Aortic Diseases

Aortic Aneurysms
An Immune Disease With a Strong Genetic Component

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In the United States, aortic aneurysms are the 13th leading cause of death. Approximately 15,000 individuals die every year because of the rupture of aortic aneurysms. On the basis of autopsy studies, it has been estimated that 1% to 2% of the population harbor aneurysms in their aorta, with up to 10% prevalence in older age groups. Most aortic aneurysms go undetected until rupture, and the mortality from ruptured aneurysms is as high as 90%. Along the length of the aorta, significant heterogeneity occurs in the distribution of aneurysm disease. The prevalence of abdominal aortic aneurysms (AAAs) located in the infrarenal section of the aorta is at least 3 times higher than that of thoracic aortic aneurysms and dissections (TAADs). In TAADs, ≈50% involve the ascending aorta, 10% the arch, and 40% the descending thoracic aorta. Only ≈25% of patients with TAAD have a concomitant AAA, and multisegmental disease is found in only ≈10% of cases.

There are also other differences between TAAD and AAA: (1) Age at onset for TAAD (65 years) is 10 years earlier than for AAA (75 years), and (2) AAAs are predominantly a disease of white men, with a 6:1 male-to-female ratio, whereas TAADs occur only slightly more frequently in men (1.7:1). Additional differences can be found in the pathobiology of these aneurysms. AAAs are characterized by signs of local chronic inflammation of the aortic wall, decrease in the number of smooth muscle cells in the aortic media layer, and fragmentation of the extracellular matrix of the aorta at the site of the aneurysm. Increased local expression of proinflammatory cytokines and matrix metalloproteinases (MMPs) have also been demonstrated. Furthermore, AAAs can be induced in a surgical experimental model in which elastases are infused into rodent aorta. TAADs are characterized by medial necrosis, also known as “Erdheim’s cystic medial necrosis” and more recently referred to as “medial degeneration,” mucoid infiltration, and cyst formation with elastin degradation and vascular smooth muscle cell apoptosis.

Both TAAD and AAA are silent diseases, often without symptoms. They can, however, be readily identified through imaging techniques. TAADs can be detected by echocardiography or computed tomography, and family members of TAAD patients will benefit from imaging by identifying their TAADs before catastrophic consequences. Presently, there are no recommendations about large-scale screening of populations for TAAD. On the other hand, for AAA, ultrasonography screening studies have demonstrated the cost-effectiveness of population-based ultrasonography screening programs and a decrease in the number of aneurysm-related deaths. Several recommendations have been made, including a recent consensus statement in which Kent et al recommended ultrasonography screening for AAA for all individuals aged >60 years and for those aged >50 years with family history of AAA and the recommendation by the US Preventive Service Task Force to screen for AAA in men aged 65 to 75 years who have ever smoked.

Aneurysms Are a Complex Disease
Aortic aneurysms are a complex multifactorial disease with genetic and environmental risk factors. Genetic factors have been shown to play a role in the etiology of TAAD and AAA even when they are not associated with Marfan syndrome, Ehlers-Danlos syndrome, Loeys-Dietz syndrome, or other rare aortic syndromes. As shown in Table 1, the TAAD and AAA susceptibility loci identified thus far do not overlap, suggesting that different genetic risk factors contribute to these 2 forms of aneurysmal disease. Furthermore, both TAAD and AAA demonstrate genetic heterogeneity, as has been shown to be the case for yet another form of aneurysms, the intracranial aneurysms. Elucidation of the genetic risk factors for aneurysmal diseases will require multidisciplinary approaches (Figure), in which animal studies, although not discussed here, will play a key role.

Genetics of TAADs
First reports on familial occurrence of Erdheim’s cystic medial necrosis date back >60 years, although it is not possible to establish whether these cases were syndromic or nonsyndromic TAADs. In 1967, Hanley and Jones reported on TAAD in 2 sisters and the son of 1 of them and...
noted that the patients did not fit the diagnostic criteria of Marfan syndrome. Many reports have been published since then, and systematic studies have established that 20% of nonsyndromic TAAD patients have a positive family history for aneurysms.12 Most TAAD families appear to be consistent with the autosomal dominant inheritance pattern.

