Complement-Dependent Neutrophil Recruitment Is Critical for the Development of Elastase-Induced Abdominal Aortic Aneurysm

Monica B. Pagano, MD*; Hui-fang Zhou, PhD*; Terri L. Ennis, BS*; Xiaobo Wu, MD; John D. Lambris, PhD; John P. Atkinson, MD; Robert W. Thompson, MD; Dennis E. Hourcade, PhD; Christine T.N. Pham, MD

Background—We previously established that neutrophils play a critical role in the development of experimental abdominal aortic aneurysm (AAA). The signal that initiates the influx of neutrophils to the aortic wall, however, remains unknown. In this study, we tested the hypothesis that complement participates in the development of AAA by providing the necessary chemotactic signal that recruits neutrophils to the aortic wall.

Methods and Results—Using an elastase-induced model of AAA, we showed that pretreatment of C57BL/6 mice with cobra venom factor, which depleted serum of complement activity, protected mice from AAA development. Whereas control mice exhibited a mean aortic diameter of 156±2% on day 14 after elastase perfusion, mice treated with cobra venom factor exhibited a mean aortic diameter of 90±4% (P<0.001). Examination of mice deficient in factor B further indicated that the alternative pathway of complement played a major role in this process (mean aortic diameter of 105±4% in factor B–deficient mice, P<0.001 compared with controls). Activation of the alternative pathway led to generation of the anaphylatoxins C3a and C5a, which recruited neutrophils to the aortic wall. Moreover, antagonism of both C3a and C5a activity was required to block AAA, which suggests that each can independently promote the aneurysmal phenotype. In addition, we demonstrated that complement alternative-pathway involvement was not restricted to this experimental model but was also evident in human AAAs.

Conclusions—The identification of involvement of the complement system in the pathophysiology of AAA provides a new target for therapeutic intervention in this common disease. (Circulation. 2009;119:1805-1813.)

Key Words: aneurysm ■ immune system ■ inflammation ■ leukocytes ■ complement

Clinical Perspective p 1813

The complement system plays a central role in innate immunity and is an effector arm of humoral immunity. The role of complement in host defense, including clearance of immune complexes, opsonization, and lytic activity, is well recognized.8,9 It is now understood that complement activity extends beyond host defense, because complement participates in autoimmunity, debris removal, and response to tissue injury.10 Complement activation generates the anaphylatoxins C3a and C5a, which are potent leukocyte chemoattractants.11 In the present study, we tested the hypothesis that complement participates in the development of elastase-induced...
AAA by providing the necessary chemotactic signal that recruits neutrophils to the aortic wall.

**Methods**

**Animals**
Wild-type (WT; C57BL/6 and C57BL/10Sn) and C5^−/− (B10.D2-Hc^0 H2^d H2-T18c) mice were obtained from The Jackson Laboratory (Bar Harbor, Me). C5^−/− and factor B−/− (BB^−/−) mice were backcrossed to C57BL/6 for 11 and 9 generations, respectively. C57BL/6 C4^−/− mice were obtained from Dr Michael Carroll (Harvard Medical School, Boston, Mass). Mice were kept in a pathogen-free condition at the Washington University Specialized Research Facility, and all experiments were performed according to protocols approved by the Division of Comparative Medicine.

**Elastase-Induced Model of AAA**
AAA was induced in 8- to 12-week-old mice as described previously. Briefly, mice were anesthetized with sodium pentobarbital 55 to 60 mg/kg IP. A laparotomy was performed under sterile conditions. With the assistance of an operating stereomicroscope, the abdominal aorta was isolated, and the preperfused aortic diameter (AD) was measured with a calibrated ocular grid. Temporary 7-0 silk ligatures were placed around the proximal and distal aorta. The proximal ligature was closed to interrupt proximal flow. An aortotomy was created at the inferior ligature with the tip of a 30-gauge needle, and the perfused segment of the abdominal aorta was reexposed and measured in situ before euthanasia and tissue procurement.

**Complement Depletion**
To achieve maximum reduction of serum complement, mice were injected intravenously with 9 U of cobra venom factor (CVF; Quidel) and factor B−/− (fB^−/−) mice. Mice were injected intravenously with 200 µL of WT or BB^−/− pooled sera immediately after surgery and 24 hours after surgery.

