Matrix metalloproteinase and tissue inhibitor expression in atherosclerotic and nonatherosclerotic thoracic aortic aneurysms

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Objectives: The altered expression of matrix metalloproteinases and their inhibitors influences the formation of atherosclerotic abdominal aortic aneurysms. Their association with thoracic aneurysms is less clear. This study describes the expression of metalloproteinases and their inhibitors in atherosclerotic and nonatherosclerotic thoracic aneurysms, and compares these with age-matched controls.

Methods: Matrix metalloproteinase-2 and 9 activity were measured by antibody capture, and tissue inhibitor-1 and 2 levels were measured by enzyme-linked immunosorbent assay in 24 patients with atherosclerotic aneurysms and in 63 patients with nonatherosclerotic aneurysms. Gene expression was assessed with reverse transcriptase polymerase chain reaction. The results were compared with 17 controls.

Results: Data are in nanograms per milligram of protein. Matrix metalloproteinase-2 activity was greater in controls than in the atherosclerotic and nonatherosclerotic groups (80 ± 67 vs 49 ± 50 and 35 ± 44 , $P = .002$). Matrix metalloproteinase-9 activity was greater in the atherosclerotic group than in the nonatherosclerotic group and controls (11.7 ± 15.7 vs 2.5 ± 2.2 and 1.7 ± 1.9 , $P = .001$). Tissue inhibitor-1 and 2 levels were greater in controls than in either aneurysm group (tissue inhibitor of metalloproteinase-1: 376 ± 192 vs 234 ± 233 and 174 ± 148 , $P = .003$; tissue inhibitor of metalloproteinase-2: 143 ± 74 vs 14 ± 13 and 27 ± 43 , $P < .001$). Atherosclerotic aneurysms expressed more matrix metalloproteinase mRNA than controls.

Conclusions: The metalloproteinase/tissue inhibitor phenotype of atherosclerotic thoracic aneurysms is similar to that of abdominal aneurysms. The diminished expression of metalloproteinases and tissue inhibitors in nonatherosclerotic thoracic aneurysms relative to aged controls may represent a loss of smooth muscle cells.

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Thoracic aortic aneurysms are characterized by extracellular matrix destruction involving the fragmentation of elastic lamellae within the media, ineffective elastogenesis, and disordered collagen deposition. Similar changes are seen in abdominal aortic aneurysms, where increases in the elastolytic and collagenolytic activity of matrix metalloproteinases (MMPs) are well documented.¹⁻³ The MMPs, along with their inhibitors, the tissue inhibitor of metalloproteinases (TIMP), are constitutively expressed and are key components in the pathway toward normal and pathologic extracellular matrix turnover.⁴ An imbalance in the proteolytic equilibrium between MMPs and TIMP may contribute to abdominal aortic aneurysm formation.⁵

Thoracic aortic aneurysms often have histopathologic features that differ from abdominal aortic aneurysms. Abdominal aortic aneurysms are commonly of ath-

Abbreviations and Acronyms

MMP = matrix metalloproteinase

TIMP = tissue inhibitor of metalloproteinases

erosclerotic cause and contain a chronic inflammatory cell infiltrate.^{2,3} Atherosclerotic thoracic aortic aneurysms have similar histopathologic characteristics. Many types of thoracic aortic aneurysms, however, are not associated with atherosclerosis. They have no associated inflammatory cell infiltrate and are characterized by extensive cystic medial necrosis, represented by disruption of medial elastin and collagen with focal loss of smooth muscle cells.⁶ Thoracic aneurysms of this type are commonly associated with anuloaortic ectasia, bicuspid aortic valves, or heritable connective tissue disorders, such as Marfan syndrome. Therefore, atherosclerotic and nonatherosclerotic thoracic aneurysms develop from different inciting events and may have divergent extracellular matrix proteolytic phenotypes.

The purpose of this study was to characterize the proteolytic phenotype of these 2 subtypes of thoracic aneurysms by measuring MMP-2 and MMP-9 enzyme activity, TIMP-1 and TIMP-2 protein levels, and MMP/TIMP gene expression, and compare these with nonaneurysmal control thoracic aorta. We hypothesized that atherosclerotic thoracic aneurysms are associated with increased expression of MMP-9 when compared with nonatherosclerotic thoracic aneurysms and nonaneurysmal aorta. We further hypothesized that both subtypes of thoracic aneurysms would express an enhanced proteolytic phenotype when compared with nonaneurysmal aorta.

