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LOOKING AHEAD

The field of abdominal aortic aneurysm research desperately needs therapeutic interventions to delay and prevent the growth of the aneurysm.

Basic Research Studies to Understand Aneurysm Disease

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Summary

Abdominal aortic aneurysm (AAA) is a complex multifactorial disease with life-threatening implications. Aneurysms typically have no signs or symptoms, and rupture of AAA has a high mortality rate. Multiple environmental and genetic risk factors are involved in aneurysm formation and progression making it a complicated disease to study. Little is understood about the mechanisms in disease initiation, thus there are currently no therapeutic approaches to prevent AAA, leaving patients with surgery as their only option. Ongoing research into the genetic components of AAA using a candidate gene approach has been overall unsuccessful. A more promising approach to study complex diseases involves genome-wide techniques such as DNA linkage analysis, genetic association studies and microarray expression profiling. Furthermore, studies involving inhibition of AAA progression, rather than formation, have a potentially promising outcome. Targeting biological pathways in AAA pathogenesis may benefit patients by slowing the growth and possibly preventing the rupture of AAA. Critical pathways involved in AAA pathogenesis include immunological processes, such as T-cell and natural killer cell pathways, oxidative stress, depletion of vascular smooth muscle cells through the process of apoptosis and the destruction of the extracellular matrix by matrix metalloproteinases.

Abdominal aortic aneurysm (AAA), defined as a focal dilation greater than 3 cm in diameter, is a common degenerative condition typically affecting men over the age of 65.[1] As many as 15,000 individuals die each year due to ruptured AAA, making AAA the 17th leading cause of death in the United States.[2] Most aneurysms are asymptomatic until rupture, and the mortality rate of ruptured cases is approximately 65–85%, with half of the deaths occurring before the patient reaches the hospital.[3] Currently, there are no pharmacologic therapeutics to treat this disease, and the only effective method of treatment is surgical intervention. Characteristics of AAA include local inflammation, vascular smooth muscle cell (VSMC) apoptosis and destruction of the extracellular matrix (ECM). Risk factors for AAA include smoking, positive family history, old age, Caucasian ethnicity and male gender.[1,3]

AAA is a genetic disease

Evidence suggesting a genetic role in AAA began with Clifton in 1977 when he studied three brothers diagnosed with AAA.[3] Since then there has been convincing indication of AAA clustering in families (Table I). Reports suggest that 12–19% of AAA patients have one or more first-degree relatives with the disease. Typically, familial cases of AAA are approximately 5 years younger than nonfamilial, a common characteristic of genetic diseases. Furthermore, aneurysms among familial cases grow in

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TABLE I. CLINICAL AND GENETIC EVIDENCE SUPPORTING GENETIC MODEL OF ABDOMINAL AORTIC ANEURYSM (AAA)

CLINICAL FINDINGS
Case report on three brothers with AAA in 1977
13% of AAA patients have positive family history of AAA based on interviews
17% of brothers and 4% of sisters have AAA based on ultrasonography screening of relatives
Age at diagnosis higher in sporadic AAA cases than familial AAA cases
Age at rupture higher in sporadic AAA cases than familial AAA cases
Incidence of rupture lower in sporadic AAA cases than in familial AAA cases
Male:female ratio higher in sporadic AAA cases than in familial AAA cases
Prevalence of AAA higher in Caucasians
Operative mortality higher in females than males
Rupture rate higher in females than males
First-degree relatives of AAA patients have up to 18-fold higher risk of getting an AAA than general population
GENETIC STUDIES
Formal segregation analyses suggested genetic model best fit for familial clustering of AAA
DNA linkage study identified two genetic loci: AAA1 and AAA2
Genetic association studies

For original studies, see references (2) and (3).

size more rapidly and have a higher incidence of rupture. However, the morphology of familial versus nonfamilial aneurysms shows no significant differences.[3] The prevalence of AAA among siblings of AAA patients is estimated to be eight-fold higher than among individuals without family history of AAA.[4]