**Western Blot**
Serum samples (1:100 dilution) were fractionated by SDS-PAGE under reducing and nonreducing conditions and blotted with goat anti-mouse C3 (1:10 000 dilution; Valeant Pharmaceuticals International, Aliso Viejo, Calif) or anti-mouse C5a (500 ng/mL) monoclonal antibody (BD Pharmingen). After incubation with streptavidin–horseradish peroxidase (400 ng/mL; Sigma), 100 µL of 1-Step Turbo TMB-ELISA (Pierce) was added to each well, and color development was read at 450 nm with a SpectraMax Plus reader (Molecular Devices, Sunnyvale, Calif). Mouse recombinant C3a and C5a (BD Pharmingen) were used to establish the standard curve.

**Immunohistochemistry**
Mouse abdominal aorta was dissected, snap-frozen in OCT compound, and sectioned at 5 µm. Elastin was stained with Verhoeff by use of an Accustain Elastic Stain kit (Sigma). Elastin degradation was graded on a scale of 1 to 4, where 1 = less than 25% degradation, 2 = 25% to 50% degradation, 3 = 50% to 75% degradation, and 4 = greater than 75% degradation. Smooth muscle cell content was evaluated with an alkaline phosphatase–conjugated antibody to α-actin (1:200 dilution; Sigma). Color was visualized with an alkaline phosphatase substrate kit (Vector Laboratories, Burlingame, Calif). Smooth muscle cell content was graded on a scale of 1 to 4, where 1 = less than 25% loss, 2 = 25% to 50% loss, 3 = 50% to 75% loss, and 4 = greater than 75% loss. Macrophages and neutrophils were visualized with biotinylated anti-Mac-3 (1:200 dilution; Cedarlane Laboratories, Burlington, NC) and anti-Gr-1 monoclonal antibody (1:100 dilution; BD Biosciences), respectively. After incubation in 9% BSA and streptavidin–horseradish peroxidase–conjugated antibody to Gr-1, color development was revealed with the Histomark orange peroxidase substrate kit (Kirkegaard & Perry Laboratories, Gaithersburg, Md). All sections were counterstained with 1% methyl green.

**Immunofluorescence**
Human abdominal aorta specimens were obtained at the time of elective surgery through a protocol approved by the Washington University School of Medicine Institutional Review Board. Cross sections of aortic tissues (5 µm) were fixed in methanol, blocked in 3% dry milk in PBS, and incubated with a goat polyclonal antibody to human C5 (1:1600 dilution; CompTech Complement Technology, Tyler, Tex), human C5a (1:800 dilution; CompTech), human fB (1:800 dilution; CompTech), human properdin (1:800 dilution; CompTech), or monoclonal antibody to C5-9 neointagion (1:100 dilution; Quidel) for 1 hour at room temperature, washed, then incubated with a biotinylated anti-goat antibody (Vector Laboratories) for 1 hour at room temperature, followed by incubation with streptavidin–phycocerythrin (BD Biosciences) or rhodamine-conjugated goat anti-mouse antibody (Jackson ImmunoResearch). Normal goat serum at the same dilutions as above was used for control and showed no specific fluorescence on normal or AAA tissues.

**Statistical Analysis**
Comparisons between groups were made by 1-way ANOVA followed by Bonferroni post hoc test to compare all groups of data. Data are presented as the mean±SEM. P values <0.05 were considered significant.
The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results
Elastase-Induced AAA Is Complement Dependent
Although no single animal model reproduces all aspects of human AAA, the elastase-induced experimental AAA model recapitulates many features of this disease. Transient perfusion of the abdominal aorta with a porcine elastase solution reproducibly leads to AAA in 100% of C57BL/6 WT animals. Mild aortic dilatation was observed immediately after the elastase perfusion. The AD remained relatively stable until day 3, after which a rapid secondary increase in AD occurred up to a maximum difference in AD of 0.82 ± 0.01 mm on day 14 (Figure 1A). In this model, AAA is defined on day 14 as an overall increase in the AD of at least 100% over the preperfused parameters. WT animals exhibited a mean increase in AD of 156 ± 2% on day 14 after elastase perfusion (Figure 1B).

CVF, a functional analogue of C3b, forms a stable convertase that within hours depletes serum of its key complement activation component, C3, an effect that lasts up to 5 days after treatment (Figure 1C). Pretreatment with CVF 24 hours before elastase perfusion consistently depleted serum of complement activity (Figure 1D) and prevented AAA development in all animals (increase in AD of 0.47 ± 0.02 mm or 90 ± 4%, P < 0.001; Figure 1E and 1F). Mice that received CVF 24 hours after elastase perfusion developed smaller aneurysms (increase in AD of 0.63 ± 0.05 mm or 120 ± 10%, P < 0.001). However, complement depletion on day 3 (or later) after elastase perfusion had no effect on the extent of aortic dilatation (Figure 1D and 1E). These results confirm that complement activation plays a pivotal role in elastase-induced AAA development.

