Soluble CR1 Therapy Improves Complement Regulation in C3 Glomerulopathy

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Supplementary Materials

Figure S1

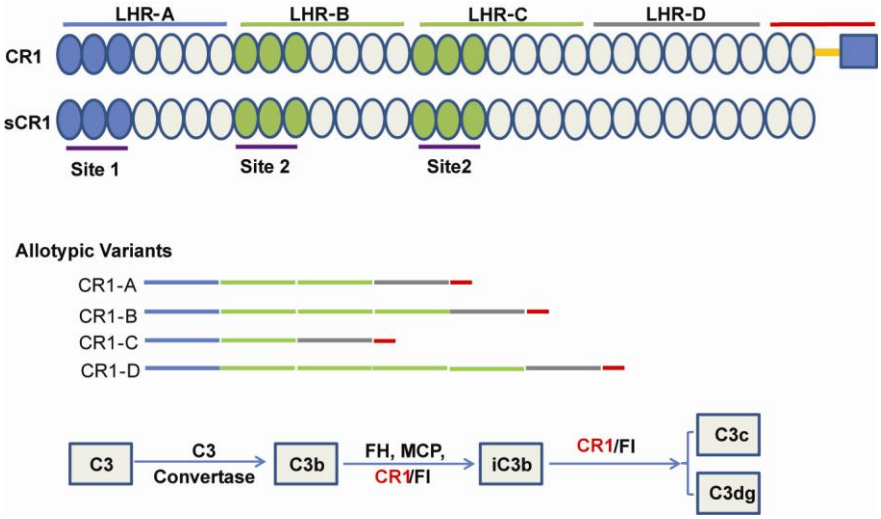

Figure S1. Complement Receptor 1 (CR1) is a monomeric single-pass type I membrane glycoprotein. Its four long homologous repeats (LHRs) are each comprised of seven short consensus repeats (SCRs), represented by ovals, which are followed by two additional SCRs, a transmembrane sequence and a cytosolic domain. The SCRs are also called complement control protein-repeat modules (CCPs) or sushi domains, and have sequence homology that ranges from 60-99%. The clusters of complement-inhibitory SCRs are referred to as sites. Site 1 (SCR 1-3) binds to C4b while Site 2 (SCR 8-10 and SCR 15-17) binds to C3b and C4b. Large insertions and deletions of the LHRs give rise to four variants of CR1, the most common of which is CR1-A. Soluble CR1 (sCR1) is similar to CR1-A, but lacks the transmembrane and cytoplasmic domains. CR1 controls C3 convertase formation and is the only cofactor that enables cleavage of iC3b into C3c and C3dg.

