Supporting Information

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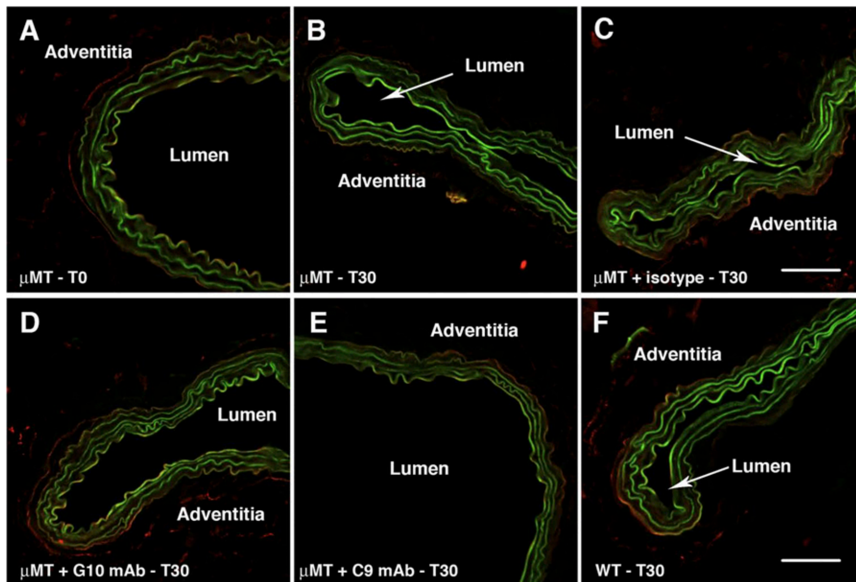


Fig. S1. Absence of C1q deposition in elastase-perfused aortas. Mice deficient in B cells called μMT (*A*–*E*) or WT (*F*) mice were perfused with elastase and administered the indicated antibody i.v. Immediately after elastase perfusion (T0) or 30 min later (T30), aortas were harvested and stained for C1q (sc-27661; Santa Cruz Biotechnology). There was minimal C1q detected above background staining (μMT at T0) in any of the tested conditions. (Scale bar, 100 μm.)