Materials and Methods**Patients**

Thoracic aortic aneurysm tissue was collected at operation from 87 patients undergoing elective repair at a single tertiary care institution (Fletcher Allen Health Care, Burlington, Vt) between December of 2000 and April of 2005. Ascending aortic samples were taken from the anterior aspect of the aorta in the region of the greatest dilatation, which was usually within 2 cm of the sinotubular ridge. Samples from the descending thoracic aorta were taken from the anterior aspect of the aneurysm, also in the region of the greatest dilatation. Gross and histologic analyses were used to designate each aneurysm as atherosclerotic ($n = 24$) or nonatherosclerotic ($n = 63$) in origin. Atherosclerosis was distinguished by the presence of chronic inflammatory cells, disruption of the intimal layer, and deposition of atheromatous plaque within the media. Nonatherosclerotic aneurysms were characterized by the absence of chronic inflammatory cells, the fragmentation of the elastic lamellae within the media, mucopolysaccharide pooling, and the loss of smooth muscle cells. Control tissue was obtained from discarded aortic punch biopsies of the nonaneurysmal ascending aorta of patients undergoing routine coronary artery bypass grafting ($n = 17$). Control tissue was not used if there was evidence of gross

atheromatous disease. Tissue was placed in phosphate-buffered saline solution at 4°C, transported to the laboratory, snap-frozen in liquid nitrogen, and stored at -80°C until the time of preparation and analysis.

The medical record of each patient was reviewed, and important clinical and demographic variables were recorded, including age, sex, vascular disease risk factors, antihypertensive drug therapy, and presence or absence of a bicuspid valve. Informed consent was obtained, and the study met the guidelines set forth by the institutional review board at this institution.

Tissue Preparation

Tissue (0.15-0.40 g wet weight) was homogenized in 1 mL of 50 mmol/L Tris-HCL buffer solution (1.5 mmol/L NaCl, 0.5 mmol/L CaCl₂, 1 μ mol/L ZnCl₂, and 0.01% [v/v] BRIJ 35, pH 7.4) at 4°C and centrifuged at 8000 rpm for 5 minutes, and the supernatant was collected. The pellets were resuspended in 1 mL of 50 mmol/L Tris-HCL, homogenized a second time, and centrifuged at 8000 rpm for 5 minutes. Combined supernatants were spun at 10,000 rpm for 10 minutes, and the final supernatant was collected and used for analysis. Total protein concentration (milligrams/milliliter) was measured in each final supernatant using a protein assay kit (BIO-RAD, Hercules, Calif) for standardization.

MMP Activity and TIMP Protein Analysis

The endogenous and total activity of MMP-2 and MMP-9 were quantified in the supernatants by activity assays (MMP-2 and MMP-9 Biotrak Activity Assay System, Amersham Biosciences Corp, Piscataway, NJ). The activity assay is based on an antibody-capture technique and has been described.⁷ The term "endogenous activity" refers to extractable bioactive MMP, whereas the term "total activity" refers to the detectable endogenous activity plus the artificially activated proenzyme. The levels of TIMP-1 and 2 were quantified in the supernatants by enzyme-linked immunosorbent assay (TIMP-1 and TIMP-2 Biotrak enzyme-linked immunosorbent assay, Amersham Biosciences Corp). Because of the limiting factor of tissue quantity in each control patient (aortic punch), not all control tissue underwent both MMP and TIMP analyses. MMP analysis was performed in all 17 control patients. Nine of these 17 patients also underwent TIMP analysis and proteolytic index calculation. In the remaining 8 patients, limited tissue quantity excluded TIMP analysis. Both MMP and TIMP levels were standardized to total protein. Values are expressed as nanograms of MMP or TIMP per milligram of protein.