To date, 5 susceptibility loci for TAAD have been identified in DNA linkage studies with family-based approaches,13 and a sixth locus was found by the candidate gene approach.14 The loci have been designated as AAT1 through AAT6 (Table 1) and include 3p22–25,13 5q13–14,13 9q33–q34,14 11q23–24,15q24–26,13 and 16p13.13–p13.12.15 Two of the loci (3p22–25 and 9q33–q34) are exactly the same as the genetic loci for Loeys-Dietz syndrome, a rare autosomal dominant disease characterized by hypertelorism, craniosynostosis, structural brain abnormalities, mental retardation, congenital heart disease, bifid uvula with or without cleft palate, and generalized arterial tortuosity with ascending aortic aneurysm and dissection. In addition, the family used for the identification of the AAT4 locus on 16p13.13–p13.12 was a large 178-member French family with TAAD and patent ductus arteriosus. These findings suggest that there is some overlap between the syndromic and nonsyndromic forms of TAAD on the molecular level. On the basis of the 6 TAAD loci identified thus far, genetic heterogeneity of TAAD is already obvious. However, they do not explain the familial aggregation of TAAD in all the families that have been studied, suggesting that additional loci will be found.13

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OMIM indicates Online Mendelian Inheritance in Man; ID, identification; AD, autosomal dominant; COL3A1, gene symbol for type III procollagen; TGFBR1 and 2, gene symbols for transforming growth factor-β receptors 1 and 2; and MYH11, gene symbol for smooth muscle myosin heavy chain. All loci except the EDS4 and AAT5 were identified by DNA linkage studies with either family-based or “affected relative pair” approaches. For a complete list of the original studies, please see http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=search&DB=OMIM. No genes harboring mutations in aneurysm patients in the AAA1, AAA2, AAT1, AAT2, or AAT6 loci have yet been identified. Official approved gene symbols were obtained from www.gene.ucl.ac.uk/nomenclature.

Figure. Methods of studying the pathogenesis and risk factors of AAA in humans, showing the approaches used to identify genetic risk factors of aortic aneurysms (AA) and the genomic and proteomic approaches used to study biological processes involved in AA. Information from one approach can facilitate another approach. Modified with permission from Kuivaniemi and Tromp (2000).89
Mutations in the Positional Candidate Genes in Patients With Syndromic and Nonsyndromic Forms of TAAD

Three genes have been found to harbor mutations in patients with TAAD (Table 1). The AAT3 locus contains the gene for transforming growth factor-β receptor 2 (TGFBR2), and mutations in it were identified in 4 of 80 TAAD families in 1 study, although another study failed to identify mutations in 70 aneurysm patients. The gene for transforming growth factor-β receptor 1 (TGFBR1) is located on the AAT5 locus, and a mutation in this gene was found in 1 patient with TAAD. Again, another research group found no mutations in this gene in 70 aneurysm patients. These findings support the hypothesis that dysregulation of TGF signaling is 1 of the potential mechanisms leading to TAAD, but it is too early to estimate the fraction of TAAD patients who have a mutation in the genes affecting this molecular pathway.

In the study by Loeys et al, mutations in the TGFBR1 and TGFBR2 genes were also found in 52 of 52 and 12 of 40 patients with Loeys-Dietz syndrome and Ehlers-Danlos syndrome, respectively, both of which are rare, syndromic forms of TAAD. Other studies have also reported mutations in patients with Loeys-Dietz syndrome and Marfan syndrome-related disorders in these 2 genes. The clinical and genetics communities are debating the interpretation of these results. Should molecular diagnosis be adopted, in which all individuals with a mutation in the same gene are classified as having the same disease, or should the clinical manifestations be the basis for diagnosis? The case for molecular diagnosis is that the spectrum of clinical manifestations of rare genetic diseases can be wide and therefore can overlap with those seen in more common diseases; consequently, the presence of a mutation in the same gene would classify the disease. For example, one could argue that all patients with mutations in TGFBR2 gene should be classified as having Loeys-Dietz syndrome. The case for diagnosis based on clinical manifestations is that genetic analyses are useful for explaining the underlying pathogenesis. Thus, mutations in the same gene could lead to different but overlapping phenotypes, e.g., nonsyndromic TAAD versus Loeys-Dietz syndrome for mutations in TGFBR2.

The AAT4 locus contains the gene for smooth muscle myosin heavy chain 11 (MYH11), and mutations in it have been identified in 2 families with TAAD and patent ductus arteriosus.

In summary, further studies are needed to establish estimates on the effect size on the population level for the 3 genes (TGFBR1, TGFBR2, and MYH11) harboring mutations in TAAD patients in some families and to identify additional susceptibility genes for TAAD in the other 3 mapped and undetermined number of unmapped genetic loci.

Genetics of AAA: From Familial Aggregation to Susceptibility Loci

Clifton, who first reported clustering of AAAs in a single family in 1977, speculated that there might be a genetic basis for some cases of “atherosclerotic AAA,” as the disease was known at that time. During the 1980s and 1990s, there were too many publications to quote in a brief review. However, 4 groups merit notation for unique conclusions or methods during the early years of AAA genetics research. Tilson and Seashore reported an initial 16 families, followed by an accumulated series of 50 families. A more recent study on 233 AAA families found that most families (72%) appeared to show an autosomal recessive inheritance pattern.