Females with AAA are more likely to have a family history of AAA. Furthermore, female AAA patients have a far worse disease prognosis, such as increased operative mortality. This is likely due to greater number of genetic and environmental risk factors.[3,5] As for racial differences, one group demonstrated that AAA occurs more often in Caucasian males than in African Americans.[6]

Two segregation studies have shown convincing support of a genetic model in AAA formation. In addition, many studies have analyzed large collections of multiplex AAA families.[3] As shown in Figure 1, genetics may be the underlying factor in many of the biological processes known to be involved in AAA, and it is likely that an individual will need multiple genetic risk factors to reach the end stage of ruptured aneurysm.

A variety of candidate genes studied in AAA include elastases, collagenases, interleukins, angiotensin-converting enzyme, nitric oxide synthase and human leukocyte antigens.[7] However, many of these studies used small numbers of individuals and therefore did not have sufficient power to show an association. Recently, a common sequence variant on 9p21 was reported to be associated with coronary artery disease, AAA and intracranial aneurysm in a large multicenter study.[8] This genetic variant is located in a gene called *ANRIL*, which is a noncoding RNA, and is the first to be described that affects the risk of AAA and intracranial aneurysm in many populations. The functional aspects of this variant still need to be investigated.[9]

Genome-wide DNA linkage analysis is another approach to identify sus-

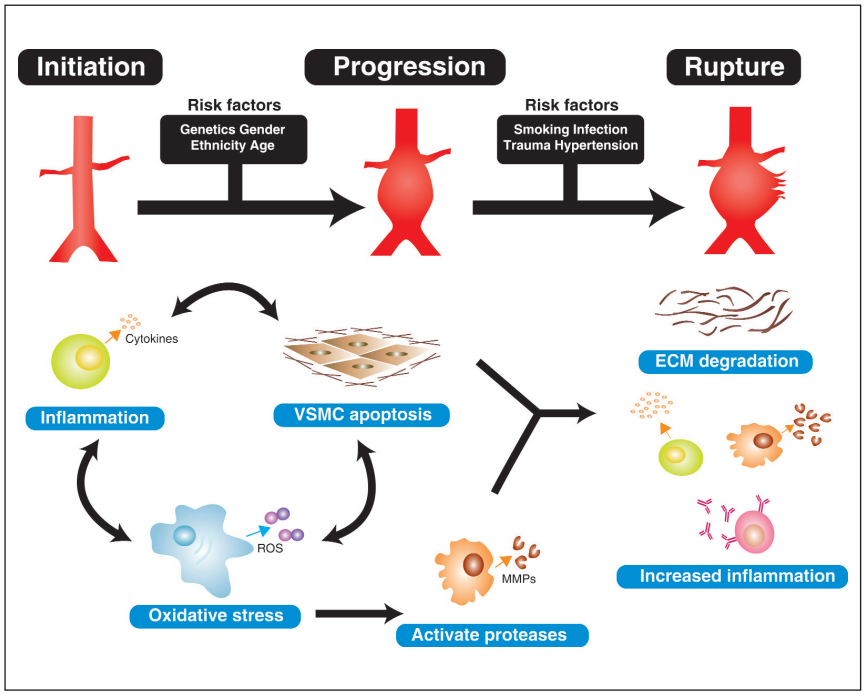

Fig. 1. Processes involved in and risk factors associated with abdominal aortic aneurysm pathogenesis. See text for detailed discussion. VSMC, vascular smooth muscle cell; ECM, extracellular matrix; ROS, reactive oxygen species; MMPs, matrix metalloproteinases.

ceptibility loci for AAA. Shibamura et al. performed a scan of the entire genome using affected-relative pair linkage analysis and identified susceptibility loci on chromosomes 19q13 and 4q31.[10] Van Vlijmen-van Keulen et al. were able to replicate the chromosome 19q13 linkage results on three Dutch families.[11]