Histological analysis of day 14 aortas from animals treated with CVF before elastase perfusion showed well-preserved elastic fibers (Figure 2A) and significantly less smooth muscle cell depletion (Figure 2B) than aortas of untreated animals. Immunohistochemistry of day 14 CVF-treated aortas showed significantly reduced numbers of macrophages (9.8 ± 1.4 cells per cross section of CVF-treated aortas versus 84.9 ± 16.7 cells per cross section of untreated aortas, P < 0.001; Figure 2C) and mast cells (6.7 ± 1.2 cells per cross section of CVF-treated aortas versus 12.6 ± 1.9 cells per cross section of untreated aortas, P < 0.001; Figure 2D). Previous studies suggested that mast cell activation modulates AAA development in mice. Consistent with these results, we found that a majority of mast cells from aortas of untreated mice had undergone degranulation, whereas only half of the
mast cells in CVF-treated aortas showed signs of degranulation \( (P<0.001; \text{Figure 2D}) \). Taken together, these results indicate a significant reduction in aortic wall inflammation after CVF treatment, which helps preserve the elastin and smooth muscle cell content.

**The Alternative Pathway Is Critical for AAA Development**

We next determined which complement pathways are responsible for the elastase-induced AAA phenotype. Three initiator pathways of complement activation can be identified: the classic, the alternative, and the lectin pathways.8,9 The importance of the standard classic and lectin pathways was assessed in mice lacking C4.13 C4/+/+/ mice developed AAA normally (increase in AD of 163\%±5\%), which indicates that the standard classic and lectin pathways are likely dispensable in this model (Figure 3A). To assess the importance of the alternative pathway, we induced AAA in mice lacking fB.14 We found that the fB/−/− mice were largely resistant to the development of AAA (increase in AD of 105\%±4\%; Figure 3A). In addition, histological analysis revealed a minimal degree of elastin degradation in day 14 fB/−/− aortas, which implies that the development of AAA requires activation of the alternative pathway.

**Alternative Pathway Activation Leads to Neutrophil Recruitment in Elastase-Induced AAA**

We next sought to determine the mechanism by which the alternative pathway contributes to AAA development. Previous studies have shown that neutrophils and mast cells modulate the development of experimental AAA.4,5,19 However, mast cells did not accumulate in significant numbers in the aortic wall until day 7, whereas neutrophil presence peaked on day 3 after elastase perfusion.5,19 Given the fact that CVF treatment 3 days after perfusion did not offer protection against AAA development (Figure 1E), we reasoned that the protective effect against AAA development seen in CVF-treated and fB/−/− mice was more likely due to an impaired influx of neutrophils to the aortic wall. To test this hypothesis, we analyzed disease induction in fB/−/− mice.

**Figure 2.** Complement depletion protects against elastin degradation and smooth muscle-cell depletion by limiting the inflammatory response. Elastin (A) was detected with Verhoeff-van Gieson staining, and smooth muscle cells (B) were detected with an antibody to α-actin. Scale bar=0.02 mm. Serial sections from untreated and CVF-treated (24 hours before elastase perfusion) aortas were stained for Mac-3 and with methylene blue to show the presence of macrophages (C) and mast cells (D), respectively. Insets from upper panels in C are shown at higher magnification in lower panels. Scale bar=0.1 mm (upper panels) and 0.02 mm (lower panels). Degranulated mast cells show abundant extracellular granules (arrows in D). Percent of degranulated mast cells was calculated by the formula: degranulated cells/compact cells+degranulated cells)×100. Scale bar=0.01 mm. Histological grading in A, B, C, and D was performed on 6 serial cross sections per aorta, 3 aortas per genotype/treatment type, by 2 independent blinded observers. D14 indicates day 14; SMC, smooth muscle cell.
neutrophil recruitment in the aortic wall (21,820 ± 7392 neutrophils per WT aorta versus 23,410 ± 8065 neutrophils per C4−/− aorta versus 12,233 ± 336 neutrophils per fB−/− aorta; Figure 3C).