Gene Expression Analysis

Real-time quantitative polymerase chain reaction analysis was performed to assess MMP and TIMP mRNA expression in randomly selected tissue (8 atherosclerotic, 11 nonatherosclerotic, and 5 control). The tissue from both aneurysm groups that underwent mRNA analysis also underwent MMP activity and TIMP protein analysis. Because of limited tissue from control subjects, mRNA analysis was performed on separate tissue that did not undergo concomitant MMP and TIMP analysis. Total RNA from tissue samples was extracted in TRIzol Reagent (Invitrogen Corp, Carlsbad, Calif) by homogenization in a Polytron (Kinematica AG, Lucerne, Switzerland) device, followed by centrifugation and further extraction with chloroform. The RNA in the aqueous layer

TABLE 1. Patient demographics

	Control N = 17 (%)	Atherosclerotic n = 24 (%)	Nonatherosclerotic N = 63 (%)	P value
Age (mean, y)	64.1 ± 13.0	69.5 ± 10.6	58.7 ± 14.0	.02
Size (mean, cm)		6.1 ± 0.9	5.6 ± 0.8	.01
Sex (male)	13 (76)	12 (50)	49 (78)	.06
Ascending aorta involvement	17 (100)	12 (50)	59 (94)	<.001
Bicuspid valve	0 (0)	3 (13)	28 (45)	.005
Hypertension	12 (71)	15 (65)	31 (49)	.18
Hyperlipidemia	13 (77)	13 (57)	21 (33)	.003
Tobacco use	2 (12)	13 (57)	17 (27)	.005
CAD	17 (100)	13 (57)	23 (37)	<.001
β-blocker	10 (59)	13 (57)	36 (57)	.99
Ca ²⁺ blocker	2 (12)	7 (30)	6 (10)	.05
ACE inhibitor	7 (41)	10 (44)	20 (32)	.54

CAD, coronary artery disease; ACE, angiotensin-converting enzyme.

was precipitated with isopropyl alcohol and washed with ethanol. The sample was resuspended in water and further purified using the RNeasy Fibrous Tissue Mini Kit from Qiagen, Inc (Valencia, Calif), according to the manufacturer's instructions. An aliquot was measured for concentration and quality analysis using an Agilent 2100 Bioanalyzer (Palo Alto, Calif) at the Vermont Cancer Center's DNA Analysis Core Facility (Burlington, Vt). Only samples with an appropriate yield of intact mRNA were used.

Real-time quantitative polymerase chain reaction was performed with the Applied Biosystems GeneAmp RNA PCR Core Kit (Foster City, Calif). Briefly, a 25-μL reaction mixture containing a 1-μg purified sample of RNA with 5 mmol/L MgCl₂ 1X PCR Buffer II, 1 mmol/L each of deoxyguanosine triphosphate, deoxyadenosine monophosphate, deoxythymidine triphosphate, and deoxycytidine triphosphate, 1 U/mL RNase inhibitor, 2.5 U/μL MuLV reverse transcriptase, and 2.5 μmol oligo d(T) was reverse transcribed at 42°C for 15 minutes, followed by an additional incubation at 99°C for 5 minutes.

Complementary DNA samples were analyzed with Applied Biosystem's Assays-on-Demand Gene Expression products. The reaction mixture consisted of 1X Taqman Universal PCR Master Mix (20X unlabeled polymerase chain reaction primers and TaqMan MGB [FAM dye labeled] probe) and 1X Assays-on-Demand Gene Expression Assay Mix (specific mRNA probes: Hs00234433_m1 [MMP-2], Hs00234579_m1 [MMP-9], Hs00171558_m1 [TIMP-1], Hs00234278_m1 [TIMP-2], and Hs00355752_m1 [hypoxanthine guanine phosphoribosyl transferase]). The reactions were performed with Applied Biosystem's Prism 7700 Sequence Detection System at 40 cycles (95°C for 15 seconds and 60°C for 60 seconds).

Relative MMP and TIMP mRNA expression was calculated by the comparative C_T method, using hypoxanthine guanine phosphoribosyl transferase as the endogenous control. Standard curves were run to validate the efficiencies of target and reference mRNA; they were approximately equal. Each target in the aneurysm groups was then normalized to the control group to obtain relative quantification values.

