Supplementary Materials and Methods

Blood from healthy donors was collected into acid-citrate dextrose-A (1:7 volume) with written consent following an Institutional Review Board-approved protocol (Duke IRB#: Pro00012901). Whole blood or plasma was used fresh on the day of experiments. Unless specified, reagents were purchased from Sigma (St. Louis, Missouri, United States) and all experiments are representative of three replicate experiments with three independent donors.

**Antigen-C3 capture enzyme-linked immunosorbent assay:** Antigen-specific monoclonal antibodies (KKO or ADA at 2 µg/mL) were incubated overnight on a microtiter plate (in phosphate-buffered saline, PBS) followed by washing and blocking with 1% bovine serum albumin in PBS for 2 hours. Antigens used in assays for complement activation included: recombinant human platelet factor 4 (PF4), protamine (PRT), unfractionated heparin (UFH; Elkins-Sinn Inc.), dextran sulphate, chondroitin sulphate A or C, dermatan sulphate, heparan sulphate and bovine intestinal heparins (E1 &E2; provided by the United States Food and Drug Administration). To activate complement, plasma was incubated with buffer or with antigens at 37°C for 1 hour followed by addition of 10 mM ethylenediaminetetraacetic acid (EDTA) to inhibit further complement generation. For studies of complement inhibition, plasma with antigen was incubated on ice or pre-incubated with 10 mM EDTA, 5 µM Cp40 (a C3 inhibitor peptide) or 5 µM control peptide prior to addition of antigens. Next, plasma containing antigen fixed by complement activation fragments was added to the capture plate for 1 hour followed by serial washes. Complement-coated antigen was detected using a biotinylated anti-C3c antibody (recognizes C3 and all C3c-containing fragments of C3, including iC3b; Quidel Corporation, San Diego, California, United States) followed by colorimetric detection as previously described.

**Complement activation studies by flow cytometry:** Complement activation studies by flow cytometry were performed as previously described using whole blood from healthy donors.