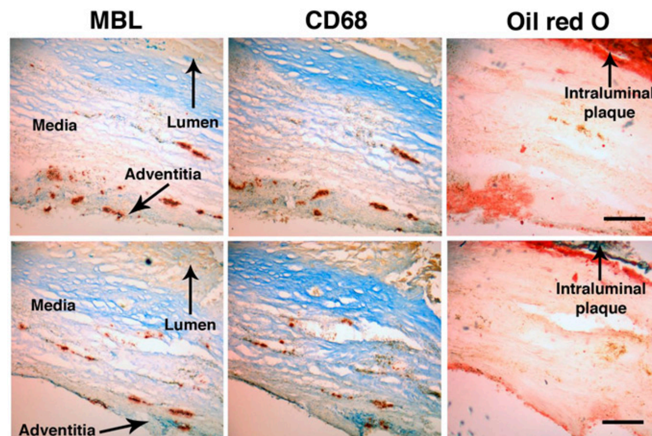
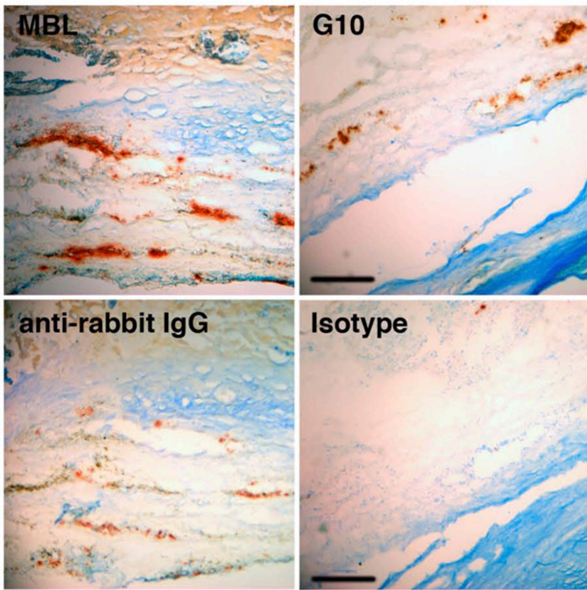
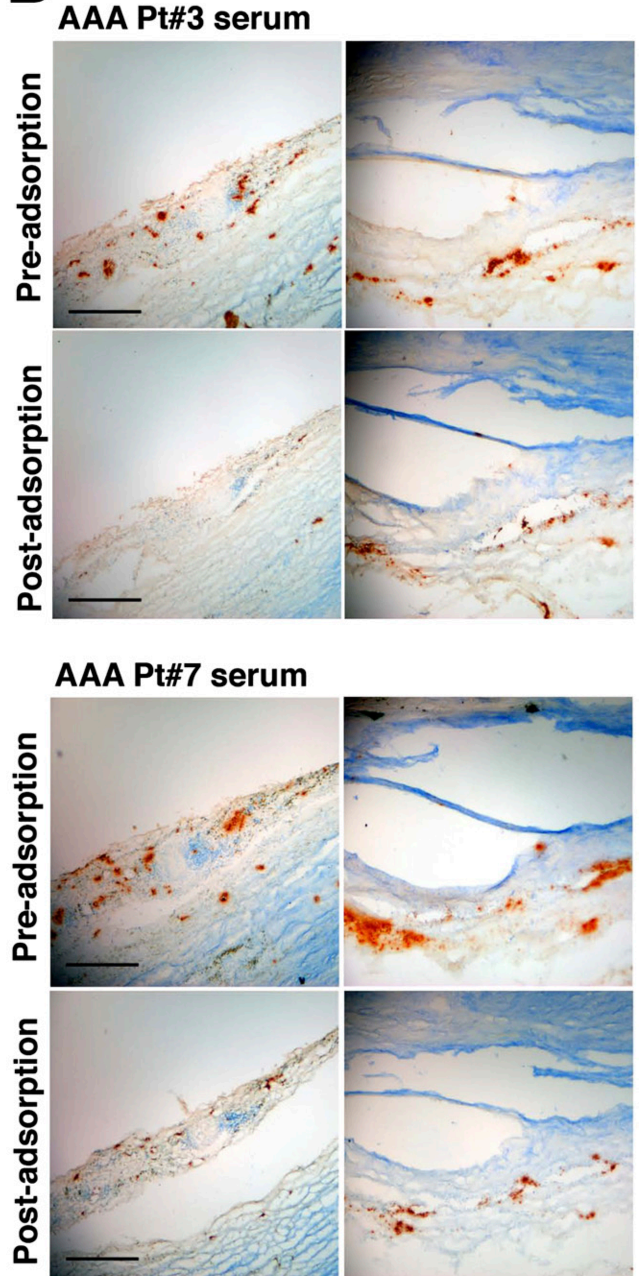


Fig. S2. Immunohistochemical localization of mannan-binding lectin (MBL), inflammatory cells, and lipid deposits in abdominal aortic aneurysm (AAA) tissues. Consecutive sections of aneurysmal tissues were stained for MBL, infiltrating leukocytes ($CD68^+$ macrophages), and lipid deposition (with Oil red O). Note the close association between MBL and macrophages that are found mainly in the adventitial layer of the aortic wall. (Scale bar, 100 μm.)