On the basis of these findings, we hypothesized that alternative-pathway complement activation led to the production of mediators that sustained the recruitment of neutrophils to the aortic wall. Therefore, restoration of fB should lead to alternative-pathway complement activation and neutrophil recruitment. To this end, animals were treated with an exogenous source of fB after elastase perfusion. Reconstitution of fB−/− mice with WT serum as a source of fB restored the influx of neutrophils to the aorta (Figure 3D) and rendered the fB−/− mice susceptible to AAA development, whereas fB−/− mice reconstituted with fB−/− serum did not develop AAA (Figure 3E). Taken together, these results suggest that activation of the alternative pathway directs the recruitment of neutrophils to the aortic wall. These neutrophils in turn propagate the inflammatory response, which leads to the aneurysmal dilatation associated with AAA.

Both C3a and C5a Contribute to AAA Development

The 3 pathways of complement activation converge at the cleavage of C3, generating C3a and C3b. C3a is an anaphylatoxin with bactericidal, cell-activating, and leukocyte chemotaxant properties, whereas C3b promotes assembly of the membrane attack complex and initiates the alternative-pathway amplification loop. Given the central role of C3, we examined AAA development in C3−/− mice (Figure 4A). Unexpectedly, AAA was not abolished (increase in AD of 133 ± 9% in C3−/− animals versus increase in AD of 105 ± 4% in fB−/− animals, P < 0.05). These results together with those derived from CVF-treated and fB−/− mice suggest the possibility of a C3-independent pathway of C5a generation. Huber-Lang et al have shown that thrombin is overexpressed in C3−/− mice and can substitute for C3 convertase in the generation of C5a in injured lung tissue. Thus, to determine whether C5a was generated in C3−/− mice, the animals were perfused with elastase, and their sera were collected and tested for the presence of C5a. Consistent with this hypothesis, C5a was detected in C3−/− mice (Figure 4B), and the mean C5a levels in C3−/− mice were comparable to the levels detected in WT animals (348 ± 189 ng/mL in WT mice versus 210 ± 92 ng/mL in C3−/− mice). In contrast, C5a levels were low in fB−/− mice 24 hours after elastase perfusion (18 ± 7 ng/mL), which suggests that porcine pan-

Figure 3. The alternative pathway is essential for AAA development. A, AD increase in WT, C4−/−, and fB−/− mice on day 14. Verhoeff-van Gieson staining showed intact elastic fibers in fB−/− mice. Scale bar = 0.02 mm. B, Neutrophils in aortic wall were stained with Gr-1 (brown) and identified by their segmented nuclei (arrows). Insets from upper panels are shown at higher magnification in lower panels. Scale bar = 0.025 mm (upper panels) and 0.01 mm (lower panels). C, The absolute number of neutrophils was calculated by multiplying the total number of cells per aorta by the percentage of CD45+ /Gr-1+ cells. D, Twenty-four hours after the last WT or fB−/− serum injection, aortas were harvested, and the number of neutrophils per aorta was calculated. Another group of mice were euthanized on day 14, and the AD was assessed (E). Reconstitution with WT sera led to AAA development and fragmentation of elastic fibers in fB−/− mice. Scale bar = 0.02 mm. The number of animals per genotype is indicated for each group.
Creactive elastase (used during perfusion) did not cleave C5. Moreover, these results indicate that no C3-independent C5a generation occurred in FB/H11002/H11002 mice. Next, we determined whether C5a activity was required for aneurysm formation by examining the susceptibility of C5/H11002/H11002 mice to AAA development. We found that C5/H11002/H11002 mice developed AAA to a similar extent as WT mice (increase in AD of 136±13% in C5/H11002/H11002 mice versus 150±4% in WT mice, P<0.05; Figure 4A), which suggests that in the absence of C5a, C3a may be sufficient to recruit neutrophils and confer susceptibility to the aneurysmal phenotype. In fact, we confirmed that C3a was produced at normal levels in C5/H11002/H11002 mice (Figure 4C), and the production of either C3a or C5a was sufficient to recruit neutrophils to the aortic wall (Figure 4D).

Blocking Both C3a and C5a Activity Protects Against AAA Development

If either C3a or C5a were sufficient to sustain AAA, then blocking the activity of both would be expected to suppress AAA. To block both C3a and C5a activities, we administered a C5aRA to C3/H11002/H11002 mice 1 day before and on days 1 and 3 after elastase perfusion. C5aRA treatment led to complete protection against AAA development in C3/H11002/H11002 mice, whereas treatment with C5aRC did not (increase in AD of 78±8% in C5aRA-treated animals versus 151±3% in C5aRC-treated animals, P<0.01; Figure 4E). However, blockade of C5a:C5aR interaction with C5aRA did not suppress AAA in WT mice (Figure 4F). These results and the data obtained from C5-deficient mice (Figure 4A) confirm that C3 and C5 activation products independently promote aneurysm formation.

