Neutrophil Depletion Inhibits Experimental Abdominal Aortic Aneurysm Formation

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Background—Neutrophils may be an important source of matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9), two matrix-degrading enzymes thought to be critical in the formation of an abdominal aortic aneurysm (AAA). The purpose of this investigation was to test the hypothesis that neutrophil depletion would limit experimental AAA formation by altering one or both of these enzymes.

Methods and Results—Control, rabbit serum–treated (RS; n = 27) or anti-neutrophil-antibody–treated (anti-PMN; n = 25) C57BL/6 mice underwent aortic elastase perfusion to induce experimental aneurysms. Anti-PMN–treated mice became neutropenic (mean, 349 cells/μL), experiencing an 84% decrease in the circulating absolute neutrophil count (P < 0.001) before elastase perfusion. Fourteen days after elastase perfusion, control mice exhibited a mean aortic diameter (AD) increase of 104±14% (P < 0.0001), and 67% developed AAAs, whereas anti-PMN–treated mice exhibited a mean AD increase of 42±33%, with 8% developing AAAs. The control group also had increased tissue neutrophils (20.3 versus 8.6 cells per 5 high-powered fields [HPFs]; P = 0.02) and macrophages (6.1 versus 2.1 cells per 5 HPFs, P = 0.005) as compared with anti-PMN–treated mice. There were no differences in monocyte chemotactic protein-1 or macrophage inflammatory protein-1α chemokine levels between groups by enzyme-linked immunosorbent assay. Neutrophil collagenase (MMP-8) expression was detected only in the 14-day control mice, with increased MMP-8 protein levels by Western blotting (P = 0.017), and MMP-8–positive neutrophils were seen almost exclusively in this group. Conversely, there were no statistical differences in MMP-2 or MMP-9 mRNA expression, protein levels, enzyme activity, or immunostaining patterns between groups. When C57BL/6 wild-type (n = 15) and MMP-8–deficient mice (n = 17) were subjected to elastase perfusion, however, ADs at 14 days were no different in size (134±7.9% versus 154±9.9%; P = 0.603), which suggests that MMP-8 serves only as a marker for the presence of neutrophils and is not critical for AAA formation.

Conclusions—Circulating neutrophils are an important initial component of experimental AAA formation. Neutrophil depletion inhibits AAA development through a non–MMP-2/9–mediated mechanism associated with attenuated inflammatory cell recruitment. (Circulation. 2005;112:232-240.)

Key Words: aneurysm ■ aorta ■ arteries ■ enzymes ■ leukocytes

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Laboratories, Wilmington, Mass). Mice were randomized into 2 groups receiving 3 daily injections of either normal rabbit serum (n = 17) or rabbit anti-mouse polymorphonuclear neutrophil (PMN) antiserum (n = 27). Mice then underwent 2% isofluorane inhalational anesthesia. A laparotomy was performed, and the abdominal aorta from just below the left renal vein to the bifurcation was isolated. Anesthesia was maintained with 1% to 1.5% isofluorane. The aorta was exsanguinated and then perfused for 5 minutes with a porcine pancreatic elastase solution (specific activity 104 U/mg protein; E1250; Sigma Chemical) containing 0.343 U/mL as described previously.11 Mice underwent repeat laparotomy for AD measurements and aortic harvest on postoperative days 2 (rabbit serum [RS], n = 3; anti-mouse PMN [AB], n = 2), 4 (RS, n = 7; AB, n = 7), 7 (RS, n = 7; AB, n = 7), or 14 (RS, n = 10; AB, n = 9). The percentage increase in AD was then calculated from the difference between the preperfusion and final measurements. An AAA was defined as an increase in AD >100%. The entire infrarenal aorta was removed at the time of aortic harvest. The aorta (or aneurysm, when present) was then divided in transverse fashion, with half of the specimen used for real-time polymerase chain reaction (PCR), Western blotting, zymography, or enzyme-linked immunosorbent assay (ELISA), and half of the specimen used for histology or immunohistochemistry. For the latter studies, the point of maximal diameter was preferentially used for analysis.