Statistical Analysis

Data are given as mean values ± standard deviation. Differences in means between groups were tested using the unpaired Student *t* test and analysis of variance. Nonparametric analysis was performed with the Kruskal–Wallis Test when there was evidence of unequal variance. Pearson chi-square analysis was used to compare clinical variables between groups. The relationship among aneurysm size, MMP activity, and TIMP levels was assessed with simple linear regression analysis.

Results

Patients

Patient demographic and clinical variables are presented in Table 1. Those in the nonatherosclerotic group were younger than those in the other 2 groups (*P* = .02). The average age of controls who underwent both MMP and TIMP analyses was 64.7 ± 4.8 years. The average age of controls who underwent mRNA analysis alone was 69 ± 3 years (atherosclerotic: 68.3 ± 4 years, nonatherosclerotic: 59 ± 4 years, *P* = .10).

Atherosclerotic aneurysms were larger than nonatherosclerotic aneurysms (*P* = .01). Nonatherosclerotic aneurysms occurred more frequently in the proximal aorta and were often associated with a bicuspid aortic valve. There were differences between groups with regard to tobacco use, hyperlipidemia, and coronary artery disease.

MMP Activity and TIMP Levels

The endogenous MMP-2 activity was greater in controls than in either aneurysm group (*P* = .002). A positive correlation existed between aneurysm size and endogenous MMP-2 activity in the atherosclerotic group (*R*² = 0.30) (*P* = .004, Figure 1). The total MMP-2 activity was greater in controls than in either aneurysm group (*P* = .002). The endogenous MMP-9 activity was greater in controls than in either aneurysm group (*P* = .002). The total MMP-9 activ-

Figure 1. Endogenous activity of MMP-2 in the atherosclerotic thoracic aortic wall relative to aortic size ($R^2 = 0.30$, $P = .004$). **MMP-2**, Matrix metalloproteinase-2.

ity was greater in the atherosclerotic group when compared with the nonatherosclerotic group and controls ($P < .001$). TIMP-1 levels were greater in controls than in either aneurysm group ($P = .003$). TIMP-2 levels were also greater in controls than in either aneurysm group ($P < .001$) (Table 2).

Subgroup analysis of the regional differences in MMP and TIMP expression in the thoracic aorta was possible in the atherosclerotic group. There were no meaningful differences in the regional expression of TIMP-1, TIMP-2, endogenous MMP-2 and MMP-9, or total MMP-9. Atherosclerotic ascending thoracic aneurysms expressed more total MMP-2 than atherosclerotic descending thoracic aneurysms ($P = .03$) (Table 3).

Proteolytic Index

The proteolytic state of the aortic wall is expressed as the ratio of total MMP activity to TIMP levels. Controls had a

TABLE 3. Regional expression of matrix metalloproteinase activity, tissue inhibitor matrix metalloproteinase levels, and proteolytic index in atherosclerotic aneurysms

	Ascending (n = 12)	Descending (n = 12)	P value
Endogenous MMP-2	0.56 ± 0.58	0.72 ± 0.49	.47
Total MMP-2	71 ± 59	26 ± 20	.03
Endogenous MMP-9	0.22 ± 0.42	0.29 ± 0.45	.69
Total MMP-9	8.5 ± 8.4	14.8 ± 20.5	.36
TIMP-1	274 ± 224	173 ± 240	.31
TIMP-2	14.5 ± 10.0	11.9 ± 15.7	.64
Total MMP-2:TIMP-1	0.52 ± 0.72	0.29 ± 0.30	.36
Total MMP-2:TIMP-2	8.5 ± 7.8	4.8 ± 5.8	.24
Total MMP-9:TIMP-1	0.05 ± 0.07	0.12 ± 0.16	.14
Total MMP-9:TIMP-2	1.2 ± 1.5	4.6 ± 10.5	.33

MMP, Matrix metalloproteinase; TIMP, tissue inhibitor matrix metalloproteinase.

lower ratio of MMP-2 activity to TIMP-1 levels than either aneurysm group, but this could be due to chance ($P = .25$). Controls had a lower ratio of MMP-2 activity to TIMP-2 levels than either aneurysm group ($P < .001$). Controls had lower ratios of MMP-9 activity to both TIMP-1 ($P < .02$) and TIMP-2 ($P < .001$) when compared with either aneurysm group (Table 2). There was no meaningful difference in the proteolytic index between atherosclerotic ascending aneurysms and atherosclerotic descending aneurysms (Table 3).

