

## BASIC SCIENCE REVIEW

# Pathogenesis of abdominal aortic aneurysms: A multidisciplinary research program supported by the National Heart, Lung, and Blood Institute

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Abdominal aortic aneurysms (AAAs) represent a common vascular condition with life-threatening implications. Although basic research has led to a better understanding of aneurysm disease in the past two decades, fundamental knowledge about the process of aneurysmal degeneration remains limited. The prevalence of AAAs is relatively high in the general population, and current clinical options are somewhat limited, making it apparent that an urgent need for multidisciplinary approaches to investigate the cellular and molecular nature of aneurysm disease and its component mechanisms and transitional research to develop new therapeutic strategies exist.<sup>1-5</sup>

In recognizing these concerns, the National Heart, Lung, and Blood Institute (NHLBI) issued a Request for Applications titled "Pathogenesis of Abdominal Aortic Aneurysms" in December 1998 (RFA HL-99-007), announcing a new program to support multidisciplinary research programs on AAAs. After detailed peer review, 11 collaborative R01 research grants in seven different institutions were funded in September 1999. Each of these programs is highly integrative and synergistic, and in con-

cert with several related NHLBI research programs, they address the etiology, pathophysiology, and clinical progression of AAAs with an assortment of scientific approaches and experimental strategies (Table). The NHLBI held a meeting of Principal Investigators in Bethesda, Md, on April 10 and 11, 2000, to establish consensus on current research needs, identify additional avenues for collaboration between individuals working in diverse fields, and maximize the overall impact of the aneurysm research program. The purpose of this report is to summarize the information and perspectives discussed at that meeting for the wider community of vascular surgeons, biologists, and basic scientists, according to four broad areas of research: (1) proteolytic degradation of aortic wall connective tissue, (2) inflammation and immune responses, (3) biomechanical wall stress, and (4) molecular genetics.

### PROTEOLYTIC DEGRADATION OF AORTIC WALL CONNECTIVE TISSUE

Aneurysmal degeneration is the end result of a multifactorial process leading to the destruction of aortic wall connective tissue.<sup>6</sup> Compelling evidence indicates that the most important structural elements in the aortic wall are elastin and interstitial collagens and that AAAs are associated with increased local production of enzymes capable of degrading these fibrillar extracellular matrix proteins.<sup>7</sup> Much of this evidence is derived from studies on matrix metalloproteinases (MMPs); four MMPs, including 72-kD gelatinase (MMP-2), 92-kD gelatinase (MMP-9), matrilysin (MMP-7), and macrophage elastase (MMP-12), are capable of degrading elastic fibers, whereas at least three other MMPs are specific for interstitial collagens.<sup>8-10</sup> Other proteinases, such as plasminogen activators, serine elastases, and cathepsins, may also contribute to aneurysmal degeneration, and factors controlling connective tissue repair are undoubtedly of considerable importance. Mechanisms regulating connective tissue metabolism have,

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The views expressed herein are those of the authors and do not necessarily reflect the views of the National Institutes of Health and the National Heart, Lung, and Blood Institute.

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NHLBI multidisciplinary research programs related to Request for Applications HL-99-007\*

<i>Institution</i>	<i>Principal investigator</i>	<i>Grant number</i>	<i>Proposal topic</i>
Columbia University	M. David Tilson, MD	HL64334	Autoimmune aortic antigens in AAAs
Dartmouth University	Mark F. Fillinger, MD	HL64351	Role of wall stress distribution in AAAs
University of Nebraska	B. Timothy Baxter, MD	HL62400	MMP regulation by doxycycline in aortic aneurysms
Northwestern University	Rex L. Chisholm, PhD William H. Pearce, MD	HL64326	MMP-9 and aortic aneurysms—developing a model
Stanford University	Christopher K. Zarins, MD Ronald L. Dalman, MD	HL64327 HL64338	Quantitative biomechanical determinants of human AAAs Mechanobiologic determinants of experimental AAAs
Temple University	Christopher D. Platsoucas, PhD	HL64340	Is an AAA an antigen-driven autoimmune disease?
Texas A&M University	Jay D. Humphrey, PhD	HL54957	Saccular intracranial aneurysm—size, shape, and behavior
Washington University	Robert W. Thompson, MD Robert W. Thompson, MD Robert W. Thompson, MD Jay Heinecke, PhD William C. Parks, PhD	HL56701 HL64332 HL64333 HL64344 HL48762	Molecular mechanisms of aneurysmal degeneration Regulated expression of collagenases in AAAs Aneurysm research center Oxidative mechanisms of AAA formation Post-transcriptional regulation of tropoelastin expression
Wayne State University	Helena Kuivaniemi, MD, PhD	HL64310	Genetic factors in AAAs

\*Programs currently supported by NHLBI are listed in alphabetical order by institution.

therefore, attracted great interest within the NHLBI-supported programs on AAAs.