In a separate set of experiments designed to determine the effect of MMP-8 on aneurysmal degeneration with this model, C57BL/6 wild-type (n = 15) and MMP-8–deficient (n = 17) mice were subjected to transient aortic perfusion with elastase as described above. These mice were also 8 to 12 weeks of age and weighed between 22 and 28 g but were from a different litter and were housed in a separate facility from those used in the first set of experiments. AD measurements in this study were obtained before perfusion, after perfusion, and at 14 days.

**Generation of Neutropenia**

Mice assigned to the neutropenia group received 3 daily preoperative intraperitoneal injections (0.2 to 0.5 mL) of saline-diluted (1:5 to 1:10) rabbit anti-mouse PMN polyclonal antibody (Accurate Chemical & Scientific, Westbury, NY). This resulted in a circulating absolute neutrophil count of ≤350 cells/μL by the day of operation. Additional doses of antiserum were given the morning of operation and on postoperative days 2 and 4. The control group of mice followed the same dosage schedule, receiving intraperitoneal injections of normal rabbit serum (Accurate Chemical & Scientific, Westbury, NY) instead of the antiserum.

Peripheral blood, 0.25 to 0.5 mL, was obtained from all mice before treatment, on the day of operation, and at tissue harvest, being drawn from a prewarmed ventral tail artery by laceration. Cell counts were analyzed with a HEMAVET 1500FS multispecies hematology instrument (CDC Technologies).

**Quantitative (Real-Time) PCR**

Expression of MMP-2, MMP-8, MMP-9, and β-actin mRNA was determined by PCR. mRNA was isolated from aortic segments by treatment with TRizol reagent (Life Technologies) and reverse-transcribed by incubating with Oligo-(dT) primer and M-MLV Reverse Transcriptase (Life Technologies) for 3 minutes at 94°C, followed by 70 minutes at 40°C. The resultant cDNA was amplified by Taq Polymerase (Promega) in the SmartCycler quantitative PCR system (Cepheid). SYBR Intercalating Dye (Roche) was used to monitor levels of cDNA amplification for each gene. Primer sequences were derived using Primer Premier software (Premier Biosoft International) on the basis of primary mRNA sequences from GenBank (http://www.ncbi.nlm.nih.gov/Genbank). SmartCycler quantification used the cycle threshold (Ct) for the gene of interest normalized to the β-actin gene. Relative mRNA expression was calculated by the formula 2^(-ΔΔCt), where ΔΔCt = (Ct target gene - Ct reference gene).

**Western Blotting**

Individual MMP proteins were isolated from aortic segments using TRizol reagent and dissolved in 1% SDS. Proteins were separated electrophoretically on 7.5% polyacrylamide gels and blotted onto nitrocellulose membranes. Nonspecific binding was blocked by incubating the membrane for 1 hour in 20 mmol/L tris-HCl (pH 7.5) containing 0.5 mol/L sodium chloride, 0.1% Tween 20, and 5% nonfat milk. Electroblotting and Western blotting supplies were obtained from BioRad. Primary antibodies were diluted in the same buffer and included rabbit anti-mouse MMP-9 polyclonal antibody (Triple Point Biologics, Portland, Ore), mouse anti-human MMP-2 monoclonal antibody (Zymed, San Francisco, Calif), and rabbit anti-human MMP-8 polyclonal antibody (BIOMOL, Plymouth Meeting, Pa). Peroxidase-coupled species-appropriate secondary antibodies were then applied. Immunoreactive bands were visualized with an ECL chemiluminescence detection kit (Amersham), and the amount of each protein was measured with densitometry, as subsequently described. Densitometric
analysis was then adjusted for total cellular protein content as determined by a bicinchoninic acid protein assay (Pierce).