Immunology: A central component of AAA

One of the hallmarks of AAA is the infiltration of inflammatory cells in the vessel wall. Histological examination of the aneurysm wall shows two primary populations of infiltrating cells: intimal macrophages and T cells primarily associated with atherosclerotic plaque and lymphoid follicle-like structures in the adventitial layer.[12,13] Analysis by flow cytometry has shown most of the infiltrating lymphocytes to be T cells, with a smaller number (~20%) of B cells, present as well.[14,15] Further analysis has demonstrated that the T cells in aneurysms represent clonal populations, suggesting a possible autoimmune component to AAA.[16] Additionally, other leukocytes, such as dendritic cells, mast cells, natural killer (NK) and natural killer T (NKT) cells, have been identified in aneurysmal tissue by immunostaining.[17–20]

Several studies have shown promising results from therapies that exhibit an antiinflammatory effect, including doxycycline and statins, although neither specifically targets the immune system. Additionally, several other antiinflammatory drugs such as indomethacin and celecoxib have been tested in animal models (Table II). However, there is still great potential for identification of new immune targets in the treatment of AAA.

Two recently published array-based studies have helped to further characterize the immune system in AAA and suggest new targets for treatment. The primary advantage of these studies is that they offer an approach that is less biased with respect to the choice of genes or proteins being studied and may identify new targets to study. The only whole genome microarray comparison of AAA to nonaneurysmal tissue found statistically significant enrichment of several immunological pathways when examining upregulated genes.[21] Some pathways and genes, such as the T-cell receptor signaling pathway were expected, whereas others such as NK cell-mediated toxicity were more surprising. Although NK and NKT cells have been identified in aneurysm tissue and there are more circulating NK cells in AAA patients, the extent of activation of cytotoxicity pathways in AAA indicates that cytotoxic lymphocytes may have an important role, perhaps in apoptosis of VSMCs. Another study used a protein microarray to study the cytokines present in the aneurysm wall.[22]

In addition to tissue-based methods, genetic association studies and animal models are also being used to identify immune system targets for therapy. Association studies have been performed looking for risk alleles in several immune mediators such as *CCR5* and several interleukins (IL).[2]

TABLE II. THERAPEUTIC APPROACHES TESTED IN ABDOMINAL AORTIC ANEURYSM (AAA) ANIMAL MODELS

PROCESS/PATHWAY	TARGET	DRUG (REF.)
Apoptosis	Rho-kinase	Fasudil (41)
Cell signaling	c-Jun N-terminal kinase	SP-600125 [see (1)]
ECM degradation	MMPs	Hydroxamate-based inhibitors (53)
		Tetracycline derivatives (54)
	Elastin stabilization	Polyphenolic tannins (55)
	Histone deacetylase	Metacept-1 (56)
Inflammation	Calcium channel	Azelnidipine (57)
	Cyclooxygenase	Indomethacin (58, 59)
		Rofecoxib (58)
		Colecoxib (60)
	Rapamycin binding protein	Rapamycin (61)
	Inducible nitric oxide synthase	Aminoguanidine (62)
		L-NAME (62)
		1400W (58)
	NF-κB	Pyrrolidine dithiocarbamate (63)
	Binding sites for transcription factors ets and NF-κB	Decoy oligodeoxynucleotides (64)
Lipid metabolism	HMG-CoA reductase	Simvastatin [see (1)]
Oxidative stress	Oxidation	α-Tocopherol [see (1)]
Renin-angiotensin pathway	ACE	ACE inhibitors [see (1)]
Others	β-Adrenergic receptor	Propanolol (65)
	Hormone receptors	17β-Estradiol (66)
		Tamoxifen (67)

ECM, extracellular matrix; MMP, matrix metalloproteinase; NF-κB, nuclear factor-κB; ACE, angiotensin-converting enzyme.