**MMP-9 and aortic aneurysms—developing a model.** The long-term goal of the research group at Northwestern University is to understand the role of MMP-9 in the pathogenesis of AAAs. Preliminary studies have demonstrated a significant correlation between aneurysm size and aortic wall expression of MMP-9 messenger RNA (mRNA), and patients with AAAs also exhibit elevated plasma levels of MMP-9. On the basis of these studies, the investigators proposed that the mechanisms of arterial wall repair are abnormal in patients with AAAs, with MMP-9 overexpression resulting in matrix destruction. The experiments being conducted are designed as a means of testing this hypothesis by developing a novel in vivo system to model the pathogenesis of aneurysms, in which transgenic mice have been engineered to overexpress MMP-9 under the direction of a vascular smooth muscle cell (SMC)-specific promoter. These animals are subsequently being used with a variety of experimental “initiating events” as a means of characterizing the pathogenesis of aneurysms, including mechanical and chemical injury to the aorta, and as experimental models of “genetic susceptibility,” including hypertension and atherosclerosis (ie, apolipoprotein knockout mice). The investigators are also expanding their evaluation of plasma MMP-9 levels in patients with aneurysms, as compared with age- and sex-matched control subjects with aortic occlusive disease. They will establish the optimal sensitivity and specificity for plasma MMP-9 levels by using receiver-operating-characteristic and multiple logistic regression analyses, and plasma MMP-9 levels will be followed longitudinally in a subgroup of patients not undergoing AAA repair to help predict rapid expansion. Plasma MMP-9 levels will also be followed in patients undergoing open and endovascular repair as a means of determining the prognostic usefulness of these analyses.<sup>11-13</sup>

**MMP regulation by doxycycline in aortic aneurysms.** Complementary work is being done at the

University of Nebraska, where investigators point out that although MMP-9 may have a role in AAAs, there is also a significant increase in total MMP-2 in human AAA tissue. They found that a larger proportion of the MMP-2 in AAA tissue is in the active form, compared with the proenzyme, and that much of this enzyme is bound to the extracellular matrix, which suggests a direct and ongoing role in proteolysis. They have also demonstrated that AAA tissue contains increased levels of membrane type-1 MMP (MMP-14), the principal activator of proMMP-2, and that doxycycline inhibits MMP-2 production by aortic SMCs in culture. The investigators therefore proposed that MMP-2, through its increased activation, has a central role in aneurysm formation and that this can be inhibited by doxycycline. They will examine this hypothesis through these specific aims: (1) determine the effects of individual MMPs implicated in AAAs, including MMP-2, MT1-MMP, MMP-9, and MMP-12, on the size and rate of aneurysm formation in a murine AAA model; (2) determine the effects of doxycycline on the size and rate of murine aneurysm formation and AAA progression and correlate these effects with serum doxycycline levels; and (3) determine the mechanisms by which doxycycline downregulates MMPs in human aortic SMCs. The first of these aims will be accomplished with the use of a novel mouse model of AAAs with various knockout mice, including animals deficient in MMP-2, MMP-9, or MMP-12. They will also use a tissue inhibitor of metalloproteinases-2 knockout mouse, in which proMMP-2 cannot be activated. The second aim will examine how doxycycline influences the murine model of AAAs by correlating its effects on aortic wall MMP expression, aneurysm size, and growth with serum doxycycline concentrations. The third aim will be accomplished by determining MMP-2 mRNA levels, half-lives, and rates of transcription in vascular SMCs and by identifying the doxycycline-response elements that may exist in the MMP-2 promoter. The long-term goal of this work is to develop novel pharmacologic therapies to specifically tar-

