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Citation Information: He, Rumin; Guo, Dong-Chuan; Sun, Wei; Papke, Christina L; Duraisamy, Senthil; Estrera, Anthony L; Safi, Hazim J; Ahn, Chul; Buja, L Maximilian; Arnett, Frank C; Zhang, Jingwu; Geng, Yong-Jian; and Milewicz, Dianna M, "Characterization of the inflammatory cells in ascending thoracic aortic aneurysms in patients with Marfan syndrome, familial thoracic aortic aneurysms, and sporadic aneurysms." (2008). The Journal of Thoracic and Cardiovascular Surgery
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Characterization of the inflammatory cells in ascending thoracic aortic aneurysms in patients with Marfan syndrome, familial thoracic aortic aneurysms and sporadic aneurysms

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Abstract

\textbf{Objectives}—This study sought to characterize the inflammatory infiltrate in ascending thoracic aortic aneurysm (TAAs) in patients with Marfan syndrome (MFS), familial TAA (FTAA), and non-familial TAA cases.

\textbf{Background}—TAAs are associated with a pathologic lesion termed medial degeneration, which was described as a noninflammatory lesion. TAAs are a complication of MFS and also can be inherited in an autosomal dominant manner of FTAA.

\textbf{Methods}—Full aortic segments were collected from patients undergoing elective repair with MFS (n=5), FTAA (n=6) and TAAs (n=9), along with control aortas (n=5). Immunohistochemistry staining was performed using antibodies directed against markers of lymphocytes and macrophages. Real-time PCR analysis was performed to quantify the expression level of T cell receptor \(\beta\) chain variable region gene.

\textbf{Results}—Immunohistochemistry of TAA aortas demonstrated that the media and adventitia from MFS, FTAA and sporadic cases had increased numbers of T lymphocytes and macrophages when compared with control aortas. The number of T cells and macrophages in the aortic media of the aneurysm correlated inversely with the patient’s age at the time of prophylactic surgical repair of the aorta. Surprisingly, T cell receptor profiling indicated a similar clonal nature of the T cells in the aortic wall in a majority of aneurysms, whether the patient had MFS, FTAA or sporadic disease.

\textbf{Conclusion}—These results indicate that infiltration of inflammatory cells contributes to the pathogenesis of TAAs. Superantigen-driven stimulation of T lymphocytes in the aortic tissues of the TAA patients may contribute to the initial immune response.
Ultramini-Abstract—This study sought to investigate the infiltration of T-lymphocytes and macrophage in the aortas of patients with MFS, FTAA and sporadic TAAs. The results indicate that infiltration of inflammatory cells contributes to the pathogenesis of TAAs and superantigen-driven stimulation of T-lymphocytes may contribute to the initial immune response.

Introduction

Aortic aneurysms are classified in terms of their anatomical location and most commonly occur in the infrarenal abdominal aorta (AAA) and thoracic aorta (TAAs). AAAs affect primarily the elderly population and are characterized by atherosclerotic changes with chronic inflammation of the aortic wall. In contrast, TAAs affect a younger population and the pathology present in the aortic wall in these patients is medial degeneration (MD) which is described as a lesion characterized by the triad of loss of smooth muscle cells, fragmented and diminished number of elastic fibers, and increased accumulation of proteoglycans.1

Individuals with TAAs are referred for prophylactic surgical repair of the ascending aorta when an aneurysm reaches a diameter greater than 5 cm to prevent the life-threatening complications of aortic dissection or rupture.2

A number of genetic conditions can predispose individuals to the formation of thoracic aortic aneurysms, most notable of which is MFS.3 MFS is a pleiotropic condition inherited in an autosomal dominant manner that also has associated skeletal and ocular manifestations. A defective gene causing MFS, FBN1, encodes for an extracellular matrix protein called fibrillin-1, a component of the elastic fiber system. The fibrillin-1 deficient mouse, an accepted model of MFS that recapitulates the cardiovascular phenotype, shows MD in the aortic wall that was surprisingly associated with inflammatory infiltrates prior to dilatation or dissection.4 A second gene for MFS was recently identified as transforming growth factor β receptor type II (TGFBR2).5

A genetic predisposition for TAA can also be inherited in an autosomal dominant manner in the absence of any associated syndromic findings. Families with multiple affected members typically demonstrate autosomal dominant inheritance with decreased penetrance and variable expression, indicating that a single defective gene is responsible for the disease.6;7 Genetic heterogeneity has been demonstrated for familial TAA, and four chromosomal loci have been mapped for inherited forms of thoracic aortic disease: TAAD1 on 5q,8 TAAD2 on 3p,9 TAAD4 on 10q,10 the FAA1 locus on 11q,11 and a locus of 16p for TAA associated with patent ductus arteriosus (PDA).12 Recently, the defective gene at the TAAD2 locus has been identified as TGFBR2. Mutations in TGFBR2, TGFBR1, ACTA2, and MYH11, have been identified that lead to varied phenotypes associated with thoracic aortic aneurysms and dissections.5;13;14 Similar to MFS, the aortas of patients with familial forms of TAA also demonstrate MD.