Johansen and Koepsell wrote the second noteworthy early communication. In addition to the merit of its large scale (250 AAA probands), this article included control subjects (250 probands) with atherosclerotic occlusive disease (AOD). Approximately 19% of AAA probands had an affected first-degree relative by history, whereas only 2% of the AOD patients had a relative with AAA. After adjustments for age and sex, there was a 12-fold increase in risk for the relatives of AAA probands. These authors were the first to suggest that screening might be advisable for relatives to detect AAA disease in time to save life.

Darling and coauthors wrote the third article on this short list, and it is included because it described a situation for which there is still no molecular explanation. In a study of 542 patients undergoing AAA repair, 15% were known to have a first-degree relative with an aneurysm versus 2% of a control group of 500 patients of similar age and sex with no aneurysmal disease (P < 0.001). An analysis was performed to determine whether there were unique features among the cases known to be familial (FAAA) compared with the ones that appeared to be sporadic. The ratio of women to men in the FAAA cases was 2.5-fold higher than in the sporadic cases, in which men consistently outnumber women. In addition, the male FAAA cases tended to be 5 years younger than the non-FAAA male patients. Finally, there was an unusually high incidence of rupture (41%) in the FAAA cases. The authors inferred that women in the FAAA cases increased the likelihood of unfortunate outcomes and used the term Black Widow to caption this situation. Although this terminology is perhaps overly emotive, 1 author of this review (M.D.T.) has reached a similar conclusion on the basis of cases that have come to his attention. He is aware of 1 family in which 9 of 10 offspring of a woman, who died with rupture, developed aneurysmal disease.

The fourth and final article in this short list was by Majumder et al and addressed one of the inherent difficulties of analyzing clinical genetics in diseases of late age at onset, such as Alzheimer disease and AAA. Specifically, there is the obvious difficulty of ascertainment of the status of other first-degree relatives. The parents are usually deceased, and the children have years to live before passing through the window of peak risk (≈80 years of age in men with AAA). In addition, some siblings may have died young and been presumed to have died with myocardial infarction. Sixteen percent of 43 probands in the study by Majumder et al were already known to have an affected first-degree relative by history. Indeterminate siblings were invited to undergo ultrasound screening, and 51% agreed. Six AAAs were discovered in families that had previously been considered negative for clustering, raising the percentage of multiplex families to almost 30%. This rather remarkable finding anticipates the conclusion of Verloes and coworkers when analyzing the families of >300 Belgian AAA patients that 1 of the common
AAA susceptibility genes operates in an autosomal dominant manner with surprisingly high yet age-related penetrance. The aforementioned findings support the hypothesis that AAAs are a complex genetic disease (Table 2). Additional supportive data exist. Female AAA patients have been found to have an increased operative mortality for intact and ruptured AAAs compared with male patients. Female AAA patients have been found to have an increased operative mortality for intact and ruptured AAAs compared with male patients. This might be due to such factors as later referral of female patients to treatment or the size standards for AAAs being the same in women and men (although women are generally smaller and have smaller aortas; thus, a 5-cm aneurysm in a female patient is in a more advanced stage than a 5-cm aneurysm in a male patient). It is, however, possible that the differences in the outcomes are due to genetic factors. Because the prevalence of AAA is 6 times higher in men than in women, it would be expected that when the disease occurs in women, it is due to the presence of a larger number of liabilities, a phenomenon characteristic of multifactorial diseases and well documented in such diseases as pyloric stenosis and many congenital heart defects. Female AAA patients would then represent the more severe spectrum of the aneurysmal disease, and their offspring would be expected to have a higher risk of developing AAAs.

A whole-genome DNA linkage study using an affected relative pair approach has been performed for AAA. The study identified 2 genetic loci for AAA, designated as AAA1 on chromosome 19q13 and AAA2 on chromosome 4q31 (Table 1). Van Vlijmen-van Keulen et al reanalyzed 3 of the AAA families with a different statistical method and also found linkage on chromosome 19q13, providing further validity to the study of Shibamura et al. Both positional candidate intervals contain a large number of biologically and physiologically feasible candidate genes for AAA, and studies to identify the specific mutations are in progress.