get the MMPs important in aneurysm pathogenesis and progression.<sup>14,15</sup>

**Molecular mechanisms of aneurysmal degeneration.** Investigators at Washington University in St Louis have designed an additional program to understand the regulation of individual gene products within the aneurysmal aorta and their pathophysiologic implications in disease progression. They have characterized small animal models of elastase-induced aortic injury that recapitulate many of the critical features of human AAAs, including transmural infiltration of the aortic wall by mononuclear phagocytes, increased local production of MMPs, and progressive degradation of aortic wall matrix proteins. They have also demonstrated the feasibility of pharmacologically suppressing aneurysmal degeneration *in vivo*, by using anti-inflammatory agents and nonselective MMP inhibitors (eg, doxycycline). More recent investigations have resulted in two important observations. First, elastase-induced aneurysmal degeneration in mice is suppressed by targeted gene disruption of MMP-9, but is unaffected in animals deficient in MMP-12; furthermore, bone marrow transplantation from wild-type mice prevents the aneurysm-resistant phenotype in MMP-9-deficient animals, and wild-type mice acquire aneurysm resistance after transplantation from MMP-9-deficient donors. Second, systemic administration of doxycycline was found to reduce aortic wall expression of MMP-9 mRNA as much as fivefold in patients undergoing elective AAA repair, and physiologically relevant concentrations of doxycycline suppressed phorbol-stimulated expression of MMP-9 in cultured human THP-1 mononuclear phagocytes. To build on these observations, the investigators are pursuing four specific aims to identify the critical molecular steps involved in elastase-induced aneurysmal degeneration in the mouse and elucidating the molecular pathways by which aneurysmal degeneration might be suppressed by MMP-inhibiting tetracyclines: (1) determine whether the generation of biologically active elastin degradation peptides is responsible for leukocyte recruitment, aortic wall infiltration, and MMP expression during the initiation of elastase-induced AAAs; (2) clarify the molecular mechanisms by which targeted deletion of MMP-9 suppresses the development of elastase-induced AAAs; (3) establish whether urokinase-type plasminogen activator (u-PA), plasmin, or additional MMPs are required during the development of elastase-induced AAAs; and (4) define the molecular mechanisms by which doxycycline suppresses phorbol-stimulated expression of MMP-9 in cultured human mononuclear phagocytes.<sup>16-18</sup>

**Regulated expression of collagenases in AAAs.** A second program at Washington University is focused on the possibility that collagen-specific enzymes are responsible for the clinical progression of AAAs. The investigators point out that the progression of aneurysm disease cannot be explained solely on the basis of elastin degradation and that considerable evidence implicates the breakdown of interstitial (types I and III) collagens in diminishing the tensile strength of the aneurysmal aorta. They also note

that increased collagen synthesis may counteract collagen degradation in stable, intermediate stages of aneurysm disease, but that, at later stages, a negative balance favoring collagen degradation may precipitate rapid aneurysm expansion and rupture. The investigators reemphasized that the dissolution of interstitial collagens requires the action of collagenases that can cleave intact collagen fibers within their cross-linked triple helix domains and that three MMPs can efficiently catalyze this reaction. In preliminary studies, the investigators demonstrated that the expression of collagenase-1 (MMP-1) is highly variable in human aneurysm tissues, but that collagenase-3 (MMP-13) is more consistently expressed in human AAA tissue and by vascular SMCs in culture. Moreover, they found that SMC expression of MMP-13 is regulated in a manner that is profoundly distinct from MMP-1, consistent with MMP-13 exhibiting a relatively limited tissue distribution and often being regulated differently from other MMPs. These observations have raised the possibility that MMP-13 might have a substantial role in the pathophysiology of aortic aneurysms. The goals of this proposal are to: (1) determine the relative expression and tissue distribution of the three collagenases in human AAA tissue and define how the expression of each enzyme is influenced by the clinical stage of disease; (2) identify extracellular ligands and signaling mechanisms that differentially regulate the expression of MMP-1 and MMP-13 in cultured vascular SMCs; and (3) elucidate the transcriptional mechanisms by which MMP-13 is regulated in vascular SMCs, as compared with other cell types.<sup>19-21</sup>

**Oxidative mechanisms of AAA formation.** In a third program at Washington University, investigators reason that the elastin and collagen degradation in AAAs is likely triggered by the activation of latent MMPs. The investigators noted that MMPs possess a common structural motif, the "cysteine switch," in which the latent state is preserved by interactions between a cysteine residue in the propeptide domain and the zinc atom in the active site; thus, a variety of thiol-reactive reagents and chaotropic agents are potent activators of MMPs *in vitro*. The investigators proposed that reactive oxygen intermediates (ROIs) might expose the active site through reaction with thiol groups on the propeptide, thereby damaging the cysteine switch, and that inflammatory cells in AAA tissue might mediate such reactions. Earlier studies indicate that activated phagocytes produce  $O_2^-$  with a membrane-associated reduced nicotinamide adenine dinucleotide phosphate oxidase and that this dismutates to hydrogen peroxide ( $H_2O_2$ ), which can serve as an oxidizing substrate for myeloperoxidase (MPO), another phagocyte product. MPO greatly amplifies the toxic potential of  $H_2O_2$ , generating potent oxidants in large part by reaction with chloride [ $H_2O_2 + Cl^- + H^+ \leftrightarrow HOCl + H_2O$ ]. It has been demonstrated that HOCl generated by MPO can activate latent MMP-8 and MMP-9; although MMP-8 and MMP-9 are structurally distinct and exhibit very different substrate specificities, their behavior with HOCl suggests a common activation mechanism. The investiga-