Previous studies have documented an inflammatory infiltrate in the aortic wall of patients with TAA.15;16 In aortas of patients undergoing prophylactic repair of TAAs, there were significant increase in the number of CD3+ and CD68+ cells throughout the aortic media and adventitia when compared with control aortas.17 Other investigators have found also documented an inflammatory infiltrate in the aortic wall which was associated with IFN-gamma production production.16 In this study, we sought to further characterize the inflammatory infiltrate in aortas of patients referred for prophylactic repair of an ascending aortic aneurysm resulting from single gene defects, such as MFS and familial TAA, and sporadic, non-familial cases.
Materials and Methods

Subjects and Collection of Tissues
All patients gave signed consent to these studies, which were approved by the Institutional Review Board at the University of Texas Health Science Center at Houston. Full aortic segments were collected from patients undergoing elective repair of TAAs (N=20; twelve males and eight females, mean age 46.9 ±16.6 years, range, 21–77 years) (Table 1). Five patients had MFS (three males and two females, mean age 35.6 ± 11.2 years, range, 25–49 years), six unrelated patients had a family history of TAAs (four males and two females, mean age 44.8 ± 18.1 years, range, 21–67 years), and nine patients were sporadic TAA cases (five males and four females, mean age 54.6 ± 15.4 years, range, 39–77 years). Control ascending aortas were obtained from five individuals at autopsy who died of cause unrelated to aortic disease and who were not septic at the time of death (two males and three females, mean age 57.2 ± 12.5 years, range, 44–74 years). Aortic specimens were immediately transferred to the lab in a container with cold Waymouth’s MB 752/1 Medium (Invitrogen). Each specimen was divided into several parts for histochemistry, protein and RNA extraction.

Immunohistochemistry Analysis of Aortic Tissue
The same antibodies were used for immunohistochemistry assay as the previous study.18 The antibodies reacting to ICAM-1, VCAM-1, and S-100 were obtained from Dako, and the antibody to CD83 was obtained from Santa Cruz. Immunohistochemistry assay followed a standard protocol.18

T Cell Receptor β Chain Variable Region (TCR BV) Gene Expression by Real-time PCR Analysis
Total RNA was extracted from aortic tissue and peripheral blood mononuclear cells (PBMC) using the RNeasy Mini kit (Qiagen Valencia, CA). cDNA was synthesized with Superscript II Reverse Transcriptase (Invitrogen, Carlsbad, CA). TCR BV and TCR BJ gene expression was analyzed by real-time quantitative PCR. Real-time PCR was performed in 96-well optical PCR plates with SYBR-Green PCR Master Mix (Applied Biosystems, Foster City, CA) on an ABI 7000 Sequence Detection System (Applied Biosystems, Foster City, CA) following published protocols.19;20 Reactions were performed in duplicate.

Statistics Analysis
To test whether there were significant differences in the number of positive inflammatory cell staining in the aortas between controls and patients with MFS, FTAA and TAA, exact Wilcoxon rank-sum tests were performed. Multiple comparison corrections were made using Bonferroni corrections [significance levels were corrected for multiple comparisons by dividing them by the number of multiple comparisons]. Linear regression analyses were conducted to investigate the association between the number of positive cells and the age at surgery.

Results
Characterization of the Inflammatory Cells in TAAs of Patients with MFS, FTAA, and Sporadic TAA Referred for Prophylactic Surgical Repair
Immunohistochemistry was performed using monoclonal antibodies directed against markers for T and B lymphocytes and macrophages to characterize the cells present in the aneurysm wall in patients undergoing prophylactic repair of an ascending aortic aneurysm. CD3+ T cells were clearly observed throughout the sections of aortic media and adventitia of all patients with aneurysms but not in the control aortas (Fig. 1, CD3 staining), and confirmed by
immunostaining with a monoclonal antibody to CD45 (data not shown). Further characterization indicated that CD4+ and CD8+ T cells were both present in the aortas (Fig. 1, CD4 and CD8 staining). CD20 immunostaining detected a few B lymphocytes in the TAAs but no cells in control aortas (Fig. 1, CD20 staining). Staining with macrophage-specific antibody indicated that all aneurysm tissue sections contained CD68+ macrophages that were frequently present diffusely in the media and adventitia of the aortas from patients but were rarely found in control aortas (Fig. 1, CD68 staining).