The Alternative Pathway of Complement Is Activated in Human AAA

Previous studies demonstrated the presence of complement-fixing immunoglobulin subclasses and C3 deposition in the aortic wall of AAAs, which prompted the investigators to suggest that activation of the classic pathway may contribute to the pathogenesis of human AAA.22,23 Although the present results do not preclude classic pathway activity, our findings suggest alternative-pathway involvement in human AAA as well. Immunostaining of AAA aortic wall tissues for C3, C4, fB, properdin (a component of the alternative-pathway convertase),24 and C5-9 neoantigen (a complement lytic pathway marker) confirmed the presence of these complement proteins throughout all layers of the aortic wall (Figure 5). C3, C4, and fB showed a similar pattern of fluorescence mainly along the
medial and adventitial layers, whereas properdin and C5-9 neoantigen were also seen prominently along the luminal side. Thus, these samples obtained from patients undergoing elective surgical repair point to activation of both the classic and alternative pathways in human AAAs.

Discussion

In the present study, we identified complement as a critical mediator of neutrophil recruitment and subsequent development of elastase-induced AAA. We made several new findings: (1) AAA development in the mouse model was dependent on activation of the complement cascade; (2) the alternative pathway played a major role in the process, whereas the standard classic and lectin pathways together were not required; and (3) either C3a or C5a could independently promote the aneurysmal phenotype (Figure 6; online-only Data Supplement Table). In addition, we showed that complement alternative-pathway involvement was not restricted to our mouse model but is also seen in established human AAAs.

In view of the results with CVF, and given the central role of C3 and C3 activation products in inducing the downstream activation of C5, we expected that a deficiency in C3 would be sufficient to halt the development of AAA. The results we obtained with C3−/− mice were not as anticipated and prompted us to consider alternative means of C5a production. That C5 might be cleaved in C3−/− mice has been described recently. In a study by Huber-Lang et al, the authors showed that although thrombin had limited function in the presence of C3, it became the major C5 convertase in C3−/− mice. In the present study, we demonstrated that a compensatory pathway also exists to generate C5a in the absence of C3. Moreover, interruption of the C5a/C5aR interaction by a receptor antagonist suppressed AAA development in C3−/− mice, which confirms that C5a was indeed active in the absence of C3. However, contrary to other complement- and neutrophil-dependent animal models in which C5a is pivotal in disease induction,21,25–27 we found that elastase-induced AAA was not suppressed in C5−/− mice, which suggests that in the absence of C5a, C3-mediated activity and activation products were sufficient to sustain the inflammatory process that led to aneurysmal dilatation.

C3a and C5a have been shown to have overlapping functions, including their ability to chemotact and activate many cell types, trigger oxidative bursts, and increase endothelial permeability.11 However, the prominent role of C5a in other disease models was attributed to its unique ability to attract neutrophils, whereas C3a was unable to do so.28 Perhaps the role of C3 in AAA development can be explained by recent studies showing that C3a was required for the production of neutrophil-specific CXC chemokines, whereas C5a did not have the same effect.29 Therefore, C3a may provide the necessary signal to recruit neutrophils indirectly, through the elaboration of CXC chemokines. Taken together, these results suggest that the functions of C3a and C5a are...
important, and antagonism of both factors is required to halt progression of AAA.

How does one explain the critical involvement of complement in this model of AAA? The likely scenario is that chemical injury elicited by elastase perfusion provides a protected site for complement activation. In the presence of complement activation, C3 and C5 convertases are generated, which leads to the release of anaphylatoxins that recruit inflammatory cells, specifically neutrophils. Neutrophils may amplify complement activation through the release of properdin, which stabilizes the alternative-pathway convertase. Properdin may also bind to target cell surfaces directly, thus providing a platform for the assembly of the alternative-pathway convertase. The strong staining for properdin observed on the luminal surface of the AAA specimens suggests that properdin may indeed serve as a focal point for initiation of the alternative-pathway complement activation. The direct role of properdin in this model of AAA is currently under investigation. The alternative pathway has also been shown to proceed directly via the lectin pathway, bypassing the standard C4 requirement. The significance of this pathway in vivo and to this AAA model awaits further investigation. Regardless of the initiation mechanism, the alternative pathway plays a critical role in recruiting the neutrophils that are needed to sustain the inflammation in elastase-induced AAA.

