Incidence and Pathogenesis of Aneurysmal Disease of the Abdominal Aorta

DAVID C. PAIK, M.D.
RESEARCH FELLOW IN VASCULAR BIOLOGY AND SURGERY, DEPARTMENT OF SURGERY,
COLUMBIA UNIVERSITY, ST. LUKE'S/ROOSEVELT HOSPITAL CENTER,
NEW YORK, NY

JOSEPH F. CAPELLA, M.D.
RESEARCH FELLOW IN VASCULAR BIOLOGY AND SURGERY, DEPARTMENT OF SURGERY,
COLUMBIA UNIVERSITY, ST. LUKE'S/ROOSEVELT HOSPITAL CENTER,
NEW YORK, NY

M. DAVID TILSON, M.D.
ALISA MELLON BRUCE PROFESSOR OF SURGERY, DEPARTMENT OF SURGERY,
COLUMBIA UNIVERSITY, ST. LUKE'S/ROOSEVELT HOSPITAL CENTER,
NEW YORK, NY

Abdominal aortic aneurysms (AAAs) are a major cause of illness and death in the United States and abroad. Along with progress in the surgical management of this condition, numerous advances have been made in understanding the pathogenesis of AAAs. Since the time of Scarpa (1804), AAA disease has been associated with, and attributed to, atherosclerotic vessel changes. Excluding patients with Marfan's Syndrome and Ehlers-Danlos type IV diseases, virtually all human AAA specimens contain some degree of atherosclerosis. However, atherosclerotic changes are associated with diverse arteriopathies (ie, aorto-occlusive disease versus AAAs). Over the last 25 years, considerable research has been performed comparing aneurysmal, occlusive, and normal aortae. AAA disease is a unique process with pathogenic mechanisms that may operate independently of atherosclerosis.[1] In this chapter we discuss the incidence of AAA as well as its pathogenesis concerning genetics, molecular biology, biochemistry, and immunology.

INCIDENCE

AAA disease is the 15th leading cause of mortality in the United States, accounting for over 15,000 deaths per year.[2] Autopsy studies report the prevalence of AAAs ranges from 1.8% to 6.6%.[3-6] Ultrasound screenings report the prevalence of clinically significant AAA ranges from 2% to 3%.[7,8] Data from autopsy studies, routine mortality statistics, and hospital in-patient statistics indicate that AAA incidence and prevalence are rising.[9-11] Although much of this rise can be attributed to greater physician awareness and improved diagnostic tools (ie, computed tomography [CT] scan, ultrasound), AAA mortality is also increasing.[10] Interestingly, death rates from other conditions associated with atherosclerosis (ie, cerebrovascular and coronary artery disease) have been falling over the last 30 years.[12,13] Melton and colleagues reported a seven-fold increase in AAAs in Rochester, Minnesota residents between 1951 and 1980.[14] The same study noted age-specific increases in AAAs of all kinds: asymptomatic, symptomatic, uncomplicated, and complicated. The greatest increase, however, was among asymptomatic, uncomplicated aneurysms. An autopsy report from Malmo, Sweden found the age-specific prevalence of AAAs increased 4.7% among men and 3% among women annually from 1958 to 1986.[11]

Certain groups are at increased risk for developing AAAs. Men are from two to six times more likely to develop AAAs.[15-17] The prevalence among men increases rapidly after age 55, reaching a peak incidence of 5.9% at age 80. Women reach a peak of 4.5% at an age greater than 90.[11] In a study from North Carolina, Caucasian men were found to have a three-fold greater incidence of AAAs as compared to Black men and both Caucasian and Black women.[18] Persons with a first-degree relative with an AAA are at a significantly greater risk of developing an AAA than is the general population.[19] In addition, AAAs are more common among taller patients.[20,21]