Although none of these studies have produced conclusive evidence, there has been suggested association in *IL10* and *IL6*, perhaps making their signaling pathways worth further study in AAA. Using experimental AAA models in knockout mice, several additional immunity genes have been studied for their role in AAA development.[2] Of particular note are several recent studies implicating neutrophils and mast cells, two cell types with little information about their role in aneurysm formation.[23–25] Using such models, it is possible that additional novel targets will be identified to develop into AAA therapies.

Oxidative stress in AAA

Oxidative stress and the generation of reactive oxygen species (ROS) such as superoxide radical and hydrogen peroxide affect many biological processes and have been implicated directly and indirectly in a variety of pathological conditions such as Alzheimer's disease and cancer.[26,27] The role of ROS in vascular pathology has also been studied widely,[28] and a role for their involvement in AAA has been proposed and investigated.[29]

Oxidative stress is the result of two distinct processes, the development of ROS through processes such as NADPH (nicotinamide adenine dinucleotide phosphate) oxidase, and the cellular control and elimination of these molecules.[30] A pathological oxidative state can be caused by an imbalance on either side of this biological system.[31] In general, the proposed role of oxidative stress in AAA is thought to involve mainly two processes: the apoptosis of the VSMCs in the aorta, and the recruitment or exacerbation of immune infiltration into the aorta.

With increasing evidence of immunity and inflammation in AAA, oxidative stress could have a key role in the formation of the aneurysm.[29,32] It is well established that ROS are not only generated by resident VSMCs in the aorta, but can also be produced by infiltrating immune cells.[33] This is very interesting, since the generation of ROS leads to the production of cytokines which then attract more immune cells into the aorta that may produce more ROS, setting up a positive regulation cascade.[34] In addition, disturbed hemodynamics have been shown to lead to the upregulation of ROS.[32]

Although ROS can be involved in VSMC apoptosis, this aspect of ROS in AAA is somewhat controversial since they can also cause proliferation of VSMCs.[35] Interestingly, ROS can activate proteases such as matrix metalloproteinase 2 (MMP2) and MMP-9.[36] This is important to AAA biology, since MMPs have long been implicated in the pathobiology of AAA through their ability to degrade the ECM of the aorta.

Loss of smooth muscle cells via apoptosis

Depletion of VSMCs is one of the characteristic histological findings in the media layer of aneurysmal artery wall.[37] Several studies have come to the conclusion that this loss of cells is due to the phenomenon known as apoptosis or programmed cell death[37–39] although the underlying trigger setting off the apoptosis cascade is still unknown. VSMC apoptosis is also seen in experimental animal models of AAA such as the elastase-perfusion model[40] and in the model in which apolipoprotein E-deficient mice are infused with angiotensin II.[41]

In experiments where VSMCs were co-cultured with T cells, $CD4^+$ T cells enhanced VSMC proliferation, whereas NKT cells induced VSMC apoptosis.[18] Activation of NKT cells leads to perforin and granzyme B, as well as FasL release, which causes potent cytolytic activity. Notably, increased expression of the genes involved in the activation of NKT cells has been recently shown in a microarray-based global gene expression study providing additional support for the hypothesis that VSMC apoptosis and the activation of NKT cell pathway are closely linked to aneurysm pathogenesis.[21]

Increasing concentrations of osteoprotegrin (OPG) in cell culture systems slowed down VSMC proliferation and high levels of OPG were found in macrophages isolated from AAA tissue.[42] OPG is known to work as a decoy receptor for TRAIL, a tumor necrosis factor-related apoptosis-inducing ligand.

Another interesting study showed lymphocytes isolated from aortic tissue samples of AAA patients and activated in culture were resistant to Fas-induced apoptosis although lymphocytes isolated from healthy controls and from patients with aortic occlusive disease did go into apoptosis when activated in this culture system.[43] These results made the authors speculate that AAA resembles other autoimmune diseases in the sense that activated lymphocytes do not complete the apoptotic pathway and remain in the tissue causing damage to the aortic wall.

