Simultaneous analysis of 1176 gene products in normal human aorta and abdominal aortic aneurysms using a membrane-based complementary DNA expression array

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Background: **A number of changes in gene expression have been described in abdominal aortic aneurysms (AAAs), but the spectrum of molecular alterations in this disease is unknown. The purpose of this study was to characterize the expression of approximately 1000 gene products in human AAA tissue and to compare the profile of genes expressed in AAAs with that observed in normal aorta.**

Materials and Methods: **Total RNA was isolated from abdominal aortic wall tissues (4 AAAs and 4 normal aortas), and array-specific [^{32}P]–labeled complementary DNA (cDNA) probes were created with reverse transcription. The cDNA probes were hybridized with nylon membranes containing an array of 1176 cDNA clones (AtlasArray Human 1.2 I; Clontech, Palo Alto, Calif), and autoradiographs were scanned to identify the patterns of gene expression characteristic of each tissue type. Densitometric analysis was used to standardize the expression of individual genes to a panel of housekeeping controls, and differential gene expression was defined by a signal ratio of at least 2:1.**

Results: **One hundred forty-five (12.3%) of the 1176 genes were consistently expressed in aortic tissue. Thymosin β-4 was the most abundant of 101 transcripts detected in both AAAs and normal aorta, whereas 44 genes exhibited differential patterns of expression (39 predominant in AAAs and 5 in normal aorta). Densitometric analysis confirmed differences in expression for 20 of these gene products between AAAs and normal aorta, with the greatest increases seen for myeloid cell nuclear differentiation antigen (31-fold), cathepsin H (30-fold), platelet-derived growth factor–A (23-fold), apolipoprotein E (13-fold), gelatinase B/matrix metalloproteinase-9 (12-fold), and interleukin-8 (11-fold). The only gene products substantially decreased in AAAs were myosin light chain kinase (39-fold) and β-1 integrin (twofold). AAA tissues thereby exhibited a distinct pattern of gene expression reflecting chronic inflammation, extracellular matrix degradation, atherosclerosis, and smooth muscle cell depletion.**

Conclusions: **cDNA expression arrays provide a powerful new approach to help identify the molecular mechanisms responsible for aneurysmal degeneration. Further studies will be needed to elucidate the functional and pathophysiologic significance of the individual genes that exhibit altered levels of expression in AAA tissue. (J Vasc Surg 2001;34:143-50.)**

Degenerative abdominal aortic aneurysms (AAAs) are associated with aging, atherosclerosis, and an undefined familial tendency, and the histopathology of AAAs is distinguished by chronic transmural inflammation and destruction of the elastic media.[1] Although these processes are considered critical in the pathophysiology of aneurysmal degeneration, the exact causes of aneurysm growth and rupture are still unknown. Therefore, better understanding of molecular mechanisms is an important step toward the development of new therapeutic strategies for AAAs.

Increased expression of several genes relevant to the pathophysiology of AAAs is well documented, particularly enzymes of the matrix metalloproteinase (MMP) family and their endogenous inhibitors.[2,3] Evidence indicates that other enzymes may be important in AAAs, including plasminogen activators, serine elastases, and cysteine protease; genes encoding proinflammatory cytokines, chemotactic mediators, and cell adhesion molecules have also been implicated in aneurysmal degeneration. Despite the many pathophysiologic insights obtained in the past

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decade, the spectrum of gene products altered in AAAs remains unknown.

Traditional approaches to elucidate broad alterations in gene expression include subtractive complementary DNA (cDNA) hybridization and messenger RNA (mRNA) differential display. Each of these techniques can be used to effectively detect mRNA transcripts expressed in a given tissue over a comparable control, but they often reveal numerous (unidentified) gene products that must be further characterized by independent and laborious methods. In recent years, the development of microchip- or membrane-based cDNA arrays has made it possible to examine the simultaneous expression of multiple gene products of known identity in the same experiment.[4-7] These methods greatly facilitate the identification of altered patterns of gene expression in a given tissue, including detection of unanticipated changes. In addition to the sensitivity and specificity now achievable with microarray techniques, advances in analytical software have permitted the rapid analysis of cDNA expression in a semiquantitative fashion.[8] Because this approach has the potential to reveal novel pathophysiologic insights, the purpose of this study was to characterize the simultaneous expression of approximately 1000 gene products in human AAA tissue and to compare the expression profile of AAAs with that of normal aorta.