**SDS-PAGE Substrate Zymography**
Zymography was performed with precast 10% SDS-polyacrylamide gels containing 1 mg/mL of gelatin (unless otherwise stated, all zymography supplies were obtained from Novex). Tissue was homogenized in complete EDTA-free protease inhibitor cocktail (Roche) and centrifuged for 10 minutes at 2000 g. Equal volumes of the supernatant were diluted with tris-glycine-SDS sample buffer and electrophoretically separated under nonreducing conditions. Separated proteins were renatured in 2.7% Triton X-100 for 3 hours, and the gels were developed for 48 hours at 37°C in 50 mmol/L tris-HCl, 5 mmol/L CaCl₂, and 0.2% Brij 35. After staining with Coomassie Blue and destaining in 10% acetic acid, gelatinase activity was evident by clear bands against a dark blue background. Samples containing human recombinant MMP-2 and MMP-9 (Oncogene) served as standards. These bands have been shown to be inhibited by EDTA and thus are considered to be caused by metalloproteinase activity. Densitometry analysis was performed using a FOTO/Analyst CCD CAMERA (Fotodyne) and GEL-Pro Analyzer software version 3.1 (Media Cybernetics). Optical densities were normalized to the dry weight of the corresponding aortic segment. All bands assessed by densitometry were within the range of densitometric measurements.

**Histology and Immunohistochemistry**
Harvested aortas were fixed in fresh cold 4% paraformaldehyde for 6 hours followed by 70% ethanol. Segments were then paraffin embedded, and 5-μm sections were mounted onto slides. Aortic sections were stained with hematoxylin and eosin, Masson’s Trichrome for collagen, and Verhoeff–Van Gieson for elastin. Immunohistochemical sections were treated for 10 minutes with H₂O₂ to block endogenous peroxidase activity and then with 1.5% normal horse serum (Vector Laboratories) in PBS for at least 20 minutes at room temperature. Sections were then incubated for 30 minutes at room temperature with primary antibodies, including mouse anti-rat MMP-9 monoclonal antibody-8 (Neomarker, Fremont, Calif), mouse anti-human MMP-2 monoclonal antibody-4 (Neomarker), rat anti-mouse monocyte/macrophage monoclonal antibody (Sertotec, Oxford, UK), rat anti-mouse Mac-3 monoclonal antibody (BD Biosciences Pharmingen, San Francisco, Calif), rat anti-mouse neutrophil monoclonal antibody (Sertotec), rabbit anti-rat PMN antibody (Accurate Chemical & Scientific, Westbury, NY), and rabbit anti-human MMP-8 polyclonal antibodies AB81016 and AB8115 (Chemicon International, Inc, Temecula, Calif). Epitope unmasking was performed, when necessary, with 0.1% trypsin in 0.1% CaCl₂ for 20 minutes or Trilogy in a Princess model pressure cooker (Cell Marque Corporation). The appropriate secondary antibodies from the mouse, rat, or rabbit Vectastain ABC-AP kit (Vector Laboratories) were used, followed by a standard alkaline phosphatase staining procedure. These sections were lightly counterstained with hematoxylin. Two sets of slides per animal were used for analysis, with all of the same concentrations of solutions and equal incubation times being used for each particular staining protocol. Negative-control sections were prepared with species-specific immunoglobulin and were stained simultaneously. Only moderate or strongly reactive cells were counted. Slides were evaluated at high power and scored in a blinded fashion by dividing the artery into quartiles with concentric radial fields of view extending from the lumen to the adventitia in each quartile. A mean value for positively stained cells was determined for each animal, and a mean for each animal group was then calculated.