### Candidate Gene Analysis for AAAs

A large number of studies have been published that focus on analyzing potentially biologically relevant candidate genes for AAA in case-control–based genetic association studies or resequencing such candidate genes in AAA patients. These studies have been summarized in a recent review and will not be discussed in detail here. The candidate gene studies for AAA have focused primarily on 3 classes of genes: (1) genes for the structural components of the aortic wall (eg, collagens, proteoglycans, elastin); (2) genes for enzymes responsible for degrading the structural molecules (eg, MMPs and their inhibitors); and (3) genes for proteins involved in the immune response. The underlying hypothesis in genetic association studies is that a sequence variation in a gene can confer a higher risk to an individual to develop an AAA by influencing the activity or expression of that gene. It is important to note that such sequence changes are not usually the cause of the disease but rather contribute to the susceptibility for developing an AAA, and additional factors, genetic and environmental, are needed to manifest the disease. Although significant associations have been obtained in individual studies, efforts to replicate them in follow-up studies have failed. For example, 1 study found an association with a promoter polymorphism in the MMP9 gene, whereas another study with a slightly larger sample set did not detect any association with the same variant. The discrepancy could be due to several reasons: (1) The identified genetic risk factors have small effect sizes; (2) there are population-based differences in the genetic risk factors for AAA; or (3) the initial findings were false-positives due to small sample sizes or bias in selecting control groups. Detailed recommendations on how to design, conduct, interpret, and report on genetic association studies have been published recently.
Aneurysms as a Disease of the Immune System

Early Discovery Work Leads to Autoantigen Hypothesis for AAs

Before the introduction of biologically inert prosthetic materials as aortic replacements, the potential importance of the immune system as a mediator of the inflammatory response in the adventitia and outer media of the typical AAA in humans was recognized. Rob et al.55 who had extensive experience with the use of human aortic homografts, observed pointedly that the disintegration of some grafted human aortas had features of a severe immune rejection.36 Similar suggestions were made for the failure of bovine carotid heterographs when they were once used for femoro-popliteal bypass grafts in humans.37

The theory of immune involvement in AAA was strengthened by animal studies. Petersen et al.38 performed aortic autografts in spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats. No aneurysms were observed, and therefore the surgical procedure of transplantation alone did not initiate AAA disease. We infer that this fact rules out one of the older theories of the pathogenesis of AAA, specifically, that AAA results from deprivation of the outer media from oxygen provided by vasa vasorum, at least in the rat. When the authors transplanted SHR aortas to the WKY rats, aneurysms did occur, with infiltration of inflammatory cells and smooth muscle cell apoptosis. However, when WKY aortas were transplanted to SHR hosts, no aneurysms were seen. The authors concluded that “immunologic rejection but not abnormal hemodynamics is necessary for development of allograft aneurysm in this model.” Because the SHR mutation is on the WKY strain, the transplants were not allografts (in a strict sense). If the SHR and WKY strains were of different evolutionary lineage, it would be tempting to speculate that the use of different rat histocompatibility antigens might explain the results, but that was not the case here. Instead, it may be more reasonable to hypothesize that there has been a stable phenotypic alteration in the aortas that have been exposed to hypertension in the SHR donors, perhaps unmasking an epitope of an extracellular matrix protein detected as “nonself” by the WKY recipient.

Another important discovery was through the work of Anidjar, Dobrin, et al.39 who developed the widely used elastase-infusion model. One of the most interesting aspects of this model is that 24 hours after injury with elastase, aortic dilation is minimal. Aneurysms develop about a week later, during which time a major influx of inflammatory cells has occurred. Halpern et al.40 studied this model in more detail to characterize the histopathological and enzymatic events that were detectable during the latency period, confirming that there was a dramatic increase in all subsets of immune/inflammatory cells by day 7. Immunoglobulins colocalizing with extracellular matrix were also evident by day 7. The authors concluded that autoimmune processes played a role in the pathogenesis of the Anidjar-Dobrin model, perhaps because the initial injury by elastase unmasked reactive epitopes in the aortic matrix. This and other models have been used extensively for the study of AAA, and the results have been reviewed recently.4

Interest in the role of autoimmunity in human AAA began independently by 2 research groups. One of them was initially interested in the possibility of mutations that might lead to instability of collagen cross-linking in load-bearing fibrils of the aortic wall, and the cross-link deoxyxypyrudinol (d-Pyr) was assayed.51 However, instead of being deficient as expected, d-Pyr was significantly elevated. Because d-Pyr is a hallmark of immature collagen, the question arose of whether it was elevated in a reparative adaptation to collagen destruction by an inflammatory process. This led to an analysis of differences in the site and characteristics of the inflammatory infiltrate in AAA versus AOD.42 Russell bodies, aggregates of immunoglobulins that are hallmarks of autoimmune diseases such as Hashimoto’s thyroiditis, were noted in the adventitia and outer media of AAA specimens. Immunoglobulins from AAA specimens were purified and were shown to react with the adventitial microfibril associated with elastin and collagen.53 Purification of an immunoreactive protein led to partial characterization of the first putative autoantigen, named aneurysm-associated antigenic protein—40 kDa (AAAP-40).41 Another research group found extensive inflammatory cell infiltration in AAA tissue samples.45,46