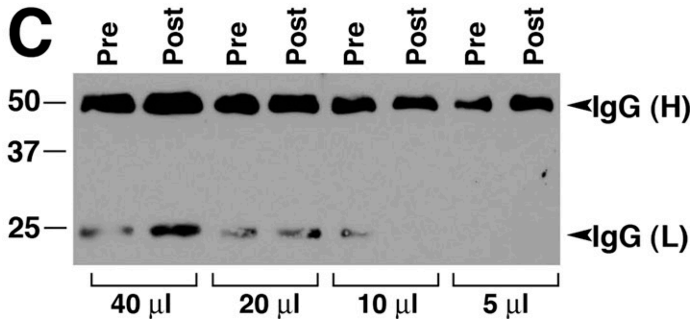


Fig. S3. (*A*) AAA tissues were stained with anti-MBL (2 μg/mL; sc-25615; Santa Cruz Biotechnology) or the anti-rabbit secondary antibody (0.16 μg/mL; 711–035-152; Jackson ImmunoResearch Laboratories) and the mAb G10 generated in-house (10 μg/mL) or IgG2b, κ-isotype (10 μg/mL; 400339; Biolegend) as a control for the G10 mAb. Most of the MBL and fibrinogen (G10) staining (*Upper*) was eliminated, suggesting that the antibodies are specific for the indicated protein. There is residual low level of reactivity detected in aneurysmal tissue stained with the anti-rabbit secondary antibody, suggesting recognition of endogenous human IgG (Fig. 8, main text). (Scale bar, 100 μm.) (*B*) Sera from AAA patients Pt 3 and Pt 7 (numbering from Fig. 8*A* in main text) were preadsorbed against human fibrinogen (5 μg/mL) before performing immunohistochemistry as described in *Materials and Methods*. Staining was performed on serial sections and was repeated on two independent sections. Preadsorption eliminated most of the staining in AAA tissues, suggesting that in these patients circulating antibodies recognize fibrinogen and/or fibrinogen-associated epitopes in aneurysmal tissues. (Scale bar, 100 μm.) (*C*) Adsorption against fibrinogen did not significantly change the serum concentration of total IgG. Indicated volumes of serum (at 1:5,000 dilution) from Pt 3 pre- and postadsorption were fractionated on SDS/PAGE gel and immunoblotted for IgG. H, IgG heavy chain; L, IgG light chain.