PATHOGENESIS

Genetics

Over the last 15 years, the concept of a genetic basis for AAAs has become more firmly established. In 1981, four years after Clifton described three brothers who had spontaneous rupture of AAAs,[22] Tilson and Stansel noted a preponderance of males to females for the condition and suggested an x-linked inheritance pattern.[20] In 1984, Tilson reported on 16 families and later 50 families with two or more first-order relatives affected by aneurysms. The most common association was among brothers (22 of 50 families). The male-to-female ratio appeared to support an x-linked pattern of inheritance; however, it was also noted that in eight families the father apparently transmitted the susceptibility to his sons. This finding prompted the hypothesis of autosomal dominant inheritance with unexplained sex limitation. In addition, a multi-gene pattern of inheritance could not be excluded.[23,24]

Numerous papers followed confirming the familial tendency of AAA disease. In 1986, Johonsen and Koepsell compared the family histories of 250 patients with 250 controls.[19] The AAA patients reported first-degree relatives with AAAs in 19.2% of cases, while the controls reported first-degree relatives with AAAs in only 2.4% of cases. A prospective study of 542 patients by Darling and colleagues reported 15.1% of probands having AAA surgery to have first-degree relatives with AAAs, compared with 1.8% for controls.[25] Interestingly, probands with a female first-degree relative with AAA disease had a significant risk of rupture (63% versus 37%). Darling and coworkers hypothesized a "black widow syndrome." Gregory and colleagues speculated that female members in these families may be a marker for a more severe genetic variant.[26] One of the more recent pedigree studies on families with AAAs favors a recessive gene at an autosomal major locus. The segregation analysis of first-degree relatives of 91 probands in this study suggested that a single autosomal diallelic autosomal locus, without a multifactorial component, accounts for the genetic susceptibility to AAA disease.[27]

Molecular Biology

A number of genetic loci have been studied with regards to AAA pathogenesis and include genes specific for blood born proteins/antigens (haptoglobin, ABO, Rh, human lymphocyte antigens [HLAs], cholesterol ester transfer protein [CETP] as well as genes specific for matrix proteins (type III collagen, tissue inhibitor of metalloproteinases [TIMP], fibrillin).[28-34]

Attempts have been made to find haptoglobin and blood groups as well as HLAs that might be associated with AAA disease. In a study of haptoglobin groups and AAAs, a significant increase in haptoglobin 2-1 over controls was noted, but no direct effect on AAA pathogenesis could be concluded.[35] In a study on blood groups and HLAs, Norrgard and colleagues found no significant increase in blood group A, as had been found by other authors.[36,37] However, a decrease in Rh factor and an increased frequency of the MN group and Kell-positive subjects were found.[37,38]

As a follow up to the study noting the association between the haptoglobin 2-1 phenotype and AAAs, Powell and colleagues investigated the polymorphisms of the haptoglobin gene and a neighboring cholesterol ester transfer protein (CETP) gene, both on the long arm of chromosome 16.[28] The protein polymorphism of haptoglobin results from a variant of alpha chains, a-1 and a-2. The frequency of the a-1 haptoglobin allele, as well as a rare polymorphism of the CETP gene, is increased in patients with AAA disease. These two hypothesized markers act independently. *In vitro* studies show that haptoglobin containing alpha-1 chains accelerate aortic elastin degradation by two- to four-fold. The CETP polymorphism is in linkage equilibrium with the haptoglobin alpha allele and may have a separate effect. The rare CETP polymorphism could alter plasma CETP levels, modify CETP function in plasma lipid exchange, or act as a marker of another closely linked gene. Alternatively, the gene associated with AAAs could be in linkage disequilibrium with both.[35,39]

The genes specific to the synthesis and degradation of matrix proteins may dictate the extensive connective tissue destruction characteristic of AAA disease. A defect in the gene for procollagen type III may be responsible for AAAs in a small number of patients. Kontusaari and colleagues studied a 37-year-old healthy woman who had several family members die from ruptured AAAs.[29] Deoxyribosenucleic acid (DNA) analysis showed the woman to be heterozygous for a single-base mutation that converted the codon for glycine in the gene for the type III procollagen molecule to a codon for arginine. Cultured skin fibroblasts demonstrated that the mutation caused synthesis of procollagen that had a lower temperature for thermal unfolding of the protein. A similar mutation was found in the DNA of family members who had died from ruptured AAAs. Despite initial enthusiasm, a

follow-up study of 50 patients from AAA families showed only one person with a functionally significant change in the coding sequences of the triple helical domain of type III procollagen.[39]

The reported deficiency of tissue inhibitor of matrix metalloprotease (TIMP) in the walls of AAAs prompted Tilson and colleagues to explore the possibility of a genetic mutation at the previously sequenced TIMP gene in AAA patients.[30] Although an identical polymorphism was detected in two of six patients, the alteration did not result in change at the protein level because it was in the third position of the codon.