tors plan to test the hypothesis that ROIs activate proMMPs by covalently modifying the sulfur group of the cysteine switch. They will first examine the effect of ROIs on a model peptide that mimics the cysteine switch. This cysteine switch peptide will be incubated with various ROIs in conditions associated with MMP activation *in vitro*, and the reaction mixture will be subjected to high-performance liquid chromatography and mass spectrometric analysis. If the peptide has been covalently modified, its apparent molecular mass will increase. The investigators will then use tandem mass spectrometry as a means of confirming the specific nature of the modification and identifying the precise site at which it occurs. They will then determine whether similar changes occur when ROIs activate intact proMMPs *in vitro*. Collectively, the proposed studies should determine whether oxidants convert proMMPs into active MMPs by covalently modifying the thiol group of the cysteine switch. This will provide important insight into the mechanisms of MMP activation and lay the groundwork for determining whether similar events trigger MMP activation in human AAAs.<sup>22-24</sup>

**Posttranscriptional regulation of tropoelastin expression.** Other investigators at Washington University have focused on elastogenesis as a potential mechanism of connective tissue repair in AAAs. They point out that elastin is composed of cross-linked tropoelastin monomers and that the production of elastin is unique among connective tissue proteins in that tropoelastin expression is limited to a brief period of development; thus, elastic fiber assembly is complete by maturity, and tropoelastin synthesis ceases. The molecular control of elastogenesis needs to be delineated to understand the mechanism of aberrant production, but insufficient information exists on the normal regulation of tropoelastin expression. On the basis of preliminary data indicating that the downregulation of tropoelastin expression is uniquely controlled at a post-transcriptional level, the investigators proposed to undertake a detailed molecular characterization of this mechanism. They will test the hypotheses that the cessation of elastogenesis is normally controlled by accelerated decay of tropoelastin mRNA and that this regulatory mechanism involves specific sequences in the transcript. The generality of this mechanism will be determined by assessing the regulation of tropoelastin production in different conditions, such as hormone treatment, time in culture, and age of the cell donor, and with models of *in vivo* elastin production during development. The effect on tropoelastin transcription, which is expected to be minimal, will be assessed by means of nuclear runoff assays, reverse transcription/polymerase chain amplification of tropoelastin pre-mRNA, and transfection with tropoelastin promoter-plasmid constructs. Because transcript turnover can be affected by diverse pathways, a detailed characterization of the accelerated degradation of tropoelastin mRNA will be performed with different methods. Specific enzymatic decay of tropoelastin mRNA will be examined as a means of determining whether such mechanisms are activated in response to inhibitors of elastin production. A nuclease protection

assay will be used as a means of determining whether the status of polyadenylation correlates with the turnover of tropoelastin mRNA, and regulatory sequences in the mRNA will be identified by monitoring the response of reporter constructs containing defined sequences coding for tropoelastin mRNA. The interaction of these and other sequences with cellular factors will be determined by means of gel retardation assays with synthetic fragments of tropoelastin mRNA. Information from these studies will provide new and valuable information on the control of elastogenesis and will eventually lead to characterization of specific cellular factors regulating elastin production in development and disease.<sup>25-29</sup>

