

Chapter 2

Inflammatory Pathogenesis and Pathophysiology of Abdominal Aortic Aneurysms

William H. Pearce and Vera P. Shively

Abstract The aorta is a high-pressure conduit that also conserves the energy output of the heart (elastic recoil). The main structural proteins of the aorta – collagen and elastin – serve these functions. The aortic architecture is not uniform and varies from the thoracic aorta to the infrarenal aorta. With these basic understandings of the aortic structure and function in mind, it is clear that inflammation, genetics, and mechanical forces play important roles in the pathogenesis of abdominal aortic aneurysms. Additional research is needed to further understand the relationship between these factors.

Keywords Abdominal aortic aneurysm, Pathophysiology, Pathogenesis

Introduction

The aorta is a complex structure in which form follows function. The aorta is a high-pressure conduit that also conserves the energy output of the heart (elastic recoil). The main structural proteins of the aorta – collagen (strength) and elastin – serve these functions. In addition to these proteins, there are thousands of other proteins that make up the extracellular matrix (ECM) of the aorta. In recent years, it has been discovered that the ECM is not a passive structure but contains growth factors and encrypted protein fragments. The packaging of growth factors and the dysregulation of transforming growth factor-beta (TGF- β) play a central role in the pathogenesis of Marfan's disease.^{1,2} The ECM, therefore, may also play a central role in healing and disease.


Aortic architecture is not uniform and varies from the thoracic aorta to the infrarenal aorta.³ This variation in aortic structure may be explained in part by differences in regional hemodynamics. The abdominal aorta is the source of visceral blood flow and, below the renal arteries, the muscles of the lower extremity. The infrarenal aorta, thus, experiences large oscillations in blood flow, depending on lower extremity activity.⁴

With these basic understandings of the aortic structure and function in mind, the role that inflammation plays in the pathogenesis of abdominal aortic aneurysms (AAAs) becomes more apparent. In 1950, Dubost reported the first repair of an AAA using a human homograft.⁵ In his report, he also describes clearly the inflammation found in AAAs: "The main feature, together with the destruction of the elastic tissue is the presence throughout the outer half of the aorta of masses of lymphocytic cells accompanying the vaso vasorum; these cells form concentric sheets but are also seen sometimes to follow the vessels at right angles to all layers of the wall. ... In summary ... this endarteritis and periarteritis would suggest an inflammatory process without any specific indication as to its nature." This observation was not studied in detail until the mid-1980s. Rizzo et al. described an inflammatory infiltrate in the outer walls of the aorta in biochemical studies of patients with aneurysms and occlusive disease.⁶ This inflammatory infiltrate was later characterized by Koch who found that the majority of cells within the median and adventitia of AAAs were CD3-positive T lymphocytes ranging from 67% to 80%.⁷ In addition, CD19-positive B cells were also found within the arterial wall. Further, there was a spectrum of disease progressing from occlusive disease to AAA and to inflammatory AAA with increasing inflammation. This data suggested that aortic aneurysms may represent an immune-mediated event rather than simple degeneration of the arterial wall. Brophy et al. also described the presence of Russell bodies, which are the accumulation of large amounts of immunoglobulins in the arterial wall.⁸ Tilson et al. purified these immunoglobulins and characterized a potential antigenic source, aneurysm-associated protein-40.⁹

The histological and cytokine profiles of the inflammatory infiltrate suggest both an adaptive and an innate immune response. Platsoucas et al.¹⁰ examined whether the infiltrate represented a monoclonal or a polyclonal T-cell amplification. The β -chain T-cell receptor transcripts from patients with AAAs were amplified and characterized. In nine of the ten patients studied, there was an oligoclonal population of T cells. In addition, they found that many of the mononuclear cells found in AAAs expressed early, intermediate, and late activation antigens. They concluded that AAAs are a specific antigen-driven T-cell disease with evidence of an ongoing inflammation.

The relationship between inflammatory infiltrate and the subsequent damage to the arterial wall has been evaluated in many animal models.¹¹ Using an elastase-induced aneurysm model, Thompson et al. studied the progression of disease.¹² Three days following an acute response, a transitional inflammatory state exists. By days 10–14, the inflammation becomes chronic. (See Fig. 1.) During this period, there are alterations in the cytokine proteins as well as the expression of metalloproteinases and their inhibitors. However, experimental aneurysms may occur without an initial injury. APO E knockout mice when exposed to angiotensin II spontaneously develop aortic aneurysms without the sequence of events described above.¹³ Thus, the role that inflammation plays in the initiation of AAA is uncertain.