MATERIALS AND METHODS

Aortic tissues. Full-thickness aortic wall specimens were obtained from four patients undergoing elective surgical repair for infrarenal AAAs (2 men [aged 84 and 80 years] and 2 women [aged 76 and 57 years]; mean age, 74.3 ± 6.0 years). The mean aneurysm size was 5.7 ± 0.3 cm as measured with computed tomography (5.5 and 5.1 cm [male patients] 6.3 and 6.0 cm [female patients]), and all patients were symptom free before repair. For comparison, normal infrarenal aortic wall without visible evidence of atherosclerosis was obtained from four transplant donors at the time of organ harvest (3 men [aged 43, 18, and 14 years] and 1 woman [aged 36 years]; mean age 27.8 ± 7.0 years). Tissue specimens were snap-frozen in liquid nitrogen immediately on procurement and were stored at –70°C before nucleic acid extraction. All tissues were obtained with approval by the Washington University School of Medicine Human Research Subjects Committee.

Isolation of RNA and preparation of labeled cDNA probes. Aortic tissue samples were pulverized under liquid nitrogen, and total RNA was isolated with Trizol reagent (Gibco BRL, Grand Island, NY), as described.[9] Each RNA sample was further extracted to eliminate excess protein and treated with RQ1 ribonuclease–free deoxyribonuclease (Promega, Madison, Wis) to eliminate residual DNA contamination. Labeled cDNA probes were prepared with reagents and protocols provided with the AtlasArray Human 1.2 I cDNA Expression Array kit from Clontech Laboratories, Inc (Palo Alto, Calif). For each specimen, 5 μg of total RNA was incubated with a mixture of array-specific oligonucleotide primers and 200 units of Moloney murine leukemia virus (MMLV) reverse transcriptase (Clontech) in the presence of [α-^{32}P]dATP (Amersham Pharmacia Biotech, Piscataway, NJ). In some cases, reverse transcription was performed with 400 units of Superscript II (Gibco BRL), a ribonuclease H MMLV mutant that appeared to produce equal or superior labeling. The labeled cDNA probes were purified with column chromatography to remove unincorporated isotope before use (Chromospin-20; Clontech).

Membrane hybridization. Each labeled cDNA probe was mixed into hybridization buffer (ExpressHyb; Clontech), then incubated overnight (68°C) with a nylon membrane containing bound cDNA clones corresponding to 1176 different human genes (AtlasArray Human 1.2 I; Clontech). After hybridization, membranes were washed under stringent conditions and exposed to radiographic film (Kodak Biomax MS, Rochester, NY). Membranes were stripped before reuse according to the manufacturer's instructions.

Analysis of data. Autoradiographs were scanned and analyzed with AtlasImage software (Clontech). This program allows assignment of numerical values to each point on the array in accord with the intensity of the hybridization signal on the blot and the adjacent background density. For differential gene expression between aneurysmal and normal aortic tissues to be determined, the signals on each array were standardized with a series of housekeeping genes present on the same membrane (ubiquitin, glyceraldehyde phosphate dehydrogenase, and the 60S ribosomal protein L13A), and numerical data from each array were used to generate a computationally derived consensus array for each tissue type. For each gene represented on the two consensus arrays, the ratio of gene expression levels was determined by dividing the signal intensity on the AAA array by that on the normal aorta array. Differential gene expression was considered significant when the signal ratio was greater than 2:1.

RESULTS

Fig 1 shows representative autoradiographs of the AtlasArray Human 1.2 I membrane after hybridization with cDNA probes derived from AAA tissue and normal aorta. By initial inspection, aortic tissue samples exhibited a cDNA expression profile consisting of up to 221 of 1176 genes represented on the array. Because only 145 of these genes were consistently expressed in either AAAs or normal aorta (ie, detected in at least half of the specimens examined), the remaining analysis was confined to these transcripts (12.3% of the total number of genes analyzed).