AAA is an Immuneologic Disease

The early work described above introduced the concept that autoimmunity may be responsible for the pathogenesis of AAA.42–44,46 Such a mechanism assumes the breakdown of the immunoregulatory mechanisms and of tolerance in general, which will permit the generation of an autoimmune response against self antigens. Molecular mimicry is another mechanism that may be responsible for the pathogenesis of AAA and the generation of autoimmunity.47 Molecular mimicry is defined as the sharing of common antigenic epitope(s) between a microorganism (such as bacteria and viruses) and self (host) antigens (reviewed in Oleszak et al.48). An immune response against a bacterial or viral antigenic epitope may recognize, by molecular mimicry, as nonself a self epitope, which is homologous (cross-reacting) with the antigenic epitope of the microorganism that initiated the T-cell response. This T-cell response may be propagated by the self cross-reacting epitope long after the clearance of the virus or of the microorganism. It appears that molecular mimicry is responsible for several autoimmune diseases (reviewed in Oleszak et al.48). The immune response to the antigenic epitope(s) of the microorganism may be critical for breaking tolerance and would permit the development of an immune response against self antigens. Therefore, these T cells will recognize, by molecular mimicry, both the cross-reacting antigenic epitopes, the one of the microorganism and the other of the host antigen, and may play a critical role in the pathogenesis of the disease.

Substantial evidence has been accumulated suggesting that AAA is a specific antigen-driven T-cell disease and that T cells may be responsible for the initiation and/or the propagation of the disease (Table 3), as follows.
1. Mononuclear Cell Infiltrates Containing CD3+ T Cells Are Present in AAAs

Extensive mononuclear cell infiltrates have been documented in the adventitia and, to a lesser but substantial degree, the media of AAAs in humans.46,49–52 These infiltrates comprise T and B lymphocytes, plasma cells, monocytes, and natural killer/natural killer T cells.46,49–52 CD3+ T cells account for \( \approx 50\% \) of the hemopoietic cells (CD45+), and macrophages account for \( \approx 25\% \).46,49–52 These infiltrates are suggestive of activation of vascular-associated lymphoid tissue (VALT).49 Inflammatory cell infiltrates are absent from normal aorta, which at most may contain only few cells of the immune system.46 Mono- nuclear cell inflammatory infiltrates are found also in atherosclerotic plaques of patients with AOD,53 a common feature of AAA patients. There are, however, substantial differences in the inflammatory response found in AAA versus those seen in AOD.54

2. Early-, Intermediate-, and Late-Activation Antigens Are Expressed by Mononuclear Cells Infiltrating AAAs

T lymphocytes and other mononuclear cells infiltrating AAAs express early-activation (CD69), intermediate-activation (CD25, CD38), and late-activation (CD45 RO, HLA class II) antigens.47 The expression of these activation antigens on the infiltrating inflammatory cells suggests the presence of active ongoing inflammation in these lesions. In particular, the presence of CD69+ T cells in these AAAs inflammatory sites reveals that T-cell activation is occurring in situ in AAAs. The CD69 antigen is expressed on T cells very early on activation, and it is responsible for T-cell interactions with cells of the monocyte/macrophage lineage leading to the production of interleukin-1 (reviewed in Isler et al55 and Testi et al56).

3. Association of HLA Alleles With AAA

An association of HLA-DRB1 alleles (HLA-DR2*15 and *16, *12 and *13) was described by Tilson et al57 and Rasmussen et al (HLA-DRB1*04 and *15).58 The presence of the DRβGln(70) residue was identified as a risk factor for AAA.58 These results are somewhat controversial because other larger studies have not been able to replicate these findings.59,60

4. Antigen-Presenting Cells Are Present in AAAs

Both professional and nonprofessional antigen-presenting cells (APCs) are present in AAAs and include the following: (1) infiltrating cells of the monocyte/macrophage lineage,46,49–52,61,62 (2) vascular dendritic cells that have been reported to be in contact with T and B lymphocytes in human AAAs61 and are associated with formation of lymphoid follicles and lymph node–like structures (VALT), primarily in the adventitia of these patients, suggesting that these APCs may be responsible, at least in part, for the induction of both cellular and humoral immune responses61; (3) activated endothelial cells may act as APCs in AAAs62; and (4) vascular smooth muscle cells expressing HLA class II are present in AAAs and may act as APCs.62

5. Mononuclear Cells Infiltrating Human AAAs Contain Oligoclonal αβ T-Cell Receptor+ T Cells and γδ T-Cell Receptor+ T Cells

Clonal Expansions of αβ T-Cell Receptor+ T Cells

To determine whether human T cells infiltrating AAAs contain clonally expanded populations of αβ T-cell receptor (TCR)+ T cells, α- or β-chain TCR transcripts were amplified from these AAAs by the nonpalindromic adaptor-polymerase chain reaction (NPA-PCR)/Vα- or Vβ-specific PCR.47,63,64 The NPA-PCR method was specifically developed for the amplification of transcripts with unknown or variable 5′ ends, such as the TCRs and the immunoglobulins.47,63,65 The amplified TCR transcripts were cloned and sequenced. Sequence analysis revealed the presence of substantial proportions of identical β-chain TCR transcripts in 9 of 10 patients examined. These clonal expansions were very strong. In certain patients, the proportions of identical β-chain TCR transcripts in the AAAs were as high as 60% of the transcripts sequenced.