Matrix metalloproteinases (MMPs) appear to play a critical role in the pathogenesis of aneurysms. Busuttill et al. first described collagenase-like activity in the wall of patients with AAAs in 1980.¹⁴ Since then many investigators have described the role that MMPs play in aneurysm formation. The metalloproteinases are a subfamily of the metazincin superfamily of proteinases.¹⁵ The majority of these proteins are secreted as a proenzyme,

M E D I A T O R S C E L L S	Early	Intermediate	Late
	1-7 days	7-14 days	14+ days
	ECM fragments Oxygen radicals Angiotensin Chemokines Cytokines	MMPs TIMP ECM fragments Cytokines Chemokines	Dysregulation of healing Self-sustaining immune response
	PMN Monocytes T-cells	Macrophages Th1-cells	Macrophages Th2-cells
			
	Progressive elastin degradation and AAA formation		

Th= T helper, PMN= polymorphonuclear neutrophil, ECM= extracellular matrix

Fig. 1 Arterial response following injury. Modified from Thompson et al.¹²

requiring extracellular activation. However, several of the MMPs are membrane-bound. Aortic aneurysms have been associated with a variety of MMPs, including 2, 9, and 13.¹⁶ The substrate specificity of each MMP is variable and, while at first it was thought to be only ECM proteins, other substrates have been identified. Enzyme substrates include both noncollagenase ECM proteins, such as fibronectin and laminen, as well as nonstructural components. The nonstructural components may be even more important to the pathogenesis of AAAs. Nonstructural substrates of the MMPs include interleukin-1 alpha (IL-1 α), active MMP-9, chemokine (C-X-C motif) ligand 5 (CXCL-5), IL-1 β , IL-2 receptor, TGF- β , and many of the pro-MMPs.¹⁷ Thus, MMPs may regulate the inflammatory response.

The immune response is highly regulated to prevent injury to the host. In the majority of instances, the inflammatory process leads to a healing process and tissue repair. However, in some instances, this process becomes pathologic, leading to continued tissue damage secondary to the immune response. An infectious etiology is clear in many cases; however, the initiating event may be unknown. The key intersection of MMPs in the regulations of inflammation may be the central process in the pathogenesis of AAAs. The modification of proinflammatory cytokines, chemokines, and the shedding of the membrane receptors by the MMPs are critical. CXCL-7, CXCL-12, CXCL-5 (chemokine), and TGF- β are substrates of MMP-2, MMP-3, and MMP-9.¹⁸ The lack of critical control of the immune response at multiple layers may, therefore, lead to the same phenotype (AAAs). Therefore, AAAs may arise from a variety of stimulators and the response (healing or destruction) is guided by subtle differences in the control of inflammation.