Consistency of gene expression. Twenty-one (14.5%) of the 145 genes were strongly expressed in both AAAs and normal aorta, and 80 (55.2%) were detected at lower levels in both tissue types (see the online-only supplement to this report for a complete list). The most abundant transcript expressed in all samples was thymosin β-4, a cytoplasmic protein that regulates actin fibril assembly during cell migration and chemotaxis.[10] Additional genes

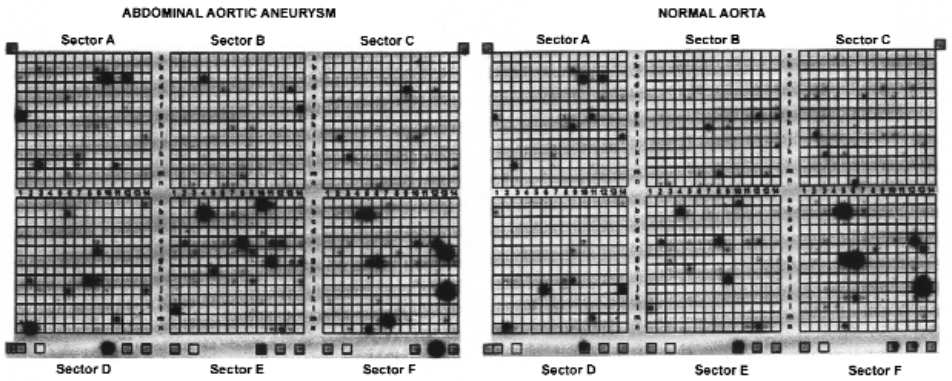

Fig 1. cDNA membrane arrays for AAAs and normal aorta. Representative autoradiographs are shown for membranes blotted with radiolabeled cDNA derived from an AAA and a normal aorta. Each blot is superimposed on an orientation grid corresponding to the AtlasArray Human 1.2 I membrane (Clontech), which contains bound cDNA clones from 1176 distinct gene products. Gene products represented by each location on grid are identified by accessing the manufacturer's interactive Web site, http://atlasinfo.clontech.com (follow link to AtlasInfo 2.0 and select Human 1.2 I).

expressed at high levels in both tissue types included proteins involved in RNA transcription, proto-oncogenes involved in cell signaling and transcription, and other thymosin family proteins. Genes encoding heat shock proteins (HSP86 and HSP27) and proteins involved in inflammation and cell growth were also expressed at high levels in both AAAs and normal aorta, as were calgranulin A and tissue inhibitor of metalloproteinases (TIMP)-1. The consistently high levels of expression for these genes suggest that they are required to maintain tissue homeostasis in the normal and diseased abdominal aorta.

Thirty-nine (26.9%) of the 145 genes were expressed exclusively or predominantly in AAAs (ie, detectable in > 75% of AAA specimens and < 25% of normal aortas). Genes expressed exclusively in AAA tissue included those involved in atherosclerosis (apolipoprotein E [apo E] and the thrombin receptor), inflammatory responses (myeloid cell nuclear differentiation antigen [MNDA], C-X-C chemokine receptor type 4, lymphocyte adaptor protein LNK, heat shock transcription factor–1, interleukin-2 [IL-2] receptor-γ, and macrophage inflammatory protein-2α), intracellular signaling (v-YES-1 sarcoma-related oncogene Lyn, protein kinase C delta, protein kinase SGK, RAL A GTP-binding protein, and transducin β-1), and extracellular matrix degradation (gelatinase B/MMP-9). The prevalence of these gene products in AAAs supports the recognized role of these biological processes in aneurysmal degeneration. In contrast, only five (3.4%) of the 145 genes were expressed exclusively in normal aorta (ie, detectable in no AAA specimens and > 75% of normal aortas). The most striking of these differences was observed for myosin light chain kinase, a cytoskeletal protein that regulates contraction and migration.[11]

Fig 2 shows representative autoradiographs of the part of the AtlasArray Human 1.2 I membrane that corresponds to extracellular matrix proteinases and their inhibitors (Sector F, Rows m and n). TIMP-1 was prominently expressed in all normal and aneurysmal tissues, whereas plasminogen activator inhibitor–1 was expressed in three of four AAAs and only two of four normal aortas. Gelatinase B/MMP-9 was substantially and exclusively increased in aneurysms, but it is notable that other MMPs and TIMPs known to be produced in AAAs (eg, MMP-2, MMP-3, MMP-12, MMP-13, and TIMP-2) were generally expressed below the limits of detection on the microarray membranes. The expression of the cysteine protease, cathepsin D, was also consistently observed in AAAs, as well as in three of four normal aortas.