Gene Expression

Atherosclerotic aneurysms expressed almost 3 times more MMP-2 mRNA and 5 times more MMP-9 mRNA than controls. Nonatherosclerotic aneurysms showed no difference in expression of MMP-2 mRNA when compared with controls, but expressed 4 times more MMP-9 mRNA. Nonatherosclerotic aneurysms expressed less TIMP mRNA than atherosclerotic aneurysms and controls (Table 4).

TABLE 2. Matrix metalloproteinase activity, tissue inhibitor matrix metalloproteinase level, and proteolytic index

	Control	Atherosclerotic	Nonatherosclerotic	P value
Endogenous MMP-2	1.54 ± 1.6	0.64 ± 0.53	0.61 ± 0.71	.002
Total MMP-2	80 ± 67	49 ± 50	35 ± 44	.002
Endogenous MMP-9	0.74 ± 1.3	0.25 ± 0.42	0.17 ± 0.19	.002
Total MMP-9	1.7 ± 1.9	11.7 ± 15.7	2.5 ± 2.2	<.001
TIMP-1	376 ± 192	234 ± 233	174 ± 148	.003
TIMP-2	143 ± 74	14 ± 13	27 ± 43	<.001
Total MMP-2:TIMP-1	0.11 ± 0.06	0.39 ± 0.55	0.33 ± 0.53	.25
Total MMP-2:TIMP-2	0.32 ± 0.27	7.0 ± 7.1	2.8 ± 5.5	<.001
Total MMP-9:TIMP-1	0.01 ± 0.009	0.08 ± 0.12	0.03 ± 0.08	.02
Total MMP-9:TIMP-2	0.03 ± 0.02	3.1 ± 7.7	0.24 ± 0.36	<.001

MMP, Matrix metalloproteinase; TIMP, tissue inhibitor matrix metalloproteinase.

TABLE 4. Relative quantitation of aneurysm mRNA to control aorta

Group	Target mRNA	$\Delta\Delta C_T$	Target relative to control (range)
CON	MMP-2	0.0 ± 1.4	1.0 (0.4-2.6)
ATH		-1.5 ± 3.6	2.7 (0.2-33.1)
NOATH		-0.2 ± 2.3	1.1 (0.2-5.5)
CON	MMP-9	0.0 ± 1.5	1.0 (0.4-2.8)
ATH		-2.3 ± 3.3	5.1 (0.5-51.3)
NOATH		2.0 ± 1.6	4.1 (0.3-12.5)
CON	TIMP-1	0.0 ± 1.5	1.0 (0.4-3.0)
ATH		-1.0 ± 2.7	2.0 (0.3-13.5)
NOATH		0.4 ± 2.5	0.8 (0.1-4.3)
CON	TIMP-2	0.0 ± 1.5	1.0 (0.3-2.9)
ATH		-1.1 ± 1.2	2.1 (0.9-4.8)
NOATH		0.7 ± 2.4	0.6 (0.1-3.4)

CON, Control; ATH, atherosclerotic; NOATH, nonatherosclerotic; MMP, matrix metalloproteinase; TIMP, tissue inhibitor metalloproteinase; $\Delta\Delta C_T$, (target mRNA – HPRT mRNA) – control group calibrator.

Discussion

There is strong evidence that enhanced MMP activity and/or reduced TIMP levels contribute to abdominal aortic aneurysm growth and rupture.^{1-3,5} The supposition that these same pathologic conditions exist in thoracic aortic aneurysms is unproven, given the differences in the histologic pattern of disease between these two types of aneurysms. Most abdominal aortic aneurysms are atherosclerotic in cause and are associated with an inflammatory cell infiltrate. The inflammatory environment associated with the atheroma results in the increased expression of MMPs.^{2,3} MMP-9 activity predominates because of its association with the tissue macrophage.⁷