Quantization of the positive immune cells in the aortic media indicated that the patients with MFS, FTAA and TAA had significantly increased amounts of CD3+, CD4+, CD8+, CD68+, and CD20+ cells when compared with control aortas (p<0.01) (Fig. 2). Furthermore, the number of CD3+ cells in aortas of patients with MFS and CD68+ cells in aortas of patients with MFS and FTAA were also significantly increased when compared to those with sporadic aneurysms (p<0.01). T cells were the predominant inflammatory cell in the media, followed by macrophages. However, there was no significant difference in degrees of aortic enlargement between the three aneurysm groups (p = 0.661), suggesting that the increased numbers of immune cells in the MFS and FTAA compared with sporadic TAA were not due to aortic expansion.

Interestingly, these studies also revealed a large number of inflammatory cells present in the adventitia, especially surrounding the vasa vasorum (Figure 1, CD3 staining in adventitia). Although both CD3+ and CD68+ cells were found in the adventitia, the cells surrounding the vasa vasorum were predominantly CD3+ (Fig. 1, CD3, in adventitia). Infiltration of inflammatory cells in the adventitia was found to a lesser degree in sporadic TAA aortas compared to those of MFS and FTAA aortas, and not found in control aortas.

Immunohistochemistry performed using antibodies directed against intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) showed that staining for ICAM-1 and VCAM-1 was increased around the vasa vasorum in the patients with FTAA and MFS compared to control aortas (Fig. 3).

We sought to determine if the number of inflammatory cells present in the aortic media at the time of surgery correlated with the patients’ age at the time of prophylactic repair. These results demonstrated that both the number of T lymphocytes and macrophages in the aortic media were inversely related to the age of patients at the time of surgery with \( r = -0.71, p = 0.002, \) \( r = -0.69, p = 0.002 \), respectively (Fig. 4). In contrast, there was no correlation between the diameter of the aortic aneurysm at the time of surgical repair and the number of CD3+ and CD68+ cells \( r = -0.2045, p = 0.447, r = -0.1917, p = 0.477 \), respectively.

**Characterization of the T cell Receptors in T Lymphocytes in the Aortic Media**

To further characterize the genetic properties of the T cells in the aortic wall, RNA was extracted from the aortic tissues and used to assess the T cell receptors. Real-time quantitative PCR assay for RNA expression demonstrated restricted usage of the TCR BV gene in T cells derived from diseased aortas. A series of real-time PCR analyses revealed over-expression of BV22 and BV25 in more than a majority of the aortic specimens from patients with thoracic aortic aneurysm (10 out of 14 subjects) with one TCR BV gene contributing to over 20% of the total usage (Fig. E1). Over-expression of one gene was present in 5 samples for BV22 and 8 samples for BV25. Combination of expression level of BV22 and BV25 contributed to more than 50% of the whole TCRBV gene usage in 7 specimens. In contrast, BV gene distribution appeared highly heterogeneous in the remainder of aneurysm aortas (3 out of 14) and control aortas. BV gene distribution also appeared highly heterogeneous in the paired peripheral blood mononuclear cells (PBMC) obtained from patients with MFS and TAA, respectively.
Clonality of the over-expressed BV22 and BV25 of T cells derived from selected aortic specimens by TCRBJ gene usage was also examined. The TCRBJ profile of BV22 and BV25 was analyzed by using BV22 or BV25 forward primers and reverse primers for 13 BJ genes with real-time PCR. Both BV22 and BV25 genes exhibited heterogeneous TCRBJ gene profiles in aortic specimens derived from controls and patients with thoracic aortic aneurysm. These analyses revealed that no BJ genes were preferentially used in the context of the over-expressed BV22 and BV25 (data not shown).

The clonality of the T cell receptors suggested that the T lymphocytes may be replicating in response to specific antigens presented by activated dendritic cells. Staining of the aneurysm aortas with a marker of activated dendritic cells, CD83, showed rare CD83+ cells located at the adventitial/medial border, whereas CD83+ cells were not found in control aortas (Fig. 1, CD83).