**Figure 2.** Comparative histology after aortic elastase perfusion. Aortic tissue sections were prepared 14 d after undergoing elastase perfusion; all images original magnification >200, with L denoting arterial lumen and A signifying outer border of adventitia. Tissues stained with hematoxylin and eosin (A, D), Masson’s trichrome (B, E), or Verhoeff–Van Gieson (C, F). A, Aneurysmal rabbit serum–treated control mouse aortas revealed loss of normal arterial morphology, characterized by dilation, increased wall thickness, and formation of neointima. B, Masson’s trichrome staining for collagen demonstrates replacement of medial elastin fibers by collagen (red subintimal staining). C, Verhoeff–Van Gieson staining for elastin reveals fragmentation of remaining internal elastic lamellar structures (arrows). D, E, and F, Arterial morphology was not altered in the corresponding anti-PMN–treated aortic sections.
Chemokine ELISA
Aortic specimens were placed in complete EDTA-free lysis buffer at 0°C (Roche Diagnostics), homogenized, sonicated for 10 seconds, centrifuged at 10,000 g for 5 minutes, and the supernatant collected. Quantified peptide mediators were normalized to total protein in the sample. Total protein was determined using a modified Bradford assay per the manufacturer’s instructions (Pierce) with serial dilutions of bovine serum albumin (Sigma Chemical) for protein standards. Tissue homogenate ELISAs for mouse macrophage inflammatory protein-1α (MIP-1α) and monocyte chemotactic protein-1 (MCP-1) were performed with species-specific primary antibodies quantified with a double-ligand technique, as has been described for similar chemokines.17

Statistical Analysis
With each experimental condition, the entire group of animals was studied at the same time, and mice in each group were the same age and housed together. All data are expressed as mean±SE. A 2-tailed nonparametric (unpaired t test) was used for statistical testing. Probability values of $P<0.05$ were considered statistically significant.

Results
Cytotoxic Antibody Results in Systemic Neutropenia
Intraperitoneal injections of rabbit anti-neutrophil antibody (anti-PMN) significantly diminished the number of circulating neutrophils compared with the control rabbit serum–treated animals. The mean neutrophil count in the anti-PMN–treated mice dropped from 2217 cells/mL before treatment to 349 cells/mL just before operation and elastase perfusion of the aorta, representing an 84% decrease in the absolute neutrophil number ($P<0.0001$) and a decrease in the neutrophil percentage of total white blood cells (WBCs) from 25% to 8%. By 14 days, however, mice in the anti-PMN–treated group were no longer neutropenic, with the number of circulating neutrophils returning to at least baseline. No decrease in circulating neutrophils occurred in the rabbit serum–treated control group. Although a decrease in the absolute WBC and lymphocyte numbers were noted in the anti-PMN group, as has been described with antibody-mediated neutrophil depletion,18 the absolute WBC count and the total WBC percentages of lymphocytes, monocytes, and eosinophils did fall below normal after anti-PMN antibody treatment.

Aneurysm Formation Suppressed by Neutrophil Depletion
Aneurysm formation in control mice 14 days after elastase perfusion was a consistent finding, with a mean AD increase of 104±14% ($P<0.0001$) and 67% of the animals developing AAAs. In contrast, the anti-PMN–treated group had a mean AD increase of 42±33%, and only 8% developed AAAs (Figure 1, A and B). Similar trends were seen as early as 4 days after elastase perfusion (mean AD increase 74±5% versus 54±5%, $P=0.024$). Half of control animals 7 days after elastase perfusion formed aneurysms, compared with none in the anti-PMN–treated group, although the difference in mean aortic enlargement did not reach statistical significance (mean AD increase 87±17% versus 64±7%, $P=0.22$). In all mice, a typical profile of $\approx40\%$ aortic enlargement occurred immediately after perfusion with elastase. Control aneurysmal aortas revealed an increased wall thickness and loss of normal arterial architecture (Figure 2A), with replacement of the medial elastic fibers by

![Figure 3. Neutrophil and monocyte/macrophage immunostaining. Aortic tissue sections prepared 14 d after undergoing elastase perfusion and immunostained for tissue PMNs and monocyte/macrophages. A, Tissue neutrophils and macrophages significantly decreased in anti-PMN group compared with controls (8.6 cells/5 HPFs anti-PMN versus 20.2 cells/5 HPFs control, *$P=0.02$; 2.1 cells/5 HPFs ANAB versus 6.1 cells/5 HPFs control, **$P=0.005$, respectively). Representative images (original magnification $\times1000$) demonstrating a tissue neutrophil (B) and monocyte/macrophage (C); arrows denote immunopositivity.]
collagen (Figure 2B) and residual elastic fiber fragmentation (Figure 2C). These structures remained preserved in anti-PMN–treated mice (Figure 2, D through F).