A mutation in the gene for fibrillin has been found in patients with Marfan's Syndrome.[34] Fibrillin makes up the microfibrillar structure of the extracellular matrix of aortas and appears to act as the scaffolding for the deposition of elastin during elastogenesis.[31-33] The significance of fibrillin in AAA disease is under investigation.

Biochemistry

The major areas of interest concerning AAA biochemistry are:

- Elastin and elastolytic proteases
- Collagen and collagenolytic proteases

These biochemical considerations provide a foundation for discussion of an immunologic pathogenesis of AAAs.

Elastin and Elastolytic Proteases

Elastin is produced in a soluble precursor form, pro-elastin (Mw, 72 kDa). It is abundant in skin, ligament, and lung alveoli in addition to the media of arterial walls and is produced by smooth muscle cells, chondroblasts, mesothelial cells, fibroblasts, and myofibroblasts.[40] Similar to collagen, one third of its amino acid composition is glycine although not in the collagen-characteristic gly-x-y amino acid pattern. Glycine's small size allows for close inter-peptide strand apposition.[41]

Elastin's hallmark insolubility and remarkable elastic recoil capacity (up to three times its resting length) have been attributed to the numerous mono- (lysinonorleucine) and tetra- (desmosine) cross-links.[41] Its extreme chemical stability is evidenced by its half-life, which is approximately 70 years.[42] The cross-link reaction is catalyzed by the copper-requiring enzyme lysyl oxidase. Copper deficiency (Menkes' kinky hair syndrome in humans and the Blotchy mouse animal model) results in poor elastin/collagen cross-linking and the development of arterial aneurysms.[43-50] Under normal circumstances, elastin fibers are arranged in layers or lamellae.

Since the documentation of decreased elastin/collagen content in the walls of AAA specimens 25 years ago by Sumner and colleagues,[51] research efforts concerning elastin and AAAs have been numerous. In 1988, using Verhoeff's technique for iron hematoxylin staining with a van Gieson counterstain (ie, EVG or "elastin-van Geison" stain), Tilson demonstrated a marked depletion of elastic lamellae in four of nine AAA specimens.[52] Such histochemical findings were supported by biochemical studies in which depleted elastin content was confirmed by high-performance liquid chromatographic as well as two-step paper chromatographic techniques. A deficiency of stable cross-links could not be shown.[53,54] Recent studies by White and colleagues indicate that elastin depletion occurs early in AAA pathogenesis.[55]

The knowledge regarding elastin depletion and AAAs has stimulated an exhaustive search for a culpable proteolytic mechanism. The four known classes of proteolytic enzymes are: (1) serine proteases, (2) metallo (or zinc) proteases, (3) thiol proteases, and (4) carboxyl (or acid) proteases.[41] Of these, serine and metallo (or zinc) proteases have most commonly been implicated in AAA research.