Discussion

An inflammatory component in medial degeneration, composed primarily of T cells and macrophages, was identified in TAAs due to single gene disorders, MFS and familial TAA, and non-genetic, sporadic cases of TAA. Surprisingly, more inflammatory cells were present in the aortas of patients with single gene defects than in patients with sporadic TAAs. The T cell associated vasculitis of the vasa vasorum and the increased expression of leukocyte adhesion molecules by the endothelial cells of the vasa vasorum suggest that a pathway for T cell migration into the media is from the adventitia. The number of both inflammatory T lymphocytes and macrophages in the aortic media negatively correlated with patients’ age at referral for prophylactic surgical repair, thus, raising the possibility that the inflammatory infiltrate may contribute to disease progression. Alternatively, the inflammatory infiltrate could correlate with the aortic dilatation and corresponding pathologic changes, but not contribute to the disease progression. In contrast, there was no correlation between the size of the aorta and the number of inflammatory cells, implying that the inflammatory infiltrate did not simply result from irritation of the tissues from the progressive enlargement of the aorta. It is interesting to note that recent studies have suggested that dysregulation in TGF-β signaling contributes to aortic disease in both MFS and familial TAA due to TGFBR2 mutations.21,22 TGF-β signaling plays a critical role in controlling T cell homeostasis and is required to prevent undesirable self-targeted responses, suggesting that TGF-β dysregulation may contribute to the aortic T cell immune response.23

Tang and colleagues also observed that leukocyte infiltration increased in the media of aortas with TAAs compared to control aortas. They suggested dividing TAAs into two subgroups, bland and infiltrated aneurysms, based on whether leukocytes infiltrate into the inner half of aortic media.16 T and B lymphocytes were significantly increased in the aortas with infiltrated aneurysms compared to those of bland aneurysms and controls. Macrophages were significantly increased in the aortic media with infiltrated aneurysms compared to those of bland aneurysms and controls. Although insufficient information is provided to determine the clinical etiology of infiltrated and bland TAAs, it may be that the infiltrative aneurysms came from patients with a genetic cause to their aneurysms and the bland were from patient with sporadic TAAs.

There are similarities between the characteristics of the inflammatory infiltrate present in TAAs and the inflammatory infiltrate described in giant-cell arteritis and Takayasu’s arteritis. These disorders demonstrate a similar cellular immune response involving T cells, primarily CD4+ cells and macrophages, without evidence of an autoantibody component contributing to the pathology.24 Histology studies indicate that T cells enter tissue with activation of endothelial cells in the vasa vasorum and not through the intimal endothelium. Furthermore, a limited T
cell receptor repertoire has been shown to infiltrate the aortic tissue in Takayasu’s arteritis, suggesting that an aortic tissue antigen presented in the cleft of certain major histocompatibility complex molecules in the aortic tissue was targeted by the T cells. Restricted usage of T cell receptors on the T cells was also identified in TAAs, specifically TCRBV18, TCRBV22, and TCRBV25. Although the direct link between dendritic cell function and T cell activation remains unclear in the TAA tissue, the presence of activated dendritic cells capable of presenting antigen in the adventitia of TAAs but not in the control aortas provides further support that a tissue antigen is targeted by the T cells.

Once activated, CD4+ T cells can polarize into type I (Th1) or type 2 (Th2) T helper cells, which in turn control the inflammatory cascades in different diseases. A recent study of transmural inflammation of TAAs indicated that Th1-type or pro-inflammatory process is the dominant immune response occurring in aneurysms. Upregulation of IFN-γ significantly correlated with both outward vascular remodeling and intimal expansion of TAAs. Th1 responses favor effective clearance of intracellular pathogens and play crucial roles in the pathophysiology of organ-specific autoimmune diseases. Autoimmune encephalomyelitis and thyroiditis are two good examples that autoimmune diseases are initiated by Th1-mediated responses. The prominent autoimmune etiology of multiple sclerosis is also considered to be the aberrant activation of IFN-gamma-producing Th1 cells that recognize self-peptides of the myelin sheath. The restricted usages of TCRBV and infiltration of predominant Th1-type cells in TAA lesions suggest that a Th1 mediated pro-inflammatory response could be a mechanism that contributes to the pathogenesis of TAA.

The results of our study here also parallel results with a novel mouse model of thoracic aortic aneurysms, the IL-1 receptor antagonist deficient mouse (IL1-Ra−/−). An inflammatory aortitis of the ascending aorta develops in the deficient mice that are not present in wild type mice, involving medial infiltration of T lymphocytes and macrophages. Furthermore, peripheral T cells from the IL1-Ra−/− mouse cause aortic aneurysm in nu/nu mice, suggesting that activated and/or memory T cells are generated and involved in the development of the ascending aortic disease. Just as in the case for giant cell and Takayasu’s arteritis, the pathogenic antigens in the aorta in this mouse model require further investigation.

