether some SMCs were expressing the CD3 antigen, and double staining using monoclonal anti-

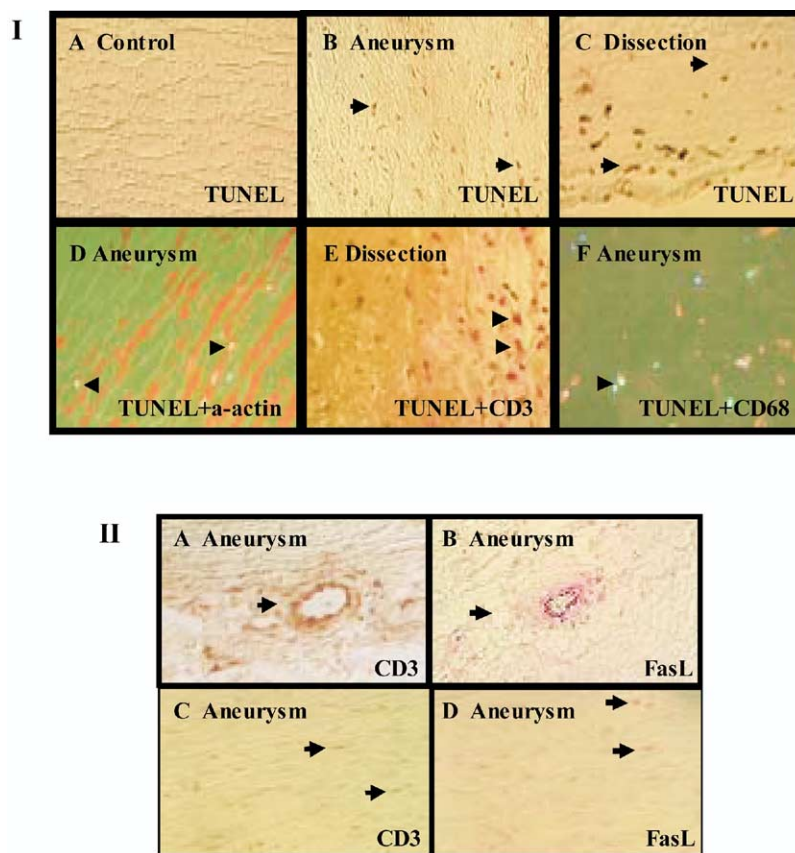


Figure 3. Increased TUNEL-positive and FasL staining in media of aneurysmal and dissected aortas. *Top:* A, TUNEL-positive cells (arrows) are rarely present in control aorta. B and C, Media from patients with TAA and TAD demonstrated TUNEL-positive cells (arrows). D through F, Double staining with combination of TUNEL and immunohistochemical assay with α -actin, CD3, and CD68 antibodies demonstrated that SMCs, T lymphocytes, and macrophages were undergoing apoptosis (arrows) in aortic media from patient with TAA. TUNEL staining in panels A through C and E used DeadEnd Colorimetric TUNEL System; that in panels D and F used DeadEnd Fluorescence TUNEL System. *Bottom:* A and C, Adventitia and media in aneurysmal aorta indicate influx of CD3⁺ cells in walls of vasa vasorum and aortic media. B and D, Same vasa vasorum and media were stained with anti-FasL antibody, which indicated colocation of CD3⁺ and FasL-positive cells. Magnification 400 \times .

bodies against CD3 and SMC α -actin was done to address this question. The double staining revealed that the majority of cells positive for SMC α -actin were distinct from those staining for CD3 (Fig. 1C and G). However, in certain regions with leukocyte accumulation, an overlap of CD3 and SMC α -actin immunostaining was observed. To further address this question, total RNA was isolated from control and aneurysmal aortas and explanted SMC cultures from patients with TAA, and messenger RNA (mRNA) expression of the CD3 α polypeptide was determined with quantitative reverse transcriptase–polymerase chain reaction. Consistent with the results of immunohistochemical staining, we detected CD3 α mRNA in the total mRNA isolated from aortas of patients with TAAs but not from mRNA from the control aortas. CD3 α mRNA was not detected in the total

mRNA isolated from SMC cultures explanted from aneurysmal aortas, providing further data that the SMCs are not expressing CD3 α .

Immunohistochemical testing with a polyclonal antibody directed against CD68 was used to determine whether macrophages were also present in the diseased aortic tissue. Numbers of macrophages were significantly increased in the media in both aneurysmal (5.5 ± 1.8 cells/mm², $P = .01$) and dissected (6.0 ± 1.4 cells/mm², $P = .005$) aortas relative to control aortas (0.7 ± 0.2 cells/mm²).