**Tissue Leukocytes Diminished by Neutrophil Depletion**

Tissue neutrophils and macrophages 14 days after elastase perfusion were significantly decreased in the anti-PMN group as compared with controls, with 8.6 versus 20.2 cells per 5 high-powered fields (HPFs) \((P=0.02)\) and 2.1 versus 6.1 cells per 5 HPFs \((P=0.005)\), respectively (Figure 3, A through C). These cells did not appear to localize to any specific area within the aortic wall. Earlier time points (4 and 7 days) reflected these differences as well. Day 4 anti-PMN cell counts compared with the control group were 0.77 versus 12.83 cells per 5 HPFs for neutrophils and 1.67 versus 2.6 cells per 5 HPFs for macrophages, respectively. Day 7 counts were 2.43 versus 2.58 cells per 5 HPFs for neutrophils and 6.14 versus 13.3 cells per 5 HPFs for macrophages, respectively.

**Aneurysm Suppression Is Associated With Decreased MMP-8 but Not MMP-2 or MMP-9**

There were no statistical differences in MMP-9 or MMP-2 mRNA expression (Figure 4A), protein levels (Figure 4, B and C), enzyme activity (Figure 4, D and E), or immunostaining patterns between the control and anti-PMN groups (Figure 5, A through F) at 14 days. Staining patterns for MMP-2 and MMP-9 were similar for both groups, with positively stained cells residing primarily within the adventitia. Evaluation of earlier time points similarly revealed no difference in mRNA expression or protein quantities at the 2-day time point or enzyme activity at the 4- and 7-day time points. Tissue inhibitor of metalloproteinase-1 expression was also no different (data not shown).

Neutrophil collagenase (MMP-8) expression was not detected in the neutropenic mice at 14 days (Figure 6A), and Western blotting revealed diminished MMP-8 protein levels in these mice \((P=0.017)\;\text{Figure 6B}\). Neutrophils staining...
positive for MMP-8 were evident almost exclusively in the control mice (Figure 7, A through C).

**MMP-8 Deficiency Does Not Inhibit AAA Formation**

In additional experiments, undertaken to determine whether MMP-8 is required for AAA formation, the mean ADs 14 days after elastase perfusion in C57BL/6 wild-type and MMP-8–deficient mice were 133.8±7.9% and 154.3±9.9%, respectively (Table 1). The difference did not reach statistical significance ($P=0.603$).

**Chemokine Levels Unaffected by Neutrophil Depletion**

Because differences in AAA formation could not be explained by differences in MMP-9, -2, or -8, yet dramatic differences in tissue neutrophils and macrophages were seen between groups at all time points, 2 cysteine-cysteine (C-C) chemokines shown to be important mediators of aneurysm-associated leukocyte recruitment were analyzed by ELISA. At both the 4- and 7-day time points, no differences in MIP-1α or MCP-1 were detected.

**Discussion**

This study establishes that neutropenia at the time of the inciting event for AAA formation in an elastase perfusion experimental model limits AAA size and incidence. AAA development in this setting was independent of detectable alterations in MMP-2 or MMP-9 but did correlate with increased tissue inflammatory cell infiltrate, increased wall thickening, destruction of elastic lamellae, and the presence of MMP-8. When MMP-8–deficient mice were compared with controls with functional neutrophil collagenase using the same elastase perfusion model, however, there was no protection conferred against aneurysm formation in this group.