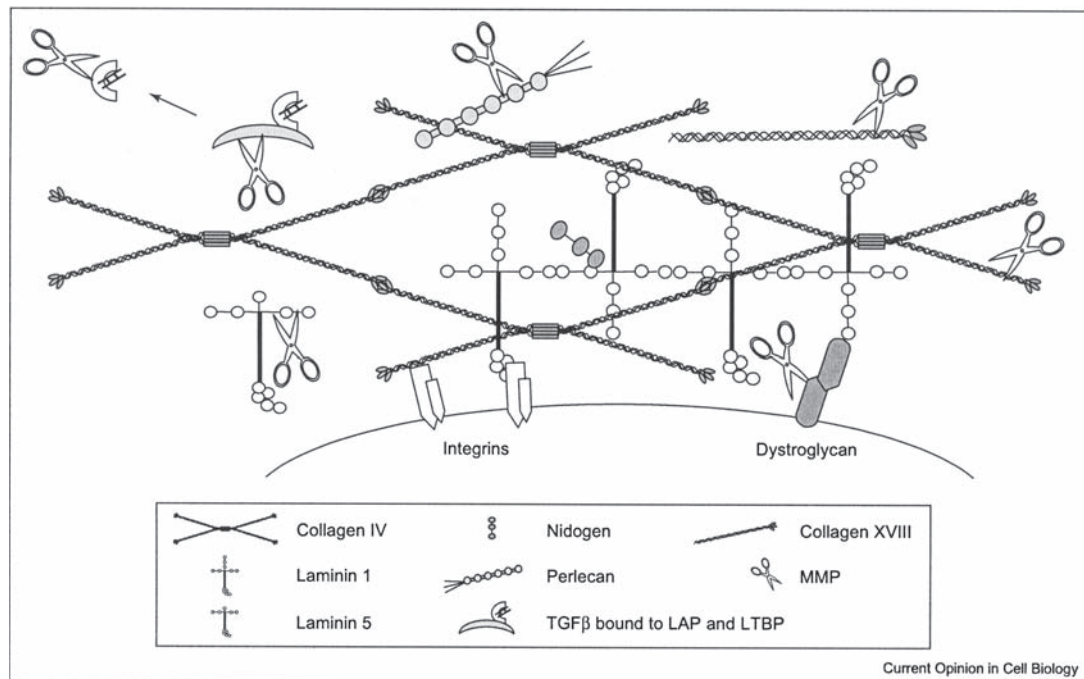


Fig. 2 A schematic diagram of some of the proteins that comprise the extracellular matrix (ECM) and the potential for matrix metalloproteinase (MMP) cleavage. The ECM is composed of both laminin and collagen networks. Within ECM network, many laminins and collagens are present as well as proteins such as nidogen, perlecan (i.e., heparin sulfate proteoglycans), and fibronectin (not shown). Cell surface receptors such as integrins and dystroglycan interact with proteins of the basement membrane network. Cleavage of ECM molecules releases bioactive fragments that have been referred to as matricryptins or matrikines. Furthermore, growth factors, such as latent transforming growth factor-beta ($TGF-\beta$), are embedded with ECM, and proteolysis of binding proteins that keep the growth factors in a latent state is a major activation pathway. In addition, some of the cell surface receptors such as dystroglycan are targets for proteolysis. Cleavage of these types of molecules breaks cell-ECM contact. Reprinted from Mott and Werb.¹⁹ Reproduced with permission from Elsevier

As MMPs digest the matrix, signals may be released to the surrounding vascular smooth muscle cells and inflammatory cells. Cryptic fragments include arrestin, deprellin, endostatin, restin, elastin degradation products, and the growth factors $TGF-\beta$ and vascular endothelial growth factor.¹⁹ Thus, a possible scenario for the progression of aneurysms is digestion of the ECM by the inflammatory infiltrate, which exposes encrypted fragments that continue to stimulate the immune response. A simple example may be elastin degradation products, which have been shown to be chemotactic for monocytes.²⁰ (Fig. 2.)

The Genetic Basis of the Inflammatory Response in AAA

The differential expression of genes involved in immune function in AAA versus control aortic tissue was recently described by Lenk et al.²¹ They used Illumina and Affymetrix microarray platforms to obtain gene expression profiles, and Gene Ontology and Kyoto Encyclopedia of Genes and Genomes database tools to analyze biological pathways. The AAA tissues showed significantly more expression of genes involved in immune function, most notably in pathways

regulating cell adhesion molecules, natural killer cell-mediated cytotoxicity, and leukocyte transendothelial migration. The authors acknowledged that a limitation of their study was sampling of AAA tissue from late-stage disease. However, their gene expression data provides valuable insight into the future development of immune-based treatment strategies, as well as direction to the search for underlying genetic factors in AAA.