TAAs and TADs Exhibit Increased Markers of Apoptosis

To test the hypothesis that infiltration of T lymphocytes leads to activation of the death-promoting pathways through

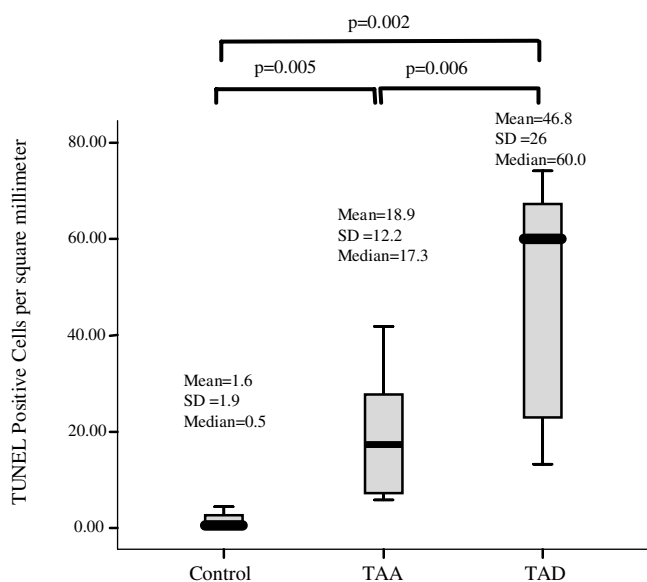


Figure 4. TUNEL-positive cells present in media from control, aneurysmal, and dissected aortas. TUNEL-positive cells in 1 mm² of the aortic media were counted from aortic tissue sections from control patients, patients with TAAs, and patients with TADs. Total areas examined were 2.2 mm². Box-whisker plots display TUNEL. Central horizontal bars indicate median, upper and lower ends of boxes indicate 75th and 25th percentiles, and error bars indicate maximum and minimum values of TUNEL values. P values were determined by Mann-Whitney Wilcoxon rank-sum tests.

Fas-FasL engagement and caspase activation, DNA fragmentation was initially analyzed on the aortic specimens with the TUNEL assays. Aortas from patients without aortic disease showed few TUNEL-positive cells (Figures 3, A top, and 4). In contrast, TUNEL-positive cells were present in aortas from both the TAA group ($P = .005$) and the TAD group ($P = .002$) at significantly higher levels than in control aortas, with significantly higher numbers of TUNEL-positive cells in the dissected aortas (Figures 3, A top through C top, and 4). The TUNEL-positive cells were primarily distributed in the regions with elastic fibril loss, and there was focal accumulation at the edges of ruptured media in the dissected aortas (Figure 3, C top). Double immunostaining with a combination of TUNEL and immunohistochemical techniques confirmed the collocation of TUNEL-positive cells with SMCs, lymphocytes, and macrophages (Figure 3, D top through F top).

Immunohistochemical testing demonstrated diffuse staining of immunoreactive Fas antigens throughout the media and adventitia of the control aorta, with increased amounts in both locations in the diseased aortas (data not shown). In contrast, staining for FasL was mainly associated with cells in the adventitia, in particular around the vasa vasorum, with occasional positive cells in the media of aneurysmal

and dissected aortas collocated with the CD3⁺ cells (Figure 3, A bottom through D bottom). Consistent with the results obtained with immunohistochemical testing, increased levels of Fas and FasL antigen were detected in the aneurysmal and dissected tissues relative to control aortas (Figure E2). In contrast, the control aortas displayed modest expressions of Fas and FasL immunoreactivity. Furthermore, both the aneurysmal and dissected aortas showed immunoreactivity to activated caspase-3, whereas control aortas showed almost no activated caspase-3 (Figure E2).

Discussion

Medial degeneration associated with TAAs and TADs was originally described by Erdheim¹ as a noninflammatory lesion associated with loss of SMCs and elastic fibers in the media. Evidence presented in this report indicates that T lymphocytes and macrophages are common features in the aortas of patients with medial degeneration. Immunohistochemical testing with two different monoclonal antibodies directed against CD3 clearly showed the presence of T lymphocytes in the media, either diffusely throughout the media or in focal accumulations. Some of the CD3⁺ cells with flattened appearance in the aortic media resided between the SMC layers, suggesting a close interaction between these inflammatory cells and the vascular cells. Flattened perforin-positive cells, presumably T lymphocytes, were previously observed in AAAs, indicating that lymphocytes may assume this morphologic appearance in the aortic media.¹¹ The infiltration of CD3⁺ cells in the walls of the vasa vasorum in this study suggests that the T cells might migrate from these blood vessels of the adventitia into the media of the aortas. This study also demonstrated increased CD68⁺ cells in the diseased aortas, indicating the presence of macrophages in this lesion.