MMP infiltration and ECM degradation

The ECM of the abdominal aorta is comprised mainly of collagen, elastin/fibrillin and proteoglycans.[44] Elastin gives the aorta its viscoelastic properties and fibrillar collagen types I and III maintain the structural integrity of the vascular wall.[45] AAA formation and expansion share the characteristics of ECM fragmentation and degradation of the aorta at the site of the aneurysm.[44,46] An early step in aneurysm formation is the loss of elastic fibers, while collagen degradation has been shown to ultimately cause the rupture of an AAA.[45]

Degradation of elastin and collagen takes place via a class of proteolytic enzymes, the MMPs.[44,45] Neutrophils, macrophages and a variety of mesenchymal cells are responsible for producing MMPs in an inactive form, known as zymogens or pro-MMPs. They can then be activated by a number of proteinases, including other MMPs.[47] Regulation of MMPs under normal physiological conditions takes place at the level of transcription, synthesis as inactive

zymogens, posttranslational activation of zymogens and by endogenous tissue inhibitors of MMPs (TIMPs).[48,49] In fact, many studies have shown an imbalance of MMPs and their inhibitors in aneurysmal tissue.[44]

Several members of the MMP family have been shown to play a role in AAAs including MMP1, MMP3, MMP2, MMP9, MMP12, MMP13 and MT1-MMP.[44] Much of the research has focused on MMP9, which functions in collagenolysis and elastolysis and has been directly implicated in the proteolytic degradation of the ECM of the aortic wall.[45,47] MMP9 is the most abundant proteinase secreted by human AAA tissue *in vitro* and the plasma of AAA patients have elevated levels of MMP9.[44,50] Animal studies have gone further to demonstrate that targeted gene disruption of *Mmp9* in mice, in which aneurysm was induced using an elastase-perfusion model, prevented AAA formation.[50]

Notably, MMP2, which also functions in collagenolysis and elastolysis, has been shown to be elevated in aortic specimens from patients with small aneurysms.[47] Additionally, Longo et al. suggested that MMP2 and MMP-9 work in concert to produce AAAs as *Mmp9* knockout mice along with *Mmp2* knockout mice each failed to produce AAAs when induced by abluminal application of calcium chloride although the other MMP was present in each model.[51]

The role MMPs play in ECM degradation along with the evidence of their involvement in AAAs make them potential targets for drug therapies for AAAs (Table II). Currently, the market lacks specific MMP inhibitors although doxycycline, a nonspecific inhibitor of the entire class of MMPs, has been shown to prevent aneurysm growth in both animal models and humans.[44,45,52] Statins have also been shown to inhibit the effects of MMPs as both simvastatin and cerivastatin downregulate the production of MMP9 and other mediators of inflammation.[44,45] Additionally, the calcium channel blocker amlodipine had an inhibitory effect on MMP9 activity.[44] Other studies have tested synthetic MMP antagonists, RS-132908 and BB-94, which were found to decrease the size of AAA in a rat model.[44,45]

Discussion

AAA is a serious cardiovascular disease and it is estimated that 1–2% of individuals harbor AAAs. The current clinical approach for AAA includes screening for detection, monitoring growth of the aneurysm, and then either surgical or endovascular repair.[44] However, there are a number of disadvantages involved in surgeries including high cost and postoperative complications. The field of AAA research desperately needs therapeutic interventions to delay and prevent the growth of the aneurysm. The problem with discovering successful therapeutic approaches is that the processes involved in AAA are complex. As shown in Figure 1, many of the processes known to be involved in AAA pathogenesis influence each other. VSMC apoptosis leads to a situation where the cells needed for synthesizing ECM are depleted making it difficult to repair the damage in the aortic wall induced by MMPs and other proteolytic enzymes. Also, ROS can induce apoptosis, and inflammatory cells in the aneurysmal wall can trigger VSMC apoptosis by secreting cytokines.[38] Of the current therapeutics studied in AAA models (Table II), a few look promising, and clinical studies are needed to confirm these results.

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