Computational analysis. Densitometric analysis confirmed semiquantitative differences in expression for 20 gene products between AAAs and normal aorta (Table). The greatest increases were seen for MNDA (31-fold), cathepsin H (30-fold), platelet-derived growth factor (PDGF)–A chain (23-fold), apo E (13-fold), gelatinase B/MMP-9 (12-fold), and IL-8 (11-fold). Additional transcripts significantly increased in AAAs included the C-X-C chemokine receptor HM89, Fli-1 oncogene, intercellular adhesion molecule (ICAM)–1 and its receptor (integrin alpha L; CD11a/-CD18), two immediate-early response genes (transcription factor ETR 101 and early growth response–1), the chondroitin/dermatan sulfate proteoglycan core protein (decorin), and RANTES (*R*egulated on *A*ctivation *N*ormal *T* cell *E*xpressed and *S*ecreted). In contrast, the only genes substantially decreased in AAAs were myosin light chain kinase (39-fold) and integrin β-1 (twofold). AAA tissues thereby exhibited a distinct pattern of gene expression reflecting the association of this disease with chronic inflammation, extracellular matrix degradation, atherosclerosis, and smooth muscle cell depletion.

DISCUSSION

For the initial application of gene expression profiling to the study of aortic aneurysms, we used a commercially available membrane array that contains cDNA sequences from 1176 known human genes (approximately 0.6%-2% of

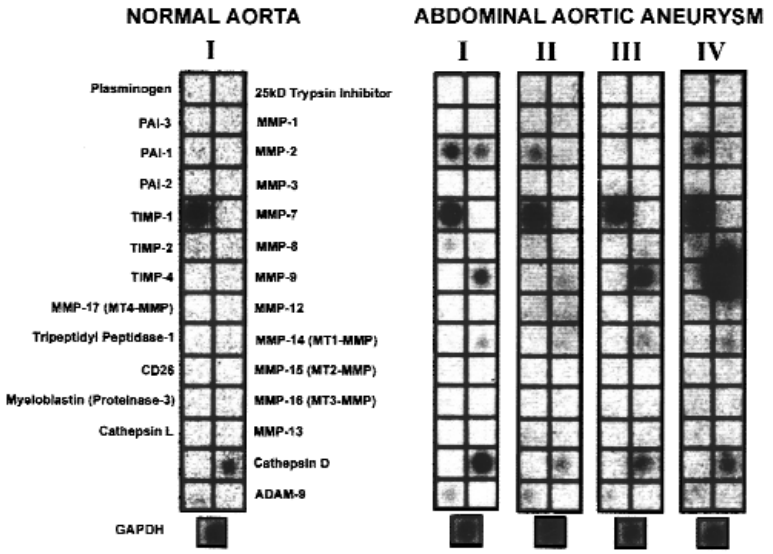

Fig 2. Expression of matrix proteinases and inhibitors in normal and AAAs. Representative autoradiographs are shown for portion of the AtlasArray Human 1.2 I membrane (Clontech) that contains bound cDNA clones for genes encoding extracellular matrix proteinases and their inhibitors (Sector F, Rows m and n), after blotting with radiolabeled cDNA derived from normal aorta (*I*) and four different AAA specimens (*I-IV*). Gene products represented by each location on membrane are identified, along with glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as housekeeping control. *PAI,* Plasminogen activator inhibitor; *TIMP,* tissue inhibitor of metalloproteinases; *MMP,* matrix metalloproteinase;