Figure S2

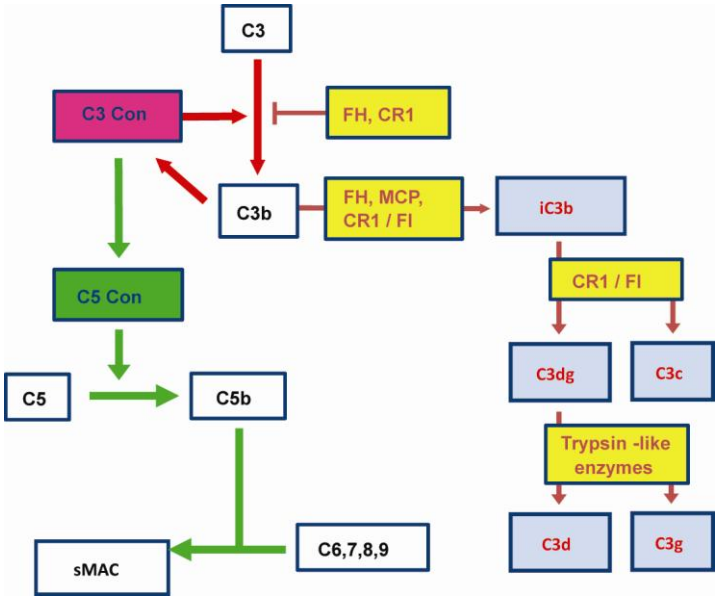

Figure S2. C3G is caused by complement dysregulation. The amplification loop of the AP is dependent on the generation of C3b from C3, shown by the red arrows. Dysregulation of the C3 convertase in the fluid phase leads to both DDD and C3GN, the two major subtypes of C3G. This dysregulation can also lead to C5 convertase dysregulation with consequent activation of the terminal complement cascade, shown by the green arrows. The most potent naturally occurring fluid-phase regulator of the AP C3 convertase is factor H. Other regulators include CR1, MCP and factor I. Targeted therapy for C3G might: 1) restore C3 convertase control; 2) replace deficiencies of regulatory proteins; 3) remove auto-antibodies that stabilize C3 convertase such as C3Nefs; 4) remove mutant proteins; or 5) remove C3b breakdown products like iC3b. CR1 has many properties that warrant its consideration as a treatment for C3G. It controls C3 and C5 convertase activity by blocking the conversion of C3 to C3b, it mediates the conversion of C3b to iC3b (as do factor H and MCP), and it serves as a cofactor for the cleavage of iC3b to C3c and C3dg.

Figure S3

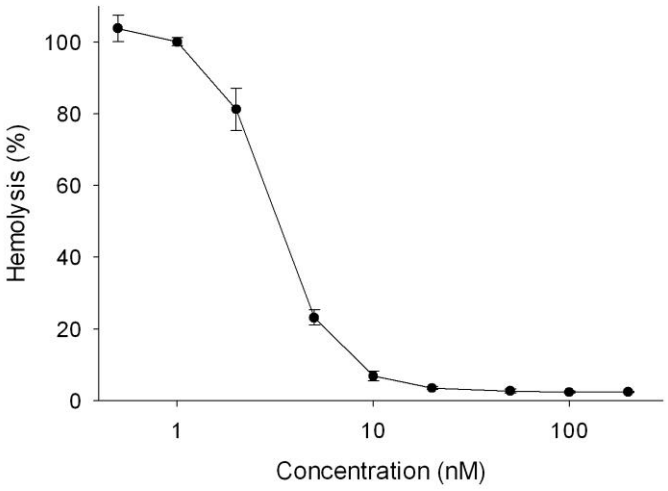

Figure S3. Human sCR1 prevents activation of murine complement cascade. Under normal circumstances, rabbit erythrocytes are an activating surface for the murine AP complement cascade. However, as the concentration of sCR1 is increased, C3 convertase-mediated generation of C3b is prevented and the AP is not activated. As a result, hemolysis does not occur (IC50 = 6.15 ± 0.18 nM). Data represent mean ± SD of triplicate assays.

Figure S4

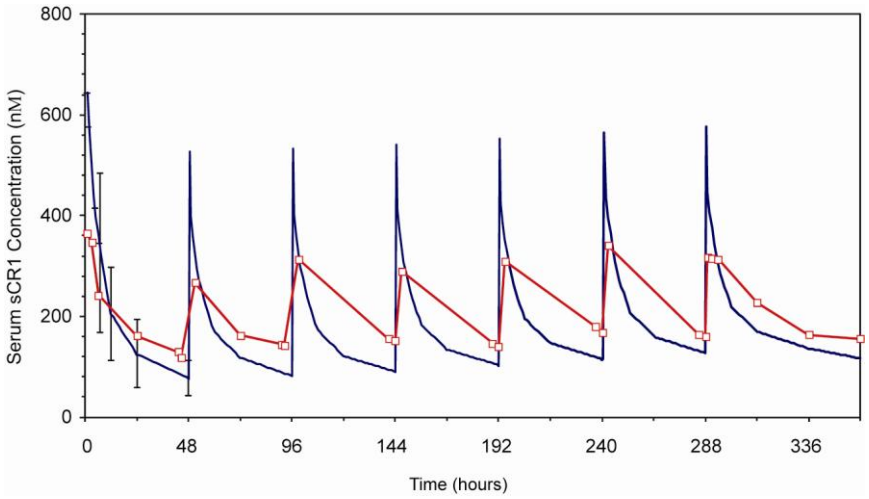

Figure S4. Simulated sCR1 concentrations are based on pharmacokinetic data from 26 cardiac surgery patients (blue line). These data compare favorably to measured serum sCR1 concentrations from the single DDD patient (red squares). Error bars included in the first 48 hours of the simulation represent standard deviations in mean concentrations at the indicated time points.