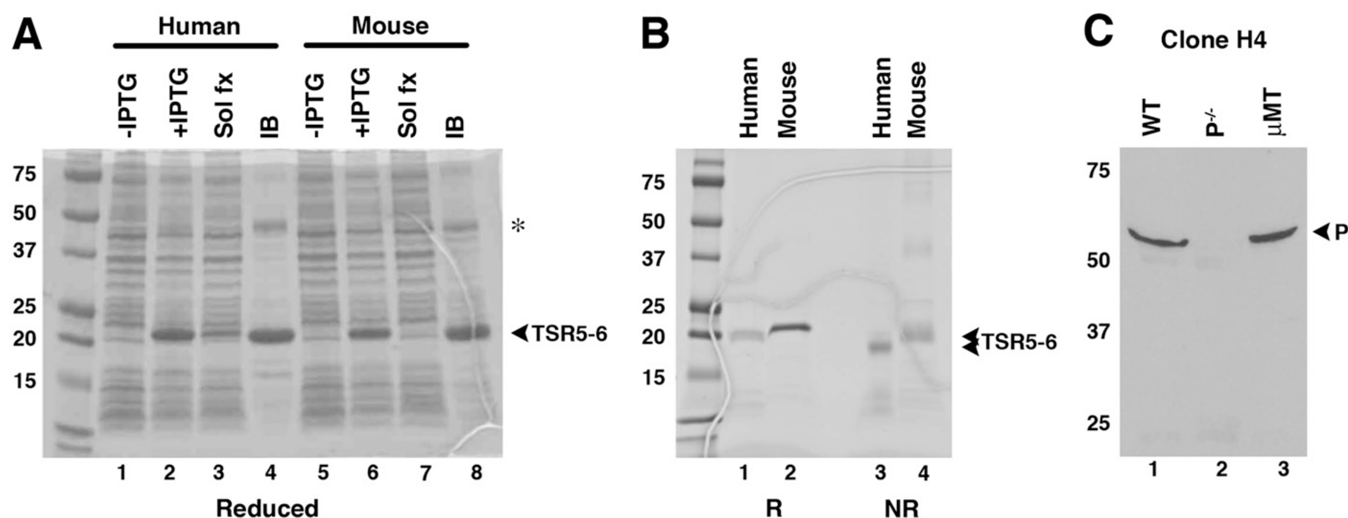


Fig. S4. Generation of anti-mouse properdin monoclonal antibody. The properdin monomer is composed of seven thrombospondin type 1 repeats (TSRs) numbered in order from the N terminus TSR0 followed by TSR1–6 (1, 2). Higgins et al. had shown that properdin constructs lacking TSRs 5 or 6 were deficient in binding to C3b and to sulfatide, a sulfated glycoconjugate (3). In another study, Perdikoulis et al. reported the expression of each of these TSRs individually in a bacterial system (4). Although the TSR5 construct did not have detectable C3b-binding activity, antibodies raised to it were capable of blocking hemolytic activity. Recombinant mouse properdin TSR5–6 cDNA was amplified by PCR from one of our existing cDNA templates (provided by Dirk Spitzer, Washington University School of Medicine, St. Louis, MO) using the following oligos: 5′-CCGGAATTCATGGTAAACGGGGAGTGGGAGG-3′ and 5′-CCCAAGCTTTTAGG-GTTTCTTCTCTTCTGGGT-3′. The resulting PCR fragment was ligated into the EcoR1 and HindIII sites of pET28a+ (EMD/Novagen). For expression of the recombinant protein, plasmids were transformed into BL21(DE3) RIL codon plus (Stratagene). Cultures were grown to log phase at 37 °C in Luria Broth (LB) containing 30 μg/mL kanamycin and 34 μg/mL chloramphenicol and induced for 4 h at 37 °C following the addition of 1 mM isopropyl beta-D-1 thio-galactopyranoside. Harvested cells were resuspended in inclusion body (IB) Prep Buffer containing 1 mM tris(2-carboxyethyl)phosphine (EMD/Novagen). Following incubation with Lysonase (EMD/Novagen) according to the manufacturer's directions, Triton X-100 was added and the suspension sonicated. The inclusion body pellet was recovered by centrifugation at 8,000 × *g* for 15 min at 10 °C and washed three times with IB Prep Buffer containing 1% Triton X-100. Inclusion bodies were dissolved in iFOLD Guanidine Denaturation Buffer (EMD/Novagen) and centrifuged at 25,000 × *g* for 15 min at 4 °C to remove insoluble materials. Denatured protein was refolded in 50 mM Mops pH 7.0, 0.5 M nondetergents sulfobetaines (EMD/Novagen), 6 mM reduced glutathione, and 4 mM oxidized glutathione at 4 °C overnight followed by concentration and dialysis against 50 mM Mops pH 7.0. The mouse (and human) TSR5–6 proteins are shown in *A* and *B*. Mouse recombinant TSR5–6 protein was used to immunize Armenian hamsters. Spleens from immunized animals were fused with Sp20 myeloma cells by standard protocol established by the Hybridoma Center at Washington University. Clones were selected by an ELISA-based assay: supernatants were added to plate-bound mouse TSR5–6 and incubated for 1 h at 37 °C and washed with 1× tris-buffered saline (TBS), 0.05% Tween-20. Secondary antibody HRP-conjugated goat anti-hamster IgG (Jackson ImmunoResearch; 127-035-099) was added for 1 h at 1:10,000 dilution in 1% BSA, 0.05% Tween-20, 0.25% Nonidet P-40, and 1× TBS, washed and developed using TMB substrate (Pierce). Several reactive clones were selected for further subcloning and characterization. To obtain purified mAbs, the hybridoma cell lines were grown until 95% cell death to produce hybridoma exhausted supernatants. These exhausted supernatants were purified on a protein-G column. Clone H4 (*C*) was selected for the studies herein.

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2. Sun Z, Reid KB, Perkins SJ (2004) The dimeric and trimeric solution structures of the multidomain complement protein properdin by X-ray scattering, analytical ultracentrifugation and constrained modelling. *J Mol Biol* 343(5):1327–1343.
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4. Perdikoulis MV, Kishore U, Reid KB (2001) Expression and characterisation of the thrombospondin type I repeats of human properdin. *Biochim Biophys Acta* 1548(2):265–277.