**Cysteine proteases in AAAs.** In addition to programs concentrating on MMPs, investigators from Harvard University/Brigham and Women's Hospital have called attention to the possibility that certain cysteine-dependent elastolytic enzymes might also play a role in aneurysm disease. Cathepsins are broadly distributed members of the papain superfamily with pH optima in the acidic range. Because they act intracellularly within acidic lysosomes, cathepsins have not been accorded a substantial role in extracellular matrix degradation and have therefore been overlooked in earlier studies on AAAs. The investigators emphasized that cathepsin S differs from other cysteine proteases because of its restricted tissue distribution, high elastolytic activity at neutral pH, and its capacity to degrade proteins outside the intracellular domain. Preliminary studies have demonstrated that cathepsins S and K are potent elastases and that the genes encoding these enzymes are expressed in human atheroma. Tissue extracts from atheroma also have elevated elastolytic activity sensitive to cysteine protease inhibitors, and vascular SMCs have been established as a novel source of cathepsins S and K *in vivo* and *in vitro*. The investigators have also shown that cultured human SMCs respond to interferon- $\gamma$  stimulation by secreting an active cysteine protease that can degrade insoluble elastin, and increased cathepsin S expression has been documented in atherosclerotic plaques and aortic aneurysms with immunohistochemistry and Western blot analysis. The investigators also point out that cystatin C is the most abundant endogenous cathepsin inhibitor and that a reciprocal decrease of cystatin C occurs in atherosclerotic plaques and AAAs compared with normal aorta. Moreover, serum levels of cystatin C are markedly decreased in patients with subclinical aortic dilatation. Cathepsin S, therefore, shows a shift of enzyme/inhibitor balance in atherosclerosis and AAAs, indicating the need for further study of aneurysm formation, progression, and rupture. Further studies in low-density lipoprotein receptor-deficient mice that lack either cathepsin S or cystatin C will test the role of cathepsins in experimental atherogenesis and aneurysm formation.<sup>30,31</sup>

## INFLAMMATION AND IMMUNE RESPONSES

Chronic inflammation is a prominent feature of AAAs, and inflammatory cells likely mediate much of the connective tissue destruction in the aortic wall.<sup>10</sup> The factors stim-

ulating inflammatory cell recruitment and activation in the outer aortic wall are therefore critical in understanding the pathogenesis of aneurysm disease.<sup>32</sup> In addition, there are compelling reasons to believe that the immune response contributes to aneurysmal degeneration, raising the possibility that autoimmunity may even be responsible for the pathogenesis and progression of aneurysmal disease.<sup>33,34</sup> This possibility is supported by the presence of inflammatory infiltrates in AAAs comprised of T-cells, monocyte/macrophages, B lymphocytes, and plasma cells; the presence of human lymphocyte antigen (HLA)-DR<sup>+</sup> T-cells and monocytes in AAA tissue; an association between particular HLA alleles and susceptibility to AAAs; and the demonstration that immunoglobulin G purified from AAA specimens is immunoreactive with proteins isolated from normal aortas. Understanding the nature of the immune response in AAAs is therefore an important component of the NHLBI research program.

#### **Is AAA an antigen-driven autoimmune disease?**

Investigators at Temple University School of Medicine are addressing the possibility that AAAs may be initiated by an antigen-driven T-cell response directed against self or non-self antigens. Several cell types present in AAAs may function as antigen-presenting cells to T-lymphocytes, including macrophages, activated endothelium, and SMC expressing HLA-DR. Although it is not yet known whether the inflammatory response is antigen driven, a number of putative antigens, including elastin, interstitial collagens, oxidized low-density lipoprotein, aortic aneurysm antigenic protein-40, and cytomegalovirus, have been suggested. Despite the prevalence of T-cells in AAAs, there is very little known about their role in the initiation and propagation of the disease, and it is possible that specific antigen recognition by T-cells might trigger a cascade of events leading to extracellular matrix destruction within the aorta. It is also possible that T-cells infiltrating AAA lesions become activated by a process known as "molecular mimicry," in which common epitopes shared between microorganisms and host proteins cause a form of immunological cross-reactivity. Because molecular mimicry may result in autoimmune disease, this mechanism may play a role in the pathogenesis of AAAs. The hypothesis to be tested in these studies is whether an antigen-driven T-cell response may be responsible for the initiation and propagation of AAAs and whether the T-cells present in AAAs recognize host antigens. The investigators will first determine whether mononuclear cells within AAA tissue contain oligoclonal populations of T-cells by studying the T-cell receptor (TCR) sequences of the infiltrating T-cells; although TCRs are highly polymorphic, each individual clone of T-cells expresses a unique TCR, which can serve as a "fingerprint" of T-cell (clonal) populations. In the second part of the study, the investigators will examine whether these T-cells recognize certain putative antigens in AAA tissue.<sup>35</sup>

**Autoimmune aortic antigens in AAAs.** Investigators from Columbia University/St Luke's-Roosevelt Hospital have partially characterized several proteins that may serve as autoantigens in some patients with