Family history, or genetics, is a significant and independent risk factor for AAA.²² Which genes predispose to AAA is yet to be determined, but several candidate gene polymorphism studies have shown an association between AAA and genes mediating inflammation. A recent review by Sandford et al. includes a comprehensive evaluation of genetic research in AAA, based on familial, segregation, and candidate gene studies.²³ In a case-control study of 100 AAA patients and age- and gender-matched controls, Bown et al. reported cytokine gene polymorphism data for the proinflammatory IL-1 β + 3953, IL-6 – 174, and tumor necrosis factor- α (TNF- α) – 308, and the anti-inflammatory IL-10 promoter polymorphisms at positions –1082 and –592. The A allele for IL-10 – 1082 was found in association with AAA, while the others failed to reach statistical significance.²⁴ This allele is associated with low IL-10 secretion, and may contribute to the chronic inflammation that is characteristic of AAA. In another study, Ghilardi et al. reported the association of chemokine receptor CCR5 Δ 32 deletion and AAA when compared to patients with peripheral arterial occlusive disease, carotid artery disease, and controls. Within the AAA subjects, CCR5 Δ 32 was also more commonly found in patients with ruptured aneurysms.²⁵ The deletion results in the production of fewer receptors and, in theory, less cytokine activation through this receptor pathway. This would appear contrary to increased cytokine levels found in AAA tissue, although the mechanism of this receptor has not been fully described.

Our laboratory compared five inflammatory mediator gene polymorphisms in a population of 79 elderly male Caucasian AAA patients versus 71 case-matched healthy controls free of inflammatory conditions. Proinflammatory cytokine polymorphisms included IL-1 β Taq I fragment in exon 5 and TNF- α – 308, and the anti-inflammatory cytotoxic T-lymphocyte antigen-4 (CTLA-4) at positions –318 and +49, and IL-1 receptor antagonist variable nucleotide tandem repeat in intron 2. The +49 CTLA-4 A/G allele frequencies differed significantly ($p < 0.01$) between the two groups, while the others did not reach significance. The healthy controls violated Hardy-Weinberg equilibrium (75% of the subjects were heterozygous), indicating a possible bias. This may have been due to the exclusion of subjects with emphysema, autoimmune disorders, arthritis, stroke, myocardial infarction, and diabetes. However, the controls were matched to AAA cases by age, gender, ethnicity, and smoking history, so it is possible that the data support the idea that the A/G genotype is associated with protection against inflammatory diseases in our study population.

Evidence of Inflammation in the Circulation of AAA Patients

The presence of inflammatory cells and a number of cytokines within AAA tissue has been well-documented through histology, immunohistochemistry, and in situ hybridization, although reports of elevated circulating levels

of cytokines have been fewer. One exception is IL-6. In 1999, Rohde et al. reported increased plasma IL-6 concentrations with increasing abdominal aortic diameter in subjects without aortic dilatation.²⁶ Recently, Dawson et al. reported elevated levels of plasma IL-6 in AAA patients, and a positive correlation between aneurysm surface area and mean plasma IL-6 concentration.²⁷

As our understanding of inflammation moves beyond proinflammatory cytokines, a number of interesting studies have emerged. A recent paper by Duftner et al. reviewed serological markers of inflammation in AAA, and also presented data supporting a T-cell-mediated pathophysiology of AAA.²⁸

The authors review one of the most common, and controversial, serological markers to be analyzed in AAA, C-reactive protein (CRP). Data from studies of CRP levels in asymptomatic AAA have produced varying results, although data from subsets of patients have been more consistent. For example, elevations of serum CRP were found in symptomatic and ruptured AAA,²⁹ and hsCRP was positively associated with aneurysm size.³⁰ Duftner also described data from her own studies that showed an increased prevalence of circulating CD4⁺, CD8⁺, and CD28⁻ T cells in AAA patients free of preexisting immune-mediated disease, cancer, or acute infection. This subgroup of T cells is known to produce large amounts of interferon- γ , and has been previously seen in patients with a variety of autoimmune diseases.

Our own work has recently focused on T-cell costimulatory factors in AAA. In collaboration with the laboratory of the late Ann Kari Lefvert of the Karolinska Hospital in Stockholm, Sweden, plasma samples from 100 of our AAA patients and 109 normal controls were analyzed for the soluble forms of the following T-cell costimulatory factors: sCD28, sCTLA-4, sCD86, and sCD80, as well as MMP-9. Compared to controls, plasma from AAA patients showed elevated levels of the T-cell-activating sCD28 and sCD86 ($p = 0.0001$), and a decrease in an inhibitor of T-cell activation, sCTLA-4 ($p = 0.0018$). We also found a significant inverse relationship between concentrations of sCTLA-4 and sCD80 with MMP-9.³¹ Soluble costimulatory molecules of T-cell activation may provide a new set of markers of immune activation in AAA and possible therapeutic targets for future therapies.