the human genome). We chose this particular array to detect genes involved in a wide spectrum of biological processes, including tumorigenesis, signal transduction, cell cycle regulation, apoptosis, inflammation and immune responses, DNA synthesis and repair, and matrix protein turnover. A number of other membrane- or microchip-based microarrays might have been selected for this study, and in the future it will be valuable to use either broader or more disease-specific arrays to investigate additional alterations that might occur in AAAs. To simplify the scope of this initial study, we also chose to characterize the gene expression profile of large asymptomatic AAAs by comparison with normal aorta obtained from young organ transplant donors, rather than specimens obtained from patients with unstable AAAs or those with aortoiliac occlusive disease. This represents an important limitation of the study, in that it precludes drawing conclusions about the alterations in gene expression in AAAs that might otherwise be attributable to aging or atherosclerosis alone. Nevertheless, this initial approach provides a sound basis for further investigation, because the expression profile of normal human abdominal aorta has not been previously described. Moreover, it will facilitate interpretation of further studies designed to specifically compare gene expression profiles in AAAs with age-matched atherosclerotic tissue and among AAAs at various stages of disease progression.

The most abundant transcript detected in all aortic tissue specimens was thymosin β-4, a 4-kD protein originally characterized in the thymus.[10] Thymosin β-4 interacts strongly with G-actin in the cytosol and is thought to play a role in regulating actin fibril assembly during cellular migration and chemotaxis.[12,13] Recent studies have attributed a variety of additional functions to thymosin β-4 as an extracellular mediator, including the induction of endothelial cell chemotaxis and MMP expression in vitro.[14,15] Moreover, thymosin β-4 sulfoxide is induced in glucocorticoid-stimulated monocytes and macrophages, where it acts as an extremely potent anti-inflammatory agent.[16] The marked expression of thymosin β-4 in human aorta will thereby require further investigation to determine if this protein might participate in modulating inflammation in the vascular wall.

Given the complex nature of the pathologic changes in AAAs and the large number of genes examined in this study, we found that AAA tissues were characterized by a surprisingly limited repertoire of altered gene products; substantial differences in expression between AAAs and normal aorta were observed for only 20 transcripts (1.7% of the genes examined). Previous studies on aortic aneurysms have revealed increased expression for several of the gene products detected in the current analysis. For example, we measured a 12-fold increase in the expression of gelatinase B/MMP-9 in AAAs versus normal aorta, which is in agreement with the 23-fold increase in MMP-9 mRNA recorded by Tamarina et al,[2] using competitive reverse transcriptase-polymerase chain reaction, and the 10-fold increase reported by Elmore et al,[3] using Northern analysis. Although Tamarina et al[2] also reported increases in MMP-1 (12-fold), TIMP-1 (1.7-fold), and TIMP-2 (4.5-fold) in AAAs, we did not detect significant differences in these gene products in the current study. In addition, we did not detect expression of either MMP-12 or MMP-13, two gene

Differentially regulated genes in AAAs versus normal aorta (computational analysis)

*Site**	*Gene product name (GenBank reference)†*	*Normalized density ratio AAA to normal*
Genes significantly upregulated in AAAs (n = 18)		
A-1-11	MNDA (M81750)	31 to 1
F-k-8	Cathepsin H (X07549)	30 to 1
F-g-7	PDGF, A Chain (PDGF-A) (X06374)	23 to 1
B-c-4	apo E (M12529)	13 to 1
F-m-7	Gelatinase B; MMP-9 (J05070)	12 to 1
F-g-14	IL-8 (Y00787)	11 to 1
B-d-13	CXC chemokine receptor type 4 (HM89) (D10924)	8 to 1
E-b-7	Fli-1 oncogene (M93255)	7 to 1
E-i-14	Integrin alpha L (CD11a) (Y00796)	5 to 1
E-g-14	ICAM-1 (J03132)	4 to 1
E-b-5	Transcriptional regulator ISGF3, gamma subunit (M87503)	4 to 1
E-3-11	ZFP-36 (tristetraproline) (M92843)	3 to 1
E-a-11	Transcription factor ETR 101 (M62831)	3 to 1
E-g-11	Decorin (M14219)	3 to 1
D-f-9	Acyl-CoA-binding protein (M14200)	3 to 1
F-e-10	RANTES protein T-cell specific (M21121)	2 to 1
E-h-5	Cell adhesion protein SQM1 (M33374)	2 to 1
E-a-10	Transcription factor ETR103 (M62829)	2 to 1
Genes significantly downregulated in AAAs (n = 2)		
B-g-12	MLCK (U48959)	1 to 39
E-I-09	Integrin beta-1 (X07979)	1 to 2

Gene products were identified from autoradiographs of AtlasArray Human 1.2 I membranes (Clontech) that were hybridized with labeled cDNA samples from four AAAs and four normal aortas. For each autoradiograph, the signal intensities for individual gene products were standardized to a panel of four different housekeeping genes, and consensus array data were composed for each tissue type with analytical software supplied by the manufacturer. Differential expression of individual gene products was based on a standardized density intensity ratio of at least 2:1 (AAA vs normal aorta).