The broader context for these experiments was to better define the sequence of events leading to AAA formation and, in particular, the role of the neutrophil in this process. The initial events of human AAA formation are not well understood, but they appear to be multifactorial, with a poorly defined hereditary component and biomechanical wall stresses both contributing to a host of cellular and molecular events. There appears to be a consistent inflammatory component within the aneurysm wall, fragmentation and loss of elastin fibers, medial smooth muscle cell depletion, and a
degradation and turnover of the extracellular matrix. The rodent elastase-perfusion model of AAA formation exhibits all of these cellular and extracellular matrix changes.

Inflammatory cell types occurring in AAAs of humans and rodents 7 to 14 days after elastase perfusion are similar, with macrophages and lymphocytes predominating. Elastin fiber fragmentation is thought to be an important component of inflammatory cell recruitment. The latter is supported by the present study, with less overall leukocyte influx in the neutrophil-depleted animals having preserved elastic lamellae. Elastin fibers not only provide structural integrity to the aortic wall, but also become potent chemoattractants when degraded for monocytes, fibroblasts, and, in some settings, PMNs.

PMNs have also consistently been demonstrated within the wall of human AAAs, although to a lesser degree than monocytes and macrophages. Their role in the initial stages of human AAA development is undefined. In the rodent model, however, PMNs are an important part of the early inflammatory response, becoming prominent <1 week after aneurysm induction. Once inflammation is initiated, an ongoing breakdown and turnover of extracellular matrix is thought necessary for AAA enlargement.

Figure 6. MMP-8 diminished after cytotoxic anti-PMN-antibody treatment. Aortic tissues 14 d after elastase perfusion were analyzed and total neutrophil collagenase mRNA expression was determined by quantitative real-time PCR. Expression seen only in 14-d control mice (A), whereas Western blotting revealed diminished MMP-8 protein level in anti-PMN group as well. *P=0.017 vs rabbit serum control (B).

Figure 7. Decreased immunostaining for MMP-8 in neutrophil-depleted mice 14 d after elastase perfusion. Staining for MMP-8 in control aortas (A, B) and anti-PMN aortas (C) revealed immunopositive cells (arrows) almost exclusively in the control group with aneurysms. Both luminal and tissue neutrophils staining positive for MMP-8 shown in representative sections. Original magnification ×1000.
The C-C chemokines have been shown to be important intermediaries in the aneurysm inflammatory process. MCP-1 is essential for monocyte recruitment to occur in vivo with several different inflammatory models. It is upregulated in both mouse and human aortas that have become aneurysmal. MCP-1α is upregulated in macrophages in response to leukotriene LTD4 in hyperlipidemic Apoe−/− and Ldlr−/− mice that form AAAs. It was postulated that the marked attenuation of aortic wall inflammation noted in the anti-PMN-antibody–treated mice would be accompanied by a decrease in one or both of these chemokines. Both control and experimental groups had equivalent levels of MCP-1 and MCP-1α, however, which suggests that other mediators are responsible for the attenuated inflammation that follows neutrophil depletion.

The gelatinases MMP-2 (gelatinase A) and MMP-9 (gelatinase B), are the 2 best-studied MMPs implicated as having pivotal roles in AAA formation. In one study of 21 small human AAAs and 45 larger AAAs, MMP-2 was found to be the principal gelatinase upregulated in small AAAs, whereas increased MMP-9 activity was found in larger AAAs. Mice genetically deficient for MMP-9 and MMP-2, when used in topical calcium chloride and elastase perfusion models of AAA formation, have been highly resistant to aortic enlargement.

In the present study, the reduction in circulating neutrophils at the time of elastase perfusion and the resultant decrease in tissue neutrophilic and monocytic infiltrates 4 to 14 days later suggests that the gelatinases—in particular MMP-9, which is stored in neutrophil-specific granules and actively secreted by macrophages—should be diminished in the neutrophil-depleted group. This assumption was not supported by these data because no differences in MMP-9 or MMP-2 mRNA expression, protein quantity, or enzyme activity were seen between the control and experimental groups at any time point.