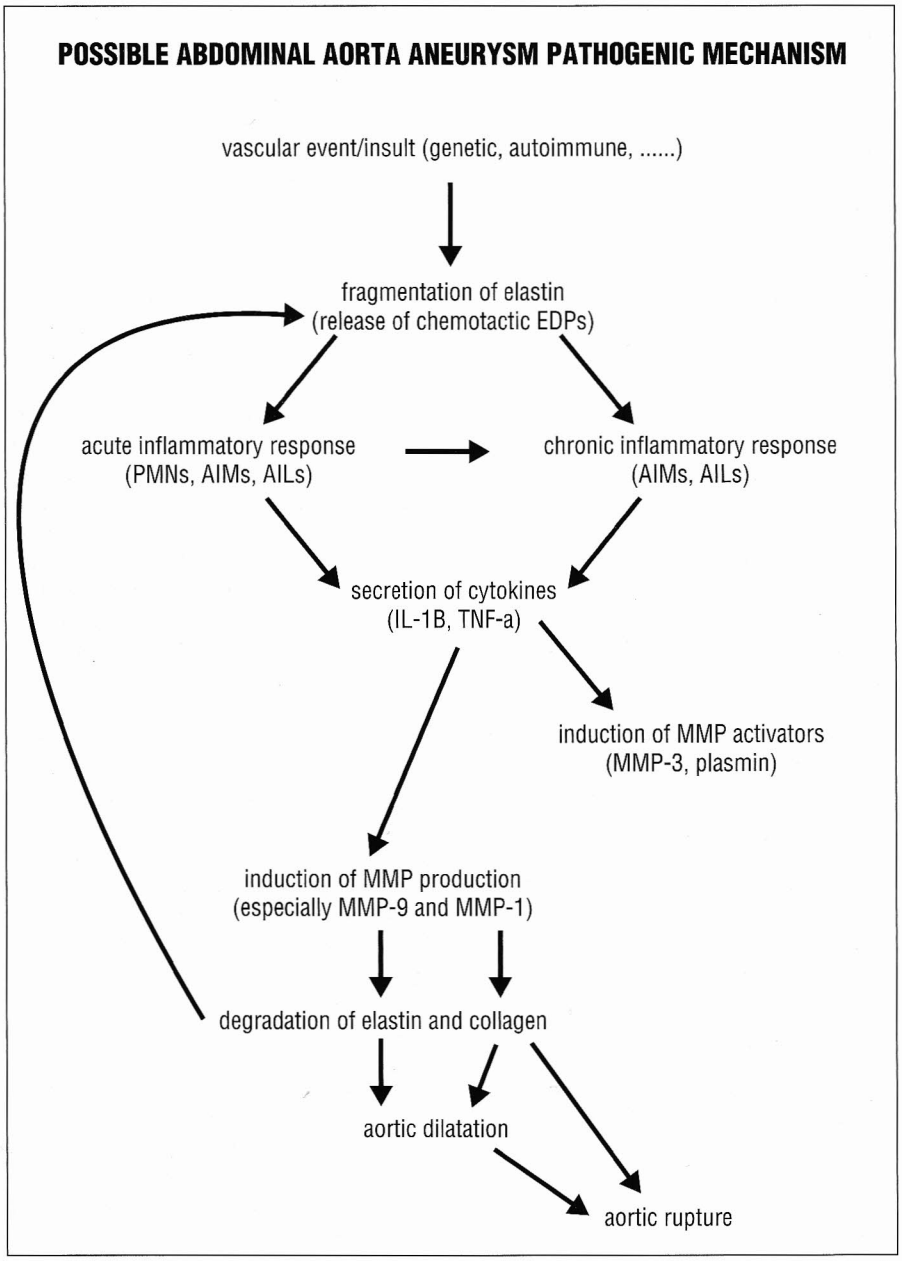

Figure 1. Schematic representation of the factors involved in abdominal aortic aneurysm pathogenesis and rupture.

In 1982, Busuttil and colleagues found increased elastolytic activity in AAA tissue.[56] In the same year, Cannon and Read reported increased elastolytic activity in the blood of AAA patients, attributing such findings to a leukocyte elastase of the serine protease family.[57] The family of serine proteases all contain a highly reactive serine residue at the 195 position, which, when reacted with organic flouro compounds, forms a stable inactive enzyme complex.[41] Several investigators, including Dubick and colleagues (pancreatic elastase), Cohen and colleagues (smooth muscle cell elastase), and Herron and colleagues,[58-60] have subsequently implicated serine proteases in AAA pathogenesis by virtue of enzyme activity inhibition by phenylmethylsulfonyl fluoride (PMSF).