AAAs. The first of these to be identified was named aneurysm-associated antigenic protein-40 kD (AAAP-40). AAAP-40 resembles the 36-kD microfibril-associated glycoprotein (MAGP-36), which has been described as an aorta-specific protein in cows and pigs. With an antibody raised against a unique AAAP-40 oligopeptide, it was revealed by means of immunohistochemistry that the immunoreactive protein is most abundant in adventitial microfibrils associated with collagen in the aorta and iliac arteries. Although it was also detected in other vessels, including the carotid and popliteal arteries, AAAP-40 appears to be an artery-specific antigenic protein (ASAP) because it was not detected in tissues outside the arterial tree. Other features of AAAP-40 include similarity to all three fibrinogen chains (suggesting a common evolutionary ancestor with the fibrinogen precursor) and a short sequence similarity to vitronectin. By screening a human aortic wall complementary DNA library with antibodies to human vitronectin, the investigators have now isolated additional sequences named recombinant clones (r-Cl)-1, -4, and -5. All have fibrinogen-like motifs, and their molecular weights are in the 28 to 30 kD range. Antibodies raised against unique sequences of r-Cl-1 and r-Cl-5 suggest that they are additional members of a "superfamily" of collagen-associated microfibrillar proteins. Protein immunoreactive with antibody against r-Cl-5 was not limited to the aortoiliac segment, but was also detected on peritoneal surfaces and in other extra-arterial locations. Because variation in the immune response against one or more of these proteins may be the basis for the observed phenotypic variability of aneurysmal diseases, the investigators plan to apply further molecular approaches with human and animal tissues to isolate, purify, and sequence ASAP family proteins and to search for common sequences and antigenic epitopes that might elicit immune reactions in the aorta. They will then relate them to other identified proteins likely to share these immunogenic properties.<sup>36-41</sup>

#### **BIOMECHANICAL WALL STRESS**

Hemodynamic stress on the aortic wall is of obvious importance in aneurysm rupture, but may also play a role in aneurysm development. In this respect, the infrarenal abdominal aorta is particularly prone to atherosclerotic plaque formation and aneurysmal enlargement, whereas the thoracic aorta is relatively resistant. The reasons for these regional differences in susceptibility are unknown, but may involve a combination of differences in structure, composition, nutrition, and biology of the aortic wall and differences in the hemodynamic flow field and biomechanical forces applied to the aortic wall in time.<sup>42</sup> Projects focusing on the analysis of biomechanical wall stress are therefore a valuable component of the NHLBI research program on AAAs.

**Quantitation of biomechanical determinants of human AAAs.** Investigators at Stanford University described their initial efforts to test the hypothesis that hemodynamic forces and cumulative biomechanical stress

and strain result in aortic wall tissue failure and aneurysmal dilation. They presented their methods to quantify blood flow and vessel strain with magnetic resonance imaging and computational modeling techniques. It has been demonstrated by means of preliminary results with magnetic resonance imaging that cyclic wall strain can be measured accurately in vitro and that in vivo methods are viable. The investigators have created computational solutions for three-dimensional (3-D) pulsatile blood flow in a model of the entire aorta and in AAAs and are now relating the differences in the pulsatile flow field to differences in aortic structure, composition, and mechanical properties between the thoracic and abdominal aorta. Computational methods to quantify blood flow and vessel wall mechanical behavior of normal human thoracoabdominal aortas and aneurysmal abdominal aortas are also in progress. The long-term goal of the investigators is to develop a quantitative computer-based information framework to integrate biological and biomechanical theories for testing hypotheses about human aneurysm pathogenesis.<sup>43,44</sup>

**Mechanobiologic determinants of experimental AAAs.** A second group at Stanford University has proposed experiments to define the molecular mechanisms by which hemodynamic and inflammatory influences facilitate AAA development, to quantitatively define the varying hemodynamic forces that act on the human aorta resulting in mechanical failure of the aortic wall and aneurysmal enlargement, and to characterize pathologic alterations in the humoral and biomechanical milieu leading to maladaptive remodeling of the vessel wall. By using animal models, the investigators hypothesize that the initiation and progression of compensatory arterial enlargement (and, consequently, aneurysmal dilatation) is regulated by the patterns of genetic expression by resident and infiltrating cells within the aortic wall. They will undertake experiments to define the patterns of gene expression associated with flow-mediated aortic enlargement, modified wall motion, and inflammatory aortic enlargement. They have also designed experiments to test the hypothesis that biomechanical factors may mediate the initiation and progression of aneurysmal degradation via transduction of specific humoral factors that influence inflammation in the degenerating aorta. To achieve these aims, the investigators will use four rat models: (1) aortic elastase infusion; (2) proximal aortic coarctations; (3) distal aortic coarctations; and (4) femoral arteriovenous fistulae (AVF). Patterns of aortic gene expression in each model will be analyzed on a custom gene chip containing representative oligonucleotide probes from all published rat genes, and confirmatory analysis of mRNA and protein synthesis will be performed. Immunohistochemistry and in situ hybridization will help to further define the role of resident versus infiltrating cells in aneurysm pathogenesis, and physical forces, such as wall motion (wall strain) and flow (shear stress), will also be analyzed. Combinations of AVF and coarctations will then be used to alter aortic wall strain and shear stress to determine how these hemodynamic changes alter the pattern of inflammatory gene

expression. Information gained on mechanobiologic factors mediating experimental aortic aneurysms will prove useful in designing novel strategies to prevent AAA expansion and enlargement in humans.<sup>45,46</sup>