A number of previous studies have documented evidence of increased apoptosis of SMCs in medial degeneration.^{12,13} Immunohistochemical testing has indicated that medial degeneration of aorta is associated with p53 accumulation, Bax upregulation, and apoptosis of SMC in the aortas of patients with TAD.¹⁴ Our study showed an increase in the amount of apoptotic cells by TUNEL staining in the media of both aneurysmal and dissected aortas. Double staining for TUNEL-positive nuclei and cell-specific markers provides evidence that SMCs are undergoing apoptosis, and to a lesser extent are lymphocytes and macrophages. Immunohistochemical results indicate collocation of FasL staining with CD3 staining, suggesting that T lymphocytes were the cells responsible for FasL production. In contrast, Fas was found diffusely throughout the media and adventitia. These results suggest apoptosis of primarily SMCs through the Fas-FasL pathway in aneurysmal tissue. The relative lack of evidence that substantial numbers of CD3⁺ cells are undergoing apoptosis suggests that the aorta is not an immune-

privileged site in which the Fas produced by SMCs leads to apoptosis of T lymphocytes to limit an inflammatory reaction.

The T lymphocyte and macrophage infiltration and TUNEL staining were more pronounced in the media of the dissected aortas than of the aneurysmal aortas. The dissection of blood into the wall of the aorta is a traumatic tissue injury, and it is therefore not surprising that inflammatory markers and apoptotic markers would be increased in the damaged tissue. Although both inflammatory and TUNEL-positive cells were observed along the margin of the dissections, the inflammatory cells and apoptotic markers were also present throughout the media. It is possible that the degree of inflammation and apoptosis in the media may be increased in an aorta before dissection. These events could potentially lead to increased rates of loss of SMCs and destruction of the tissue, setting the stage for the dissection to occur. The current recommendation is to prophylactically repair an aneurysm involving the ascending aorta to prevent dissection when it is larger than 5 cm in diameter or when the aorta is rapidly enlarging.¹⁵ Some have dissection when the aorta is minimally enlarged, however, and future studies will determine whether this premature dissection is related to the degree of inflammation and apoptosis in the aortic media.

Inflammation producing enzymatic remodeling of the vascular extracellular matrix is emerging as a shared pathologic mechanism in a variety of vascular diseases, including atherosclerosis, AAAs, and intracranial aneurysms. AAAs are characterized by atherosclerosis leading to a thinning of the tunica media and rarefaction of SMCs.^{11,16} Many medial SMCs in AAAs bear markers of apoptosis and signals capable of initiating cell death, implying that apoptotic death may contribute to the reduction of cellularity and impaired matrix homeostasis. CD4⁺ and CD8⁺ T cells are also present in AAAs, and these cells have been shown to express cytotoxic mediators, including Fas and perforin, which causes death of T cell-targeted cells. The contribution to elimination of SMCs of death-promoting products of activated immune cells was suggested by the observations made in this study of TAAs and TADs associated with medial degeneration. Thus this study provides new evidence that inflammatory infiltrate of T cells expressing Fas may contribute to TAAs in a similar fashion as to AAAs.

It is important to acknowledge the limitations of a study that uses surgically removed human aneurysmal aortas. TAAs were not removed until the diameter of the aorta reached 5.0 to 5.5 cm, and TAD represents an acute traumatic insult to the aorta. Therefore these studies involved end-stage tissues for both disease processes. Furthermore, the patients were placed on cardiopulmonary bypass and hypothermic circulatory arrest and treated with aprotinin to limit inflammation, and we cannot exclude the possibility that these procedures induced inflammatory cells in the

aortic wall. The results presented on these tissues cannot be extrapolated to earlier phases of aneurysm formation. The development of homozygous mice with a hypomorphic *fnb1* allele as an animal model of Marfan syndrome has shed some insight into the role of inflammation in the pathogenesis of aneurysms and dissections.^{17,18} Homozygous mice die at a few months of age, with half of the deaths resulting from vascular rupture. A surprising observation in these mice is the presence of a monocytic infiltration of the medial layer, evident from 8 weeks on that correlated with fragmentation of elastic lamellae, loss of elastin content, and dilation of the vessel wall. Thus the only mouse model of TAA and TAD also demonstrates an inflammatory infiltrate in the aortic wall.