Amplification of α-chain TCR transcripts from human AAAs by NPA-PCR followed by cloning and sequencing revealed strong clonal expansions in 4 of 5 patients examined.47 β-Chain TCR transcripts were cloned expanded in all 4 patients.47

Clonal expansions that were identified in AAAs with the use of NPA-PCR amplification were subsequently confirmed by Vα- or Vβ-specific PCR amplification. Identical clonal expansions of TCR transcripts were identified by these 2 different amplification approaches, followed by cloning and sequencing. Peripheral blood mononuclear cells (PBMCs) from normal donors were used as methodological controls in these experiments.47,63 Amplification of α- or β-chain TCR transcripts from these PBMCs by NPA-PCR/V-specific PCR followed by cloning and sequencing revealed unique α- or

### Table 3. Evidence for AAA Being a Specific Antigen-Driven T-Cell Disease

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<td>Enrichment of genes related to immune function seen in AAA microarray studies</td>
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See text for details.
β-chain TCR transcripts compared with each other, typical of polyclonal populations of T lymphocytes.47,63

Oligoclonal T-cell expansions were also found in the aortas of patients with 2 diseases that are related to AAA: giant cell arteritis66 and Takayasu arteritis.67,68

Clonal Expansions of γδ TCR+ T Cells
The presence of clonally expanded populations of γδ TCR+ T cells was demonstrated in AAAs.47 Sequencing analysis revealed the presence of substantial proportions of identical copies of Vγ1, Vγ2, Vδ1, and Vδ2 TCR transcripts in human AAAs in all patients examined.47

PBMCs from normal donors were used as a methodological control for these studies and were found to have almost entirely unique γ- or δ-chain TCR transcripts, in a manner typical of polyclonal populations of T cells.47 However, sequence analysis of Vδ1 TCR transcripts from PBMCs from normal donors revealed strong clonal expansions that were significant by the binomial distribution, in agreement with the reports of others.69,70 The role of these Vδ1 clonal expansions demonstrated in PBMCs from normal donors is poorly understood.

All of these clonal expansions identified in AAAs were statistically significant, as determined by the binomial distribution. Comparison of the nucleic acid and the deduced amino acid sequences of the TCR transcripts sequenced in these studies with those in the GENBANK/EMBL/SWISS PROT databases revealed that all sequences identified were novel, ie, were not described previously, and typical of the corresponding TCR transcripts.

The majority of the γδ TCR+ T cells recognize whole proteins, in a manner independent of the major histocompatibility complex. In contrast, the vast majority of αβ TCR+ T cells recognize peptides in association with major histocompatibility complex. Other γδ TCR+ T cells recognize lipids, glycolipids, carbohydrates, bacterial phosphoantigens, and other ligands in a major histocompatibility complex–independent manner. It has been suggested that γδ TCR+ T cells use their TCR as a pattern recognition receptor.71 This may be important in the event that the microorganisms that have been proposed as putative antigens in AAA (see below) indeed play a role in the pathogenesis of the disease. γδ T cells have been proposed to be a bridge between the innate and the adaptive immune system.71

The only possible explanation for the presence of substantial proportions of identical copies of α-, β-, γ-, or δ-chain TCR transcripts in AAAs is that the T-cell clones utilizing these TCR transcripts have undergone proliferation and clonal expansion in vivo in response to specific, as yet unidentified antigen(s), either self or nonself.47 T cells are composed of many different T-cell clones. Each T-cell clone recognizes antigen (antigenic epitope) through its TCR. TCRs are highly polymorphic molecules, expressed only on cells of T-cell lineage (reviewed in Boehm and Rabbitts72). Each clone of T cells expresses a different TCR molecule, which is acting as a fingerprint of that particular T-cell clone. The αβ TCR and the γδ TCR are expressed on different T-cell clones, and their expression is mutually exclusive. The maximum theoretical number of the αβ TCR heterodimers has been calculated to be 1018 and of the γδ TCR heterodimers 1019 (reviewed in Boehm and Rabbitts72). Elimination of >90% of the thymocytes by thymic selection reduces the size of the T-cell repertoire to the order of 109 different β-chain TCR polypeptide chains in the peripheral blood, each one pairing with ≥25 different α-chain TCR polypeptides.73 These estimates allow for the presence in the peripheral blood of ≈2.5×109 different T-cell clones, a number that is still very large.73 Similar estimates can be obtained for the γδ TCR. Therefore, the probability of finding by chance substantial proportions of an individual TCR transcript, either α-, β-, γ- or δ-chain, in an independent sample of T cells is negligible. The appearance of these multiple identical copies of TCR transcripts must be the result of specific antigen-driven proliferation and clonal expansion of individual T-cell clones responding to the antigenic epitopes that they recognize. This is the only possible mechanism to explain the presence of substantial proportions of identical copies of TCR transcripts found in human AAAs.