The matrix metalloproteases, or MMPs, are a group of enzymes involved in extracellular matrix remodeling. They are a subset family of metalloproteases, the prototype being the digestive enzyme carboxypeptidase, and all require a tightly bound zinc ion for activity. Metal chelators such as ethylenediamine tetraacetic acid (EDTA), inhibit their activity.[61-63]

In 1985, Brown and colleagues described a serum elastolytic protease not inhibited by PMSF.[64] Campa and colleagues supported such findings in 1987, describing a tissue protease from the media of AAA specimens that did not cross-react with leukocyte (serine) elastase antibodies and was partially inhibited by EDTA.[65] We have also isolated a principal elastolytic enzyme from AAA specimens that is partially inhibited by EDTA. In addition, this 80-kDa protease is not inhibited by PMSF, but is inhibited by recombinant TIMP. Using substrate gel enzymography (SGE), affinity isolation with r-TIMP, and specific anti-MMP-9 antibody, we believe the principal tissue metallo-elastase is MMP-9.[66] MMP-9 is known to be secreted as its 92-kDa zymogen and when activated loses approximately a 10-kDa segment.[62] This is consistent with our observation of the principal elastolytic activity at 80 kDa.

In addition, we have recently found that the 80-kDa casein substrate gel band also includes the serine protease plasmin, a known stimulator of MMP activity.[67] Plasmin is the major activator of MMP-9.

Collagen and Collagenolytic Proteases

The basic structural unit of collagen is tropocollagen, a 285-kDa molecule composed of three long peptide chains (approximately 1000 residues each) intertwined to form a long strand.[41] Variations in the composition of chains determine the type of collagen produced (>11 types). Aortic collagen (two thirds of which is type I collagen) is concentrated in the adventitia.[68-71] Similar to elastin, collagen's insolubility results from the multiple cross-links (hydroxypyridinium) between its fibers (catalyzed by copper-requiring lysyl oxidase). Although collagen lacks the recoil capacity of elastin, its tensile strength is approximately four orders of magnitude greater.[72]

Much aneurysm research has addressed both collagen/collagenolysis and elastin/elastolysis concurrently. As mentioned previously, Sumner and colleagues detected decreased elastin/collagen content in AAA specimens in 1970.[51] Since then, however, decreased collagen content has not been consistently shown in AAA samples, probably due to tissue fibroblasts' ability to synthesize new collagen throughout a lifetime (although it may lack normal tensile strength), which is not true for elastin, whose synthesis occurs predominately during the first decade of life.[73] In 1984, Dobrin and colleagues showed that elastase infusion into human arteries caused mild dilatation and collagenase infusion caused vessel rupture. Elastase infusion alone did not lead to dilatation of aneurysmal dimensions.[74] Tilson and colleagues interpreted these data to imply that collagen must also fail during aneurysm pathogenesis.[75] In addition, they noted that vessels denuded of their elastic lamellae (ie, endarterectomy), generally do not become aneurysmal, implicating adventitial collagen disruption in AAA formation.

Collagenolysis research has been numerous but somewhat inconsistent. Beginning with Busuttil and colleagues in 1980, several investigative groups (including our own) have shown increased collagenase activity from intact AAA specimens.[76-80] However, other groups have reported contrary findings,[60-81] and Menashi and colleagues detected collagenase activity only in ruptured AAAs.[82] Such inconsistencies may be attributed, in part, to the binding of MMP-1 (the major tissue collagenase) to its naturally occurring inhibitor, TIMP, masking its detection in enzyme assay. Using immunoreactive assay techniques that detect collagenase-TIMP complexes, we have found collagenase activity in AAA extracts in the 57-kDa and 52-kDa range, corresponding to the secreted zymogen isoforms of MMP-1.[80] MMP-1 degrades five different types of collagen, including types I and III (prominent in aorta).[62] Thus, MMP-1 is probably the major tissue collagenase in AAA disease.