In summary, the data in this study provide the first evidence that medial degeneration of the aorta, originally described by Erdheim,¹ is associated with T-lymphocyte and macrophage infiltration into the media of the aortic wall. Immunoblot and immunohistochemical analyses suggest that death-promoting signaling factors from the T lymphocytes may contribute to SMC apoptosis. In addition to apoptosis of the SMCs, evidence is emerging that the SMCs have an altered phenotype, which includes increased synthesis of matrix metalloproteinases and tissue inhibitors of metalloproteinases. An imbalance between matrix metalloproteinases and tissue inhibitors of metalloproteinases in the tissue may lead to elastic fiber degradation, further decreasing the anchoring of the SMCs to these matrix structures. A more detailed pathogenesis for medial degeneration is emerging, with an altered SMC phenotype, degradation of the elastic fibers, T-cell infiltration, and apoptosis of the SMCs playing roles in this pathologic lesion.

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TABLE E1. Clinical information of individuals participating in the study

Sample	Group	Age (y)	Sex	Hypertension	Smoker	Aortic diameter (cm)
2613	Control*	44	Male	NA	NA	NA
2664	Control†	48	Female	NA	NA	NA
2701	Control‡	54	Female	NA	NA	NA
2840	Control§	74	Female	NA	NA	NA
2976	Control ²	66	Male	NA	NA	NA
2478	Aneurysm	74	Female	Yes	Yes	5.6
2496	Aneurysm	77	Female	Yes	No	5.9
2761	Aneurysm	49	Male	Yes	Yes	5.0
2762	Aneurysm	65	Female	Yes	Yes	6.0
2945	Aneurysm	40	Female	No	No	5.0
2955	Aneurysm	52	Male	Yes	No	5.2
3386	Aneurysm	74	Female	Yes	No	7.0
3387	Aneurysm	64	Female	Yes	Yes	5.8
3393	Aneurysm	71	Male	Yes	Yes	5.6
2482	Dissection	71	Female	Yes	No	5.7
2789	Dissection	59	Male	No	Yes	4.5
2813	Dissection	55	Female	Yes	No	5.5
2992	Dissection	62	Female	Yes	No	8.0
2993	Dissection	57	Male	Yes	Yes	5.2
3038	Dissection	56	Male	Yes	Yes	4.3
3418	Dissection	59	Male	No	No	3.8

NA, Information not available. *Cause of death pulmonary embolism. †Cause of death systemic lupus erythematosus. ‡Cause of death diabetes. §Cause of death stroke. ²Cause of death congestive heart disease.

CSP

TABLE E2. Antibodies used for immunohistochemical and immunoblot analyses

Antibody	Cell type	Source	Manufacturer	Concentration
SMC α -actin	SMC	Mouse monoclonal	Sigma	1:50 IHC
CD3	Pan T cell	Mouse monoclonal	DAKO	1:25 IHC
CD3	Pan T cell	Mouse monoclonal	Zymed	1:1 IHC
CD4	Helper T cell	Mouse monoclonal	DAKO	1:20 IHC
CD8	Cytotoxic T cell	Mouse monoclonal	DAKO	1:20 IHC
CD1a	Dendritic cell, Langerhans cell	Mouse monoclonal	BD PharMingen	1:100 IHC
Interleukin 4	T _H 2 cell	Mouse monoclonal	R&D Systems	1:100 IHC, 1 mg/mL IB
Interleukin 10	T _H 2 cell	Goat	R&D Systems	1:1000 IHC, 0.1 mg/mL IB
Interferon γ	T _H 1 cell	Mouse monoclonal	BD PharMingen	1 mg/mL IB
CD45	Leukocyte	Mouse monoclonal	Santa Cruz*	1:100 IHC
CD68	Macrophage	Rabbit polyclonal	Santa Cruz*	1:100 IHC
Fas	Inflammatory cell, SMC	Mouse monoclonal	BD PharMingen	1:100 IHC, 1 mg/mL IBt
FasL	T cell, macrophage	Mouse monoclonal	BD PharMingen	1:100 IHC, 1 mg/mL IB
Caspase-3	Various	Rabbit polyclonal	BD PharMingen	1:100 IHC, 0.5 mg/mL IB

IHC, Immunohistochemical testing; IB, immunoblot testing. *Santa Cruz Biotechnology, Inc, Santa Cruz, Calif.