Finally, literature on the role of complement in human AAAs is scant. To date, only 2 reports have described deposition of C3 and antibodies in human AAA tissues. Presently, evidence for direct complement participation in AAA development is still lacking. The elastase-induced model of experimental AAA allowed us to definitively establish that the alternative pathway of complement directly controls aneurysmal development in mice. However, C5 cleavage in the mouse has been shown to proceed mainly via the alternative pathway. Thus, the relative importance of the alternative pathway versus the classic pathway in vivo and to this AAA model awaits further investigation.

In summary, the wall of a large blood vessel such as the aorta represents an example of a tissue site where chronic inflammation is certainly undesirable. How innate immune responses are generated, maintained, and modulated at this particular tissue site in humans is yet to be discerned. This report is an attempt to analyze the complement system in the development of AAA. The results suggest that inhibition of the chronic inflammatory response in AAA through complement-targeting strategies merits further exploration.

Sources of Funding

This work was supported by grants from the National Institutes of Health (AI086730 to Dr Lambris, AI041592 to Dr Atkinson, AI051436 to Dr Hourcade, AI049261 to Dr Pham, and HL056701 and P50HL083762 to Dr Thompson).

Disclosures

Dr Lambris is a consultant to Acusphere Inc on biomaterial-induced complement activation and has several issued and pending patents on complement inhibitors. The remaining authors report no conflicts.

References


Abdominal aortic aneurysm (AAA) is a disease characterized by chronic inflammation and remodeling of aortic wall tissue. Studies using “end-stage” human AAA tissues procured at surgery have identified a number of candidate molecules; however, these may or may not contribute to the initiation and progression of AAA. To better understand the mechanisms that promote AAA, we turned to an elastase-induced mouse model that recapitulates many features of human AAA. In this model, neutrophils are identified as critical mediators of AAA development. Neutrophil depletion or impaired neutrophil recruitment protects against AAA development; however, the signal that initiates the influx of neutrophils to the aortic wall remains undefined. We hypothesized that complement participates in the development of elastase-induced AAA, possibly by providing the chemotactic signal that recruits neutrophils to the aortic wall. In the present experiments, we showed that complement depletion abrogated AAA development. We also demonstrated that the alternative pathway of the complement system plays a major role in this process by generating the potent anaphylatoxins C3a and C5a, which recruit neutrophils to the aortic wall. Ruptured AAA is the cause of death in 1% to 3% of men over the age of 65 years. Although elective surgical repair is usually definitive, this operation is reserved for large aneurysms. At present, no therapies are available that alter the progressive growth of small aneurysms. The identification of the involvement of the complement system in the pathophysiology of AAA provides a new target for therapeutic intervention in this common disease.
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*Circulation*. 2009;119:1805-1813; originally published online March 23, 2009;
doi: 10.1161/CIRCULATIONAHA.108.832972

*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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**SUPPLEMENTAL MATERIAL**

**Supplementary Table I.** AAA phenotype in complement-deficient mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Increase in AD (mm)</th>
<th>Increase in AD (%)</th>
<th>Phenotype</th>
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<tr>
<td>WT</td>
<td>0.82 ± 0.01</td>
<td>156 ± 2</td>
<td>Susceptible</td>
</tr>
<tr>
<td>WT + CVF</td>
<td>0.47 ± 0.02</td>
<td>90 ± 4</td>
<td>Resistant</td>
</tr>
<tr>
<td>C4&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>0.81 ± 0.02</td>
<td>163 ± 5</td>
<td>Susceptible</td>
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<tr>
<td>fB&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>0.51 ± 0.02</td>
<td>105 ± 4</td>
<td>Resistant</td>
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<tr>
<td>C5&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>0.70 ± 0.06</td>
<td>136 ± 13</td>
<td>Susceptible</td>
</tr>
<tr>
<td>C3&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>0.67 ± 0.05</td>
<td>133 ± 9</td>
<td>Susceptible</td>
</tr>
<tr>
<td>C3&lt;sup&gt;-/-&lt;/sup&gt; + C5aRA</td>
<td>0.37 ± 0.04</td>
<td>78 ± 8</td>
<td>Resistant</td>
</tr>
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</table>

Mice underwent elastase perfusion in the absence or presence of CVF pre-treatment or C5aRA. Aortic diameter (AD) measurements were obtained prior to elastase perfusion and increase in AD on day 14 is presented as absolute difference (in mm) or as % increase over pre-perfused measurements.