In our study, no preferential usage of BJ gene was selected in the context of over-expressed BV22 and BV25 in the majority of TAA aortas leading to an alternative possibility that a superantigen-driven stimulation of Vβ22 and Vβ25 positive T cells in the aortic tissues of the TAA patients may be one of the major mechanisms that contributes to the initial immune response in the aortic tissue. Mitogens, superantigens, and nominal antigens use distinct mechanisms to activate T cells and induce proliferation. Mitogens and nominal antigens induce a polyclonal proliferation of T cells independent of the TCRBV family expressed. In contrast, stimulation of certain superantigens, such as toxic shock syndrome toxin-1, is based on TCRBV family and independent of CDR3 sequence. The T cell response to superantigens is oligoclonal and restricted in terms of complementary determining region 3 (CDR3).

The phenotype and biological activity of invading T cells and macrophages in TAAs should be further characterized before drawing conclusions as to their relevance to the disease process. The limited access to aortic tissue in humans impedes assessing the role of the inflammatory infiltrate in disease progression. Despite these limitations, our study results suggest that an immune mediated aortitis may contribute to pathogenesis of TAAs and that a spectrum of aortic disease processes from Takayasu’s arteritis to TAAs formation involve inflammatory infiltrates in the aortic media. Further investigations are needed to verify a role of this inflammatory infiltrate in the progressive molecular pathology of TAAs.
Acknowledgements

This work was support by the National Institutes of Health (R01HL62594 and P50HL083794 (D.M.M.), R01HL59249 and RO1HL69509 (Y.J.G.), the Doris Duke Foundation (D.M.M), and General Clinical Research Center (MO1RR02558),

Reference List


Figure 1.
Immunohistochemistry for markers of immune cells in the media and adventitia of aortas from control, MFS, FTAA and TAA. The marker recognized by the antibody used for immunohistochemistry, segment of the aorta photographed and the magnification is indicated to the left of the panel. The etiology of the aneurysm is indicated above the panel. The orientation in the photographs is the lumen of the aorta at the bottom and the adventitia at the top. Color was developed with DAB for peroxidase (brown) or Fast Red for alkaline phosphatase (red). Magnification 200x for panels except CD3 adventitia staining.
Figure 2.
Morphometric quantization of lymphocytes, subpopulations of T cells, and macrophages in ascending aortas of controls and patients with MFS, FTAA and TAA. After immunohistochemistry of aortic tissue, CD3, CD4, CD8, CD20 and CD68- positive cells in the media in 10 contiguous high-power fields (magnification 400x) were counted by two independent observers. In the aortas of patients with MFS, FTAA and TAA, there was a significant increase in the number of CD3+, CD4+, CD8+, CD20+, and CD68+ cells when compared with control aortas (* p<0.01).
Figure 3.
Increased expression of leukocyte adhesion molecules, ICAM-1 and VCAM-1, in the vasa vasorum in the adventitia of MFS and FTAA patients. Immunohistochemistry revealed positive immunostaining for ICAM-1 and VCAM-1 in the vasa vasorum from patients with MFS and FTAA. Magnification 400x.
Figure 4.
Inverse correlation between the age of patients at the time of prophylactic repair of the aorta and number of T lymphocytes (CD3+ staining) and macrophages (CD68+ staining) in the aortic media of patients. CD3+ cells (A) and CD68+ cells (B) were counted in 10 contiguous high-power fields of the aortic media of TAAs from 19 patients with MFS, FTAA, and sporadic TAA.
Figure E1.
TCR BV gene distribution profiles in the aortas of controls (CTL) and TAA patients with MFS, FTAA and sporadic TAA, and in PBMC of patients with MFS and TAA. TCR BV gene expression was analyzed quantitatively using RNA from aortic tissue and PBMC by real-time PCR using specific primers for 25 BV genes. BV gene distribution is presented as the mean percentage expression of each BV gene relative to BC expression on the y axis.
TABLE 1
Clinical information of individuals participated in the study

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* Hypertension
† Bicuspid aortic valve
‡ In the column present the samples have been used for TCR assay, counting number of various inflammatory cells present in the aortic media, and correlation assay of the number of T lymphocytes and macrophages present in the aortic media and with the patient’s age at the time of prophylactic repair.

Total cells per square millimeter.