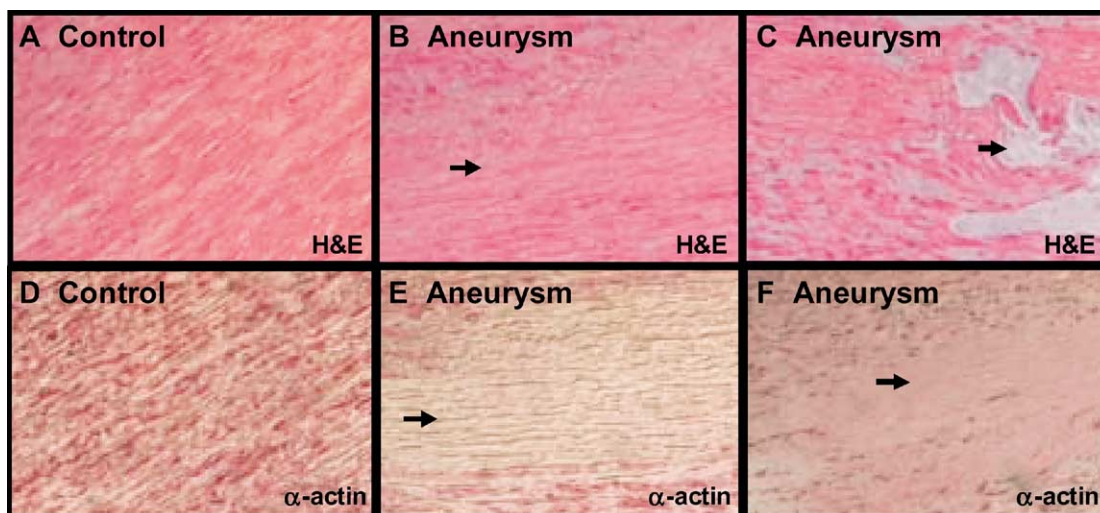


Figure E1. Medial degeneration of aorta associated with loss of SMCs and elastic fibers. Representative cross-sections of human control and aneurysmal aortic tissue were stained with hematoxylin and eosin (A-C) or with monoclonal antibody directed against SMC α -actin (D-F). A and D, Media from control aorta demonstrates nuclei and elastic fibers throughout media. B and E, Media from patient with TAA reveals loss of cell nuclei in band in media, but elastic fibers remain. C and F, Media in different field from same patient as in B and E displays areas with loss of both cells and nuclei. Magnification 100 \times .

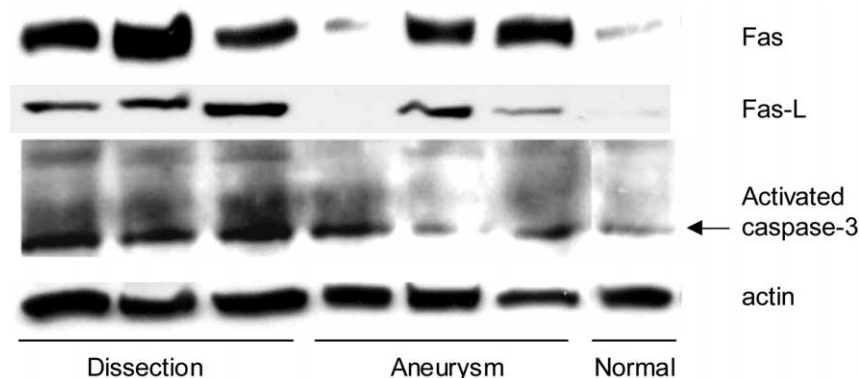


Figure E2. Immunoblot analysis showed levels of Fas, FasL, and activated caspase-3 to be increased in aneurysmal and dissected aortas. Levels of Fas and FasL were assessed relative to actin. Increased levels of Fas antigen were detected in aneurysmal (0.82 ± 0.39) and dissected (0.68 ± 0.62) tissues relative to control tissues (0.07). Increased levels of FasL antigen were detected in aneurysmal (0.71 ± 0.23) and dissected (0.42 ± 0.31) tissues relative to control tissues (0.15). Total protein loaded into each lane was 50 μ g. After separation by sodium dodecylsulfate-polyacrylamide gel electrophoresis, membranes were blocked and incubated with primary antibody against Fas, FasL, and activated caspase-3. Membranes were incubated with horseradish peroxidase-linked secondary antibodies, followed by visualization with SuperSignal West Femto kit (Pierce Biotechnology, Inc, Rockford, Ill). Bands correspond to following molecular weights of proteins: *Fas*, 40 kd; *FasL*, 37 kd; *activated caspase-3*, 20.4 kd. Results are consistent with results obtained with immunohistochemical testing.