One reason for equipoise in MMP-9 between groups could be a relative lack of tissue-specific expression of MMP-9. Although some have implicated the neutrophil and macrophage as the primary cell types storing and producing MMP-9, other studies have demonstrated that vascular smooth muscle cells can be induced, in the milieu of proinflammatory cytokines, to have significant upregulation of MMP-9 gene expression. These cells could be an important source of MMP-9 in biochemical tests run on homogenized tissue samples. Nevertheless, in the present study, immunohistochemical staining for MMP-9 was not seen within the medial smooth muscle cells of either the control or anti-PMN-antibody–treated aortas. Alternatively, the decrease in tissue inflammation seen 4 to 14 days after aortic elastase perfusion in the neutrophil-depleted group may simply have been insufficient to result in significant detectable differences in the gelatinases.

Effect of MMP-8 Deficiency on Aortic Dilation

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C57BL/6 wild-type and MMP-8−/− deficient mice (MMP-8 KO) were subjected to transient aortic perfusion with elastase to induce aneurysmal degeneration. AD measurements were obtained before (Preoperative) and immediately after (Postoperative) elastase perfusion, with final measurements obtained 14 d later (Final). Data shown represent mean ± SEM, with the overall extent of AD calculated as a percent change in parentheses. A 2-tailed nonparametric (unpaired t test) was used for statistical testing.

In contrast, MMP-8 is a highly tissue-specific MMP, limited mainly to neutrophils and chondrocytes. In neutrophils, MMP-8 is stored in secondary granules found only in the later stages of neutrophil differentiation. In the present study, neutrophil-depleted mice had undetectable MMP-8 expression and diminished protein levels. MMP-8 is among the most potent enzymes involved in the degradation of type I and type III collagen, both important contributors to the tensile strength of the abdominal aorta. Because collagen is the primary substrate of MMP-8, and qualitative assessment of collagen content by histology appears diminished rather than increased in the aorta from anti-PMN–treated mice, these observations suggest that MMP-8 is not a primary mediator of the effects of anti-PMN antibody. The possibility that elimination of MMP-8 may have afforded some protection against aneurysm formation in the neutrophil-depleted group appears even more unlikely given that isolated gene deletion of MMP-8 did not have any effect on AAA formation. Similarly, experiments using the murine elastase-perfusion model have shown that mice deficient in a related neutrophil-specific enzyme, neutrophil elastase, also had no protection from the aneurysm phenotype as compared with control animals (R.W.T., unpublished data, 2005). In the present study, MMP-8 probably served as a marker of inflammation, reflecting the loss of mature neutrophils within aortic tissues.

The current understanding of this experimental model is that aortic perfusion with elastase produces an enzymatic injury that results in leukocyte recruitment, further inflammatory degradation of the aortic wall, and finally, aneurysmal dilation. This study demonstrated that anti-PMN antibody treatment results in decreased tissue macrophages in addition to PMNs. This occurred despite any detectable decreases in MCP-1α or MCP-1 in the neutrophil-depleted animals. It is likely that other chemokines not assayed in the present study are involved and that macrophage recruitment and activation are still an important part of the difference in AAA phenotype seen between groups. Nevertheless, the macrophage contribution in these experiments does not appear to be through the more commonly understood MMP-mediated mechanisms.

This study is the first to document the relative importance of the neutrophil in the early stages of experimental AAA development. Although previous work convincingly suggests important roles for MMP-9 and MMP-2 in AAA formation, the present study shows that alterations in one or both of these enzymes are not essential for neutrophil-depletion–mediated AAA suppression. Future investigation of other neutrophil-associated mediators such as reactive oxygen intermediates, the cathepsins, or other serine proteases may improve the current understanding of aneurysm initiation and the specific functions of the neutrophil in that process.
Acknowledgments

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References


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