**Role of wall stress distribution in AAAs.** Investigators at Dartmouth-Hitchcock Medical Center will use computed tomography (CT) imaging, computer-generated 3-D reconstructions of CT data, and mathematical techniques to calculate aortic wall stress with new and sophisticated computer models. They point out that physicians have long sought the ability to identify patients with a high risk of AAA rupture and to distinguish them from those at relatively low risk. The basic premise of the proposed project is that aneurysm rupture occurs when the mechanical wall stress (ie, internal forces per unit area) exceeds the maximum stress that the aneurysmal tissue can inherently withstand (ie, failure strength); thus, when the wall stresses on a particular AAA are known, then one may make reliable predictions about its rupture risk. In addition to the recognized contributions of AAA diameter and blood pressure, aneurysm wall stress is greatly dependent on aneurysm shape, but this factor is seldom considered in the clinical setting because the technology needed to precisely determine AAA shape did not exist until recently. The proposed project will consist of three major elements. The first will test the basic hypothesis that wall stress is directly related to AAA rupture. Stress analysis will be performed on 20 AAAs near the time of rupture in which CT data were coincidentally obtained within the previous month and on 50 size-matched AAAs that did not rupture in a longer time span. The 3-D wall stress distribution (including peak, mean, and cyclic wall stresses) will be compared between the two groups. The second element of the study will involve the analysis of wall stress distribution in at least 30 AAAs that have been followed in extended periods of time; changes in wall stress distribution will be evaluated as a means of examining their role in AAA expansion and progression, including analysis of 3-D shape and how it changes with time. The third element of the project will involve refinement of the noninvasive methodology used as a means of determining AAA wall stress distribution by reanalyzing CT data from the other two elements of the study, with proposed refinements in computer modeling. These studies will provide critical insight into the role of wall stresses during AAA disease progression and rupture and may provide noninvasive tools for more accurately predicting patient outcomes.<sup>47,48</sup>

**Saccular intracranial aneurysms—size, shape, and behavior govern rupture.** Investigators from Texas A & M University discussed the similarities and differences between AAAs and intracranial aneurysms, pointing out that intracranial aneurysms are focal dilatations of the arterial wall that arise in and near the circle of Willis. These aneurysms generally occur in one of two forms: fusiform lesions, which occur primarily along the basilar artery, and saccular lesions, which usually develop at the apex of a bifurcation. Fusiform aneurysms are often referred to as space occupying because they produce symptoms by press-

ing on adjacent tissue; they tend to be atherosclerotic and affect the vessel around its entire circumference. Saccular aneurysms occur predominantly in the anterior and middle portions of the cerebral vasculature in as much as 5% of the general population; they are often asymptomatic until rupture and represent the leading cause of spontaneous subarachnoid hemorrhage. They can have either a narrow or a broad neck, but are only complicated by atherosclerosis after they have enlarged significantly. Although saccular intracranial aneurysms and AAAs have different etiologies, many aspects of their natural history and treatment are similar. It has been hypothesized that saccular aneurysms enlarge and rupture from material instabilities, because of either a limit point instability, similar to the rapid expansion of an inflated rubber balloon, or resonance, which occurs when a structure is excited at its natural frequency. Recent studies have challenged this hypothesis, showing that processes of growth and connective tissue remodeling may constitute the primary determinants of their natural history. Thus, it is now thought that mechanical stresses (both shear stress caused by altered hemodynamics and wall tension caused by blood pressure acting on the thin curved aneurysm wall) serve to signal collagen remodeling mediated by endothelial cells and fibroblasts, so as to return the biomechanical tissue environment toward homeostasis. In the case of well-balanced tissue remodeling, the lesion may stabilize once it achieves a new (optimal) configuration; in the case of an imbalance, the lesion may suffer catastrophic weakening that results in rupture. True interdisciplinary approaches to understanding the mechanisms underlying aneurysm disease will therefore have an important impact on these conditions, regardless of anatomic location.<sup>49-52</sup>