Conclusions

Inflammation appears to play a central role in the pathogenesis of AAAs. The initiating factors that begin the inflammatory cascade are unknown. It is certainly possible that atherosclerosis may be the initiating event. However, smoking is an essential component to the progression of the disease. Once the inflammatory response has been initiated, it appears to be self-perpetuating. Possible explanations for this perpetuation of the inflammation is repeated exposures of cryptic protein fragments. However, what is interesting is that once these patients are treated with endovascular grafts, the aneurysm sac regresses and continued destruction of the aneurysm ceases. If it were true that the inflammatory process was self-sustained, why is it then that the aneurysm sac shrinks when an endograft is placed? Thus, mechanical forces must also play a role in sustaining the process. From the endovascular aneurysm repair experience, it is not pulsatility in as much as its continued pressure. Much additional research must be accomplished to further understand the relationship between mechanical forces, inflammatory disease, and genetics.

References

1. Neptune ER, Frischmeyer PA, Arking DE, Myers L, Bunton TE, Gayraud B, et al. Dysregulation of TGF- β activation contributes to pathogenesis in Marfan syndrome. *Nat Genet* 2003;33:407–411.
2. Pannu H, Fadulu VT, Chang J, Lafont A, Hasham SN, Sparks E, et al. Mutations in transforming growth factor- β receptor type II cause familial thoracic aortic aneurysms and dissections. *Circulation* 2005;112:513–520.
3. Glagov S. Hemodynamic risk factors: Mechanical stress, mural architecture, medial nutrition and the vulnerability of arteries to atherosclerosis. *In: Wissler RW, Geer JC, eds. Pathogenesis of Atherosclerosis. Baltimore: Williams & Wilkins, chap 6, 1972.*
4. Taylor CA, Hughes TJ, Zarins CK. Effect of exercise on hemodynamic conditions in the abdominal aorta. *J Vasc Surg* 1999;29:1077–1089.
5. Dubost C, Allary M, Oeconomos N. Resection of an aneurysm of the abdominal aorta. Reestablishment of the continuity by a preserved human arterial graft, with a result after five months. *Arch Surg* 1952;65:405–408.
6. Rizzo RJ, McCarthy WJ, Dixit SN, Lilly MP, Shively VP, Flinn WR, et al. Collagen types and matrix protein content in human abdominal aortic aneurysms. *J Vasc Surg* 1989;10:365–373.
7. Koch AE, Haines GK, Rizzo RJ, Radosевич JA, Pope RM, Robinson PG, Pearce WH. Human abdominal aortic aneurysms: Immunophenotypic analysis suggesting an immune-mediated response. *Am J Pathol* 1990;137:1199–1213.
8. Brophy CM, Reilly JM, Smith GJ, Tilson MD. The role of inflammation in non-specific abdominal aortic aneurysm disease. *Ann Vasc Surg* 1991;5:229–233.
9. Tilson MD III, Kuivaniemi H, Upchurch GR Jr, eds. 2006;The abdominal aortic aneurysm: Genetics, pathophysiology, and molecular biology. *Ann NY Acad Sci* 1085.
10. Platsoucas CD, Lu S, Nwaneshiudu I, Solomides C, Agelan A, Ntaoula N, et al. Abdominal aortic aneurysm is a specific antigen-driven T cell disease. *Ann NY Acad Sci* 2006;1085:224–235.
11. Halpern VJ, Nackman GB, Gandhi RH, Irizarri E, Scholes JV, Ramey WG, Tilson MD. The elastase infusion model of experimental aortic aneurysms: Synchrony of induction of endogenous proteinases with matrix destruction and inflammatory cell response. *J Vasc Surg* 1994;20:51–60.
12. Thompson RW, Curci JA, Ennis TL, Mao D, Pagano MB, Pham CTN. Pathophysiology of abdominal aortic aneurysms: Insights from the elastase-induced model in mice with different genetic backgrounds. *Ann NY Acad Sci* 2006;1085:59–73.
13. Daugherty A, Rateri DL, Cassis LA. Role of the rennin-angiotensin system in the development of abdominal aortic aneurysms in animals and humans. *Ann NY Acad Sci* 2006;1085:82–91.
14. Busuttil RW, Abou-Zamzam AM, Machleder HI. Collagenase activity of the human aorta. A comparison of patients with and without abdominal aortic aneurysms. *Arch Surg* 1980;115:1373–1378.
15. Lee MH, Murphy G. Matrix metalloproteinases at a glance. *J Cell Sci* 2004;117:4015–4016.
16. Pearce WH, Shively VP. Abdominal aortic aneurysm as a complex multifactorial disease: Interactions of polymorphisms of inflammatory genes, features of autoimmunity, and current status of MMPs. *Ann NY Acad Sci* 2006;1085:117–132.
17. Somerville RP, Oblander SA, Apte SS. Matrix metalloproteinases: old dogs with new tricks. *Genome Biol* 2003;4:216.1–216.11.
18. Parks WC, Wilson CL, Lopez-Boado YS. Matrix metalloproteinases as modulators of inflammation and innate immunity. *Nat Rev Immunol* 2004;4:617–629.
19. Mott JD, Werb Z. Regulation of matrix biology by matrix metalloproteinases. *Cur Opin Cell Biol* 2004;16:558–564.