Unlike abdominal aortic aneurysms, thoracic aortic aneurysms occur as 2 distinct histopathologic subtypes: atherosclerotic and nonatherosclerotic. Attempts at defining the role of the metalloproteinases and their inhibitors in the formation of human thoracic aneurysms have been hampered by the analysis of these two subtypes of aneurysms together, the inclusion of acute aortic dissections with aneurysms in data analysis, the use of small study groups, the use of semiquantitative methodology for measuring MMP and TIMP expression, and the use of non-aged matched and non-risk factor-matched controls.^{6,8-11} Animal models have aided in the understanding of the relationship of MMPs and TIMPs to the development of thoracic aneurysms, but these models are inflammatory based^{12,13} and therefore may not reflect the pathogenesis of the nonatherosclerotic subgroup.

The inclusion of a well-matched control group is important when interpreting the role of the MMPs and TIMPs in extracellular matrix turnover. Aging is known to increase the activity of MMP-2 in the rat,¹⁴ nonhuman primate,¹⁵ and

human nonaneurysmal aorta,¹⁶ and is associated with the increased expression of both TIMP-1¹⁷ and TIMP-2¹⁵ within the nonaneurysmal aortic wall. Hypertension is associated with the increased expression of both MMP-2 and MMP-9 in the aortic wall.^{17,18} Thus, the use of an older control group with similar vascular risk factors as the experimental group is important to control for such variables related to MMP and TIMP expression.

This study used an aged control group with similar vascular risk factors as the experimental groups and is unique in its finding that both the endogenous and total MMP-2 activity were higher in the control group when compared with the aneurysm groups. Prior studies using small select groups of patients with nonatherosclerotic thoracic aneurysms, such as patients with bicuspid aortic valves,^{9,19} found higher MMP-2 expression referable to controls. Most of these studies, however, used tissue from younger patients (such as organ donors) as controls, which could confound the interpretation of MMP-2 expression relative to the experimental group.

Subgroup analysis revealed that regional differences in the expression of MMP-2 may partially account for the lower total MMP-2 activity in the atherosclerotic group when compared with controls. Ascending atherosclerotic aneurysms had higher total MMP-2 activity than descending atherosclerotic aneurysms. Regional differences in MMP expression in the aorta have been documented in both animal²⁰ and human studies.¹¹ The location of tissue sampling within the descending aneurysms may have also influenced the results, because there is evidence that MMP-2 activity is lower in the anterior wall of descending thoracic aneurysms relative to the posterior wall.²¹

Another notable finding in this study was that both TIMP-1 and TIMP-2 protein expression were greater in the control group than in either aneurysm group. The higher control TIMP levels resulted in a lower proteolytic index despite the elevated MMP-2 activity. The lower TIMP protein levels in the nonatherosclerotic group referable to controls were associated with reduced TIMP mRNA expression. The importance of inhibitor expression in the pathogenesis of thoracic aneurysms has been demonstrated in TIMP-1-deficient mouse models of inflammation-induced thoracic aneurysms.^{12,13}

The differences in MMP and TIMP expression between the controls and the nonatherosclerotic group should be interpreted relative to known age-related aortic wall remodeling. Normal aging is associated with an increase in aortic diameter and a compensatory thickening of the aortic wall as a means to normalize circumferential wall stress.^{22,23} Aortic wall thickening with aging is associated with non-inflammatory cell proliferation in the intima, medial smooth muscle cell hypertrophy, and a net increase in extracellular matrix production.¹⁵ These events are linked to increases in

smooth muscle cell expression of both MMP-2^{15,24} and TIMP-2.¹⁵ TIMP-2 can both induce and repress the activity of MMP-2¹⁵ and has been associated with induction of cell proliferation.^{15,25}

Nonatherosclerotic thoracic aneurysms, however, are associated with a progressive thinning of the aortic wall over time. These aneurysms were found to express much lower MMP-2 activity, TIMP protein, and TIMP mRNA when compared with the aged controls. It is plausible that both aortic wall thinning and a reduction in MMP/TIMP expression in nonatherosclerotic thoracic aneurysms are related to the dysfunction or loss of the smooth muscle cell, the engine that drives the normal age-related eutrophic aortic wall remodeling. Smooth muscle cell apoptosis has been documented in some types of nonatherosclerotic thoracic aneurysms.^{26,27}