6. Autoantibodies and AAA
The presence of autoantibodies in AAAs was well documented in early studies by Tilson and associates43,44 and has been described in a previous section of this review. A critical observation was that purified IgG from AAA lesions identified a host protein expressed in normal aortic tissue, demonstrating an autoimmune antibody response in AAA.43,44 B cells and plasma cells are present in AAA lesions and express the CD69 and CD80 activation markers.74 In addition, significantly higher proportions of IgA-positive and IgG-positive B cells are present in AAA lesions in comparison to those found in the peripheral blood from the same patient.74 Little attention has been paid to the clonality of B cells infiltrating AAA lesions. One report, using a genomic PCR approach, suggested that there are no restrictions in the usage of VH gene segments by B cells infiltrating atherosclerotic AAAs.75 Additional studies, however, are needed to clarify this issue.

7. Putative Self and Nonself Antigens That May Elicit Cellular and Antibody Responses in AAA
Although many questions on the pathogenesis of AAA remain to be answered to provide definite proof that AAA is a specific antigen–driven autoimmune disease, several putative self and nonself antigens have been identified (Table 4). These antigens elicit cellular and humoral immune responses in AAA. Among the nonself antigens, perhaps the most studied is Chlamydia pneumoniae, which may be involved in the initiation or the acceleration of AAA.47 C pneumoniae is frequently found in the vessel walls of AAA patients by immunohistochemical analysis, transmission electron microscopy, and tissue culture approaches. C pneumoniae–specific T lymphocytes were found in the mononuclear cell infiltrates of AAAs.47 In addition, nonself antigens may be responsible for initiating AAA by molecular mimicry (see above). These microorganisms may initiate an immune response, which is then propagated by the host cross-reacting determinants leading to clinical disease, long after the microorganism is cleared.
Table 4. Putative Antigens That May Elicit Cellular and Humoral Responses in AAA

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self antigens</td>
<td></td>
</tr>
<tr>
<td>Elastin and elastin fragments</td>
<td>47</td>
</tr>
<tr>
<td>Collagen type I</td>
<td>47</td>
</tr>
<tr>
<td>Collagen type III</td>
<td>47</td>
</tr>
<tr>
<td>AAA P-40*</td>
<td>43, 44</td>
</tr>
<tr>
<td>Oxidized low-density lipoprotein</td>
<td>47</td>
</tr>
<tr>
<td>Nonself antigens</td>
<td></td>
</tr>
<tr>
<td>Chlamydia pneumoniae</td>
<td>47</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>47</td>
</tr>
<tr>
<td>Salmonella</td>
<td>47</td>
</tr>
<tr>
<td>Treponema pallidum</td>
<td>47</td>
</tr>
</tbody>
</table>

* Also known as human microbial-associated glycoprotein-36 (MAGP-36).

8. The Role of Cytokines in the Pathogenesis of AAA

Substantial production of mostly proinflammatory cytokines in AAAs is well documented.\(^{51,76–78}\) They are produced by activated T cells, monocytes, and other infiltrating mononuclear cells, as well as by several types of cells of the aorta. Studies with a mouse AAA model developed with the use of a CD4\(^{+}\) knockout mouse have shown that CD4\(^{+}\) T cells producing interferon-\(\gamma\) (IFN-\(\gamma\)) play a critical role in matrix remodeling in AAA and the pathogenesis of the disease.\(^{78}\) These CD4\(^{+}\) knockout mice were resistant to the induction of aneurysms.\(^{78}\) However, intraperitoneal application of IFN-\(\gamma\)-producing CD4\(^{+}\) T cells partially reconstituted the development of aneurysms in these CD4\(^{+}\) mice.\(^{78}\) Targeted deletion of IFN-\(\gamma\)-producing CD4\(^{+}\) T cells (lacking the costimulatory molecule CD28) have been reported both in AAA tissue and in peripheral blood of patients with AAA.\(^{76}\) These studies demonstrate the critical role of T cells and IFN-\(\gamma\) in the pathogenesis of AAA. The overexpression of the transcription factor T-bet taken in connection with the absence of IFN-\(\gamma\) receptor expression according to this study of vascular smooth muscle cells.\(^{86}\) Intimal expansion and production of IFN-\(\gamma\) in transmural inflammation and increased expression of IFN-\(\gamma\) positively with Th1 but not with Th2 immune responses.\(^{86}\)