20. Senior RM, Griffin GL, Mecham RP. Chemotactic activity of elastin-derived peptides. *J Clin Invest* 1980;66:859–862.
21. Lenk GM, Tromp G, Weinsheimer S, Gatalica Z, Bergeur R, Kuivaniemi H. Whole genome expression profiling reveals a significant role for immune function in human abdominal aortic aneurysms. *BMC Genomics* 2007;8:237.
22. Blanchard JF, Armenian HK, Friesen PP. Risk factors for abdominal aortic aneurysm: Results of a case-control study. *Am J Epidemiol* 2000;151:575–583.
23. Sandford RM, Bown MJ, London NJ, Sayers RD. The genetic basis of abdominal aortic aneurysms: A review. *Eur J Vasc Endovasc Surg* 2007;33:381–390.
24. Bown MJ, Burton PR, Horsburgh T, Nicholson ML, Bell PRF, Sayers RD. The role of cytokine gene polymorphisms in the pathogenesis of abdominal aortic aneurysms: A case-control study. *J Vasc Surg* 2003;37:999–1005.
25. Ghilardi G, Biondi ML, Battaglioli L, Zambon A, Guagnellini E, Scorza R. Genetic risk factor characterizes abdominal aortic aneurysm from arterial occlusive disease in human beings: CCR5 Delta 32 deletion. *J Vasc Surg* 2004;40:995–1000.
26. Rohde LEP, Arroyo LH, Rifai N, Creager MA, Libby P, Ridker RM, Lee RT. Plasma concentrations of interleukin-6 and abdominal aortic diameter among subjects without aortic dilatation. *Arterioscler Thromb Vasc Biol* 1999;19:1695–1699.
27. Dawson J, Cockerill GW, Choke E, Belli AM, Loftus I, Thompson MM. Aortic aneurysms secrete interleukin-6 into the circulation. *J Vasc Surg* 2007;45:350–356.
28. Duftner C, Seiler R, DeJaco C, Fraidrich G, Schirmer M. Increasing evidence for immune-mediated processes and new therapeutic approaches in abdominal aortic aneurysms-A review. *Ann NY Acad Sci* 2006;1085:331–338.
29. Domanovits H, Schillinger M, Mullner M, Holzenbein K, Janata K, Bayegan K, Laggner AN. Acute phase reactants in patients with abdominal aortic aneurysm. *Atherosclerosis* 2002;163:297–302.
30. Vainas T, Lubbers T, Stassen FRM, Herengreen S, van Dieijen-Visser MP, Bruggeman CA, et al.. Serum C-reactive protein level is associated with abdominal aortic aneurysm size and may be produced by aneurysmal tissue. *Circulation* 2003;107:1103–1105.
31. Sakthivel P, Shively V, Kakoulidou M, Pearce W, Leffert AK. The soluble forms of CD28, CD86 and CTLA-4 constitute possible immunological markers in patients with abdominal aortic aneurysm. *J Intern Med* 2007;261:399–407.

Aortic Aneurysms

Pathogenesis and Treatment

Upchurch Jr., G.R.; Criado, E. (Eds.)

2009, XVII, 381 p., Hardcover

ISBN: 978-1-60327-203-2

A product of Humana Press