### MOLECULAR GENETICS

Familial aggregation of AAAs is widely recognized, and a major gene effect has been suggested by means of segregation studies. Other findings support the familial nature of AAAs: (1) patients with a history of familial AAAs (FAAAs) are significantly younger than those with sporadic AAAs (SAAAs) at the time of diagnosis or rupture; (2) rates of rupture are higher in patients with FAAAs than in those with SAAAs; (3) the relative risk for family members of patients with FAAAs is 18-fold higher than for family members of patients with SAAAs; (4) rupture rates are higher in women with AAAs than in men; (5) the man-to-woman ratio in patients with SAAAs is 6:1, whereas it is only 2:1 in patients with FAAAs; and (6) substantial ethnic variations exist in the prevalence of AAAs. Although alternative explanations may be proposed for the differences in outcomes between male and female patients, it is quite possible that these differences are caused by specific genetic factors. Indeed, it might be expected that when aneurysm disease does occur in women, it is caused by the presence of a larger number of inherited liabilities, a phenomenon characteristic of multifactorial diseases. Women with AAAs would then represent the more severe spectrum of the aneurysmal disease, and their first-degree

relatives would be expected to be at a higher risk of developing AAAs.

By using affected sibling pair (ASP) DNA linkage analysis, investigators from Wayne State University in Detroit are working in collaboration with scientists at Queen Elizabeth II Health Science Center (Halifax, Canada), the University of Liege (Belgium), and Case Western Reserve University (Cleveland, Ohio) to identify the chromosomal regions likely to harbor the AAA susceptibility gene(s). As of April 2000, DNA samples have been collected from 65 ASPs for this study. The investigators will identify additional affected-relative-pairs with AAAs and collect DNA to identify alleles shared as identical by descent. First-degree family members aged at least 55 years will be offered abdominal ultrasound scanning as a means of detecting asymptomatic AAAs. The linkage analyses will be performed as model-free affected sib-pair and pedigree analyses with a two-stage design. The first stage of phase I will consist of a genome scan on the 65 ASPs collected to date, with highly polymorphic markers located on average 10 cm apart (about 390 markers to scan all human chromosomes). The second stage will include genotyping additional markers from regions that warrant further investigation on the basis of the initial results. In phase II, the investigators will use additional ASPs to type markers in chromosomal regions determined to be sufficiently interesting in phase I, then perform model-free linkage analyses in those regions by using the combined data set. As a supplement to the genome scan, genetic association studies will be carried out for selected candidate loci by using DNA samples collected from unrelated patients with AAAs and control subjects; the initial candidate loci to be investigated include MMPs and HLA class II antigens. The long-term goal of this project is to identify the gene or genes that harbor mutations in patients with AAAs, yielding important information about the genetic factors contributing to the development of AAAs and potentially providing the basis for genetic testing to identify individuals at risk. The proposed work will also identify candidate genes and pathways of potential importance in the pathogenesis of AAAs, opening new avenues of research to investigate their function in transgenic animals and by pharmacologic intervention. The work will also advance related fields of research, such as statistical genetics, by providing real data sets to test new approaches for the analysis of complex diseases.<sup>53-55</sup>

### CONCLUSIONS

It is evident that current needs for basic research on AAAs encompass a broad spectrum of potential areas for investigation. Conceptual models of aortic aneurysm disease have traditionally focused on its mechanical aspects, and consequently, surgical solutions have dominated the therapeutic approach to this problem. However, with increasing recognition of the prevalence of small AAAs and the unfavorable natural history of these lesions, it is apparent that a broader base of interdisciplinary attention is needed. It is expected that rapid progress on the prob-

lem will be stimulated by attracting investigators with expertise in a variety of disciplines to studies focused on AAAs, to apply and integrate approaches involving vascular physiology, tissue-specific pathology, vascular cell biology, molecular analysis of gene expression, clinical investigations, and molecular genetics. More careful characterization and creative use of human tissue resources will expedite this effort, as will the development of more representative animal models of AAAs. A more detailed analysis of factors influencing the evolution of aneurysm disease in different clinical settings, particularly those exploring new avenues for clinical management of small AAAs, is also likely to generate new testable hypotheses. These efforts will necessarily include physicians specializing in vascular surgery, cardiology, and vascular medicine, as well as basic scientists focusing on various aspects of connective tissue biology, inflammation and immunology, biomechanics, and molecular genetics. Further efforts to foster strong collaborations between basic and clinical investigators are therefore needed to better understand the etiology, pathophysiology, and natural history of aortic aneurysms.

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