In TAADs, approximately half of the patients exhibited transmural inflammation and increased expression of IFN-\(\gamma\) in aneurysm tissue, whereas Th2 cytokines were undetectable.\(^{86}\) In this group of patients with transmural inflammation, the inner media was devoid of mononuclear cell infiltrates.\(^{86}\) However, specimens with inner media lymphocytic infiltration also exhibited increased IFN-\(\gamma\)-inducible chemokines IP-10 and Mig.\(^{86}\) Transmural inflammation and production of IFN-\(\gamma\) were associated with increased aortic diameter, intimal thickening, decreased amount of extracellular matrix proteins, and preserved density of vascular smooth muscle cells.\(^{86}\) Intimal expansion and outward vascular remodeling of these aneurysms correlated positively with Th1 but not with Th2 immune responses.\(^{86}\) Expression studies of 1185 genes revealed distinct patterns of expression between TAADs and infrarenal AAAs, TAADs above and many other reports in the literature paint a controversial picture for the type of cytokine responses, Th1 or Th2, that predominate in AAA lesions. Although many studies have reported the predominance of a Th1 response in human AAA specimens and experimental models of AAA, other studies have reported the predominance of a Th2 cytokine response.

9. Treatment With Immunosuppressive Drugs Significantly Reduces the Rate of Aneurysm Expansion in Experimental AAA Models

Immunosuppressive regimen have been shown to suppress the growth of experimental aortic aneurysms in support of the notion that aneurysmal disease is a T cell–dependent disease. Aneurysms in animals treated with rapamycin were significantly smaller in diameter versus controls, and they exhibited lower levels of nuclear factor-\(\kappa\)B and MMP-9.\(^{82}\) Similarly, treatment with methyl prednisone and cyclosporin significantly suppressed the growth of rat aortic aneurysms induced by elastase perfusion.\(^{83}\)

10. Global Gene Expression Profiles of AAA Tissue Reveal a Significant Enrichment of Genes Related to Immune Function

Microarray-based gene expression studies provide an unbiased way of obtaining a global gene expression signature for disease tissues. Analyzing the results of such profiles generated for AAA tissues showed an overrepresentation of biological pathways involved in immune response,\(^{84}\) providing further evidence that AAA is an immunologic disease.

Evidence for Involvement of the Immune System in TAADs

The immune system is likely to play a significant role also in TAADs, although the number of studies performed in TAADs compared with those in AAAs is much lower. Mononuclear cell infiltrates containing high proportions of CD3\(^{+}\) T cells and CD68\(^{+}\) monocytes have been identified in TAADs.\(^{85}\) CD3\(^{+}\) T cells were localized primarily in the media and in the adventitia (surrounding the vasa vasorum). Both CD3\(^{+}\) T cells and monocytes coexisted with vascular cell death apoptotic markers, and they may be responsible, at least in part, for the elimination of smooth muscle cells in these aneurysms.\(^{85}\)
and normal aorta from the same site, and infrarenal AAAs and normal aorta from the same site. This high degree of molecular heterogeneity of degenerative aneurysms may reflect the involvement of different mechanisms in the pathophysiology of these disorders. Genomic and proteomic studies revealed that the levels of expression of 138 genes in peripheral blood leukocytes of patients undergoing thoracoabdominal aortic aneurysm repair and the concentrations of 7 plasma proteins discriminated between patients who developed multiorgan dysfunction syndrome and those who did not.

Conclusion
Aortic aneurysms are an important cardiovascular disease, particularly in the aging population of industrialized countries. They are a complex disease with both genetic and environmental factors contributing to the disease process, which involves formation, growth, and rupture. There are even regional differences along the length of the aorta, with AAAs being much more common than TAADs, and on the basis of the pathophysiology and other features, it is reasonable to hypothesize that TAADs and AAAs are separate disease entities. Aneurysms are often silent without symptoms until rupture occurs, but they can be detected effectively via imaging techniques. First-degree relatives of aneurysm patients have an increased risk of the disease, and it is therefore important to offer appropriate advice to these individuals and counsel them to seek screening options. Although current surgical treatments give excellent results, there is a need to develop nonsurgical approaches to manage small aneurysms. Even if surgical treatments give excellent results, there is a need to develop innovative approaches as well as resources are needed. In this review article, we have discussed the fundamental research discoveries related to immunology and genetics of aneurysms but urge the readers to get more information from the proceedings of a recent meeting on “The Abdominal Aortic Aneurysm: Genetics, Pathophysiology, and Molecular Biology,” published by the Annals of the New York Academy of Sciences (Vol 1085, 2006).

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References


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