Previous animal and human studies have explored the relationship of MMP-9 expression and the pathogenesis of nonatherosclerotic thoracic aneurysms. Intense MMP-9 immunostaining was seen in the aneurysmal aortic walls of mice in a genetic knockout model of Marfan syndrome.²⁸ This expression, however, was associated with an inflammatory cell infiltrate, a histologic finding that is not characteristically seen in aortic aneurysms of patients with Marfan syndrome. MMP-9 immunoreactivity was seen in aneurysm tissue from a small study of humans with Marfan syndrome,¹⁰ but only in regions of inflammatory cell infiltration that may have occurred secondary to the trauma of acute aortic dissection in almost half of the patients. The confounding presence of an inflammatory infiltrate, therefore, may affect the conclusions in these studies. We did find increased MMP-9 mRNA expression in the nonatherosclerotic group relative to controls, but this was not associated with an increase in MMP-9 protein activity.

It was hypothesized that atherosclerotic thoracic aneurysms would have a similar MMP/TIMP phenotype with abdominal aneurysms given the presence of an inflammatory infiltrate. This was confirmed in our study by the findings that both the total MMP-9 activity and MMP-9 mRNA expression were higher when compared with controls. Because there was no meaningful difference in total MMP-9 activity between the controls and the nonatherosclerotic group, it is suggested that the enhanced MMP-9 activity associated with the inflammatory infiltrate is unique to atherosclerotic aneurysms. Although atherosclerotic aneurysm tissue expressed twice as much TIMP-1 and TIMP-2 mRNA relative to controls, this was not associated with higher TIMP protein levels. Similar findings have been noted in atherosclerotic abdominal aneurysms.²⁹ The lower protein expression relative to the higher transcriptional expression could indicate an alteration in the posttranscriptional processing of mRNA, differences in mRNA stabilization, or posttranslational control between groups. A similar relation-

ship was seen in the expression of MMP-2 mRNA and MMP-2 activity in the atherosclerotic group.

This study supports the concept that MMP/TIMP homeostasis is altered in thoracic aortic aneurysms. The specific alteration, however, is peculiar to the subtype of aneurysm. The atherosclerotic thoracic aneurysm behaves similarly to the atherosclerotic abdominal aneurysm in regard to MMP/TIMP metabolism. These aneurysms have focally thickened walls with atheroma containing an inflammatory cell infiltrate that overexpresses MMP-9, which may lead to focal wall weakening and expansion. In contrast, the nonatherosclerotic aneurysm is characterized by nonfocal thinning of the aortic wall. The lesion is associated with a paucity of cells, mainly from the loss of the smooth muscle cell. There is no inflammatory cell infiltrate. With loss or dysfunction of the smooth muscle cell and, with it, the inability to synthesize and secrete MMP-2 and TIMP, the aortic wall may lose the ability to compensate for age-related hemodynamic stress with reactive thickening. This would predispose the aortic wall to further weakening and subsequent dilatation.

There are limitations associated with this work. A cause and effect relationship between MMP and TIMP expression and aneurysm formation cannot be made, because aneurysm specimens were limited to patients with advanced disease. Because we did not perform smooth muscle cell and macrophage quantification, the supposition that the lower expression of MMP-2 and TIMP seen in the nonatherosclerotic group, or the higher MMP-9 expression seen in the atherosclerotic group, is related to these cells is purely speculative and based on past literature. The control group was limited to tissue obtained from the ascending aorta, and as noted above, regional differences in MMP/TIMP expression may occur within the aorta. Our control group, therefore, may not completely represent the entire cross-section of metalloproteinase expression within the aorta.

Conclusions

Thoracic aneurysms were found to have divergent MMP/TIMP phenotypes based on their histologic classification. Atherosclerotic thoracic aneurysms have increased transcriptional expression and activity of MMP-9 and decreased expression of TIMP protein, and are similar to abdominal aneurysms in this regard. Nonatherosclerotic thoracic aneurysms have reduced MMP-2 activity and TIMP protein and gene expression when compared with nonaneurysmal aorta, which may reflect a loss or dysfunction of the smooth muscle cell and the inability to compensate for age-related hemodynamic stress.

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