*Sites refer to the location of the target cDNA on the AtlasArray Human 1.2 I membrane by Sector Web site, http://atlasinfo.clontech.com (follow the link to AtlasInfo 2.0 and select Human 1.2 I). Each array location site is hyperlinked to the manufacturer's proprietary database for additional information about the gene and its functional properties.

†GenBank reference numbers are hyperlinked to the National Center for Biotechnology Information database maintained by the National Library of Medicine and National Institutes of Health.

apo E, Apolipoprotein E; *ICAM*, intercellular adhesion molecule; *IL*, interleukin; *MLCK*, myosin light chain kinase; *MMP*, matrix metalloproteinase; *MNDA*, myeloid cell nuclear differentiation antigen; *PDGF*, platelet-derived growth factor; *ZFP*, zinc finger protein.

products previously reported to be elevated in human AAA tissue by other techniques.[9,17] The differences in the results of these studies may be explained by several factors, particularly the higher threshold for gene expression that appears to be required for detection in cDNA expression arrays compared with other techniques. It is therefore likely that our hybridization assays failed to detect at least some mRNA species present in low abundance. In addition, we used relatively strict criteria to define differences in expression in this study, and comparison of mRNA expression levels between cDNA arrays and other techniques remains only semiquantitative and indirect. Other important considerations in interpreting our results include the heterogeneity in expression of various MMPs (and potentially other gene products) between different aneurysm tissue samples and the variable quality of RNA extracted from degenerative human aortic tissue.[9] Therefore, it is necessary to reemphasize that cDNA expression arrays are most valuable in the detection of *novel, unanticipated alterations in gene expression,* and that the strength of this method currently resides in the capacity to characterize *patterns of gene expression* rather than to quantify the expression of individual gene products. The remaining discussion is thereby limited to genes exhibiting the most prominent differential patterns of expression between AAAs and normal aorta, as organized by biological processes that relate to disease development or progression.

Extracellular matrix proteolysis. AAAs are clearly associated with accelerated degradation of aortic wall structural proteins, and our findings confirm the importance of this process in aneurysm disease. For example, gelatinase B/MMP-9 is an elastolytic proteinase known to be elevated at both the protein and mRNA levels in aneurysm tissue, where it is localized to aneurysm-infiltrating macrophages by immunohistochemistry and in situ hybridization.[18,19] Both pharmacologic inhibition and targeted gene disruption of gelatinase B/MMP-9 have been shown to specifically inhibit the development of experimental AAAs.[20] This study therefore supports the view that gelatinase B/MMP-9 plays a major role in aneurysmal degeneration. In accordance with previous studies, we found that TIMP-1 is also expressed at high levels in AAA tissues and in normal aorta.[2,3,18,19] Although TIMP-1 mRNA appeared to be expressed in AAAs at higher levels than gelatinase B/MMP-9, it is not possible to extrapolate this difference to the protein level; it therefore remains

unclear if the TIMP-1 produced in AAAs is sufficient to inhibit the activity of gelatinase B/MMP-9 or other MMPs in vivo. Finally, it is notable that two cysteine proteases (cathepsins D and H) were also prominently expressed in AAAs. Others have described increased levels of cathepsins in atherosclerosis and aneurysm tissue,[21,22] whereas low levels of cystatin C, the principle cathepsin inhibitor, appear to be associated with subclinical aortic dilatation.[23] Because cathepsin H has not been previously linked to aneurysm disease, the significantly elevated expression of this gene suggests that it may be a fruitful target for further investigation.