We have immunohistochemically identified a known activator of MMP-1 and MMP-9 zymogens, MMP-3 (or stromelysin), in AAA tissue. The MMP-3 (and the serine protease plasmin discussed earlier) may be activating an enzymatic cascade that terminates with MMP-9 and MMP-1 degradation of aortic elastin and collagen.[66]

Immunology

A striking finding on pathologic section of most human AAA specimens is an adventitial inflammatory infiltrate.[83] An animal model using hog pancreatic elastase infusion into rat aortae (Anidjar/Dobrin model) confirms the accumulation of inflammatory cells in the aneurysm wall.[84-86] Cell subsets have been determined immunohistochemically by Koch and colleagues as well as by our laboratory, using flow cytometry.[87] In human specimens, a chronic infiltrate of macrophages (aneurysm-infiltrating macrophages, or AIM cells) and lymphocytes (aneurysm-infiltrating lymphocytes, or AIL cells) is noted.[87] In the animal model, an acute infiltrate of polymorphonuclear cells (PMNs), in addition to lymphocytes and macrophages, is observed.[85]

Neutrophils, one of several types of PMNs, are known secretors of a metallo-collagenase (MMP-8) as well as a serine elastase (neutrophil elastase).[62] Although the inflammatory infiltrate seen in human AAA specimens is generally chronic and lacks neutrophils, their absence may simply be a reflection of the specimens' duration of disease. In the rat aneurysm elastase infusion model, many PMNs infiltrate the aortic wall media, particularly in the early stages of aneurysm formation.[85] Therefore, neutrophils may have an early pathogenic role in human AAA disease.

Macrophages and lymphocytes are known to secrete a number of soluble cytokines, including interleukin-1 beta (IL-1β), also known as lymphocyte activating factor, and tumor necrosis factor-alpha (TNF-α), also known as cachectin. IL-1β can be produced by macrophages and β lymphocytes. It can induce fibroblasts to produce MMPs as well as stimulate macrophages/monocytes to produce TNF-α and more IL-1β.[88] TNF-α can be produced by activated

macrophages/monocytes and lymphocytes. It can induce MMP and IL-1β production by smooth muscle cells.[89]

Pearce and colleagues have detected high IL-1β secretion from AAA explants when compared to cadaveric explants.[90] We have found increased IL-1β and TNF-α levels in AAA extracts.[91] More specifically, in recent preliminary studies, macrophages cultured from fresh AAA tissue are noted to produce MMPs, IL-1β, and TNF-α.[66] Knowing that *in vitro* immature macrophages (monocytes) produce elastolytic serine proteases and mature macrophages produce MMPs (including MMP-9),[92] we believe the tissue macrophage has a major role in AAA pathogenesis.

In the lymphocyte populations, which can secrete both IL-1β and TNF-α, T lymphocytes appear to predominate in the AAA wall, although significant numbers of β lymphocytes are also present.[66] In conjunction with the β lymphocyte infiltrate is a large amount of immunoglobulin, which is obtainable from AAA soluble extracts. We have been exploring a possible autoimmune mechanism. IgG was extracted from AAA homogenates and then used as a probe to detect a 70-kDa antigenic protein contained in fixed AAA tissue sections. We are attempting to sequence this protein (unpublished observations).

The events and signals that initiate the leukocyte migration/infiltration are unclear. Interestingly, however, elastin degradation products (EDPs) (ie, fragments of the elastin molecule) are *in vitro* chemotactic agents for inflammatory cells.[84,93] In addition, when elastase is infused into rat aortae, initial fragmentation of elastic lamellae is present accompanied by infiltration of inflammatory cells. Release of large amounts of proteases and vessel dilatation follow two to three days later.[84,86] Therefore, elastolysis may be initiating a cascade of events that include inflammation and augmented enzymatic degradation of the vessel wall by way of cellular secretion of cytokines and proteases.

SUMMARY

The area of AAA pathogenesis research has grown and diversified significantly over the last 15 years. A comprehensive discussion now includes the fields of genetics, molecular biology, biochemistry, and immunology. Continuing research activity should disclose the molecular basis for susceptibility at the gene level in due course, and this knowledge, in turn, should lead to new approaches for early detection and prevention. STI

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