Inflammation. Not surprisingly, several genes associated with chronic inflammation were found to be upregulated in AAA tissue. MNDA is a nuclear protein expressed specifically in human myelomonocytic cell types, including monocytes/macrophages and granulocytes.[24-26] Because this protein is involved in regulating gene expression uniquely associated with the differentiation process or function of mononuclear phagocytes, it appears likely that MNDA expression is a reflection of the dense infiltration of aneurysm tissue by mononuclear phagocytes and their differentiation to activated tissue macrophages. IL-8 is a C-X-C chemokine produced by various types of cells on stimulation with inflammatory stimuli.[27] It exerts a variety of effects on leukocytes, particularly neutrophils,[28] and plays a critical role in the mobilization of hematopoietic stem cells through its induction of gelatinase B/MMP-9.[29] IL-8 is localized to macrophages and endothelium in human AAA tissue by immunohistochemistry, and it has been suggested to play a role in the medial neovascularization that accompanies aneurysmal degeneration.[30,31] RANTES is a C-C chemokine expressed predominantly in T lymphocytes, and it plays a role in monocyte recruitment and other aspects of chronic inflammation.[32,33] Because increased production of RANTES has not been previously associated with aneurysm disease, the regulation and function of this protein will be of interest in future studies. Finally, transcripts encoding several additional proteins involved in leukocyte trafficking and cell adhesion were elevated in AAAs, including the immunoglobulin-like glycoprotein, ICAM-1 (CD54), and CD11a/CD18 (LFA-1), one of two leukocyte surface β2 integrins that binds ICAM-1.[34,35] ICAM-1 is prominently expressed by endothelial cells and macrophages in coronary, carotid, and aortic atherosclerotic plaques, where it is associated with neovascularization and chronic inflammation.[36-38] It has also been demonstrated in the outer aortic wall of AAAs, and its soluble form is secreted by AAA explants in vitro.[38,39] The adhesive interactions mediated by these proteins are thought to be important for transendothelial migration of leukocytes to sites of inflammation and costimulation of T cell activation, and their functional importance in vascular pathology has been recently demonstrated in ICAM-1 knockout mice.[40,41]

Atherosclerosis. Although the precise relationship between atherosclerosis and aneurysmal degeneration remains unclear, their frequent association and shared risk factors suggest common pathophysiologic mechanisms. This assertion is supported by the observation that AAAs exhibited increased expression of several gene products thought to be important in atherosclerosis, including PDGF, thrombin receptor, and apo E. Both PDGF and thrombin are potent smooth muscle cell mitogens thought to participate in the proliferative events responsible for atheroma formation,[42,43] whereas apo E, through its profound effects on cholesterol and lipid homeostasis, is thought to be protective against atherosclerosis.[44] Indeed, targeted deletion of the apo E gene in mice forms the basis of an important animal model for complex atherogenesis, and in some cases, aneurysm formation.[45] The potential role of apo E in human aneurysm disease has not been specifically investigated, and it remains unclear if its upregulated expression in AAAs is simply a consequence of the atherosclerosis present in these tissues or if it indicates a more direct relationship to aneurysmal degeneration. Further gene profiling studies comparing AAA tissue with age-matched atherosclerotic control tissues will be valuable in addressing these questions.

Smooth muscle cell depletion. We found that differential gene expression generally favored an upregulation of various genes in AAAs as compared with normal aorta. Although this may be due to the activity of biological processes involved in aneurysm disease (ie, chemotaxis, cell proliferation, inflammation, and extracellular matrix degradation), two gene products found abundantly in normal aorta, myosin light chain kinase and integrin β-1, were expressed at significantly decreased levels in AAAs. Because these proteins regulate contractile and migratory activity of vascular smooth muscle cells, their decreased expression likely reflects the pronounced depletion of medial smooth muscle cells that has been previously described in human aneurysm tissues.[46]

In summary, we have used a membrane-based cDNA microarray to characterize the expression of more than 1000 known human genes in AAA tissue. Our findings demonstrate that AAAs exhibit a distinct and relatively limited pattern of differential gene expression compared with normal aorta, reflecting the association of this disease with chronic inflammation, extracellular matrix degradation, atherosclerosis, and smooth muscle cell depletion. Although the functional significance of the individual gene products altered in aneurysm tissue will require further investigation, this study demonstrates the potential of cDNA expression arrays to help elucidate the molecular mechanisms responsible for aneurysmal degeneration.

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