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Structural recognition and functional activation of FcγR by innate pentraxins

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Summary

Pentraxins are a family of ancient innate immune mediators conserved throughout evolution. The classical pentraxins include serum amyloid P component (SAP) and C-reactive protein (CRP), that are part of acute phase proteins synthesized in response to infection1, 2. Both recognize microbial pathogens and activate the classical complement pathway through C1q3,4. More recently, members of the pentraxin family were found to interact with cell surface Fcγ receptors (FcγR) and activate leukocyte-mediated phagocytosis5-8. We now describe the structural mechanism for pentraxin binding to FcγR and its functional activation of FcγR-mediated phagocytosis and cytokine secretion. The complex structure between human SAP and FcγRIIa reveals a diagonally bound receptor on each SAP pentamer with both D1 and D2 domains of the receptor contacting the ridge helices from two SAP subunits. The 1:1 stoichiometry between SAP and FcγRIIa infers the requirement for multivalent pathogen binding for receptor aggregation. Mutational and binding studies show that pentraxins are diverse in their binding specificity to FcγR isoforms but conserved in their recognition structure. The shared binding site for SAP and IgG results in competition for FcγR binding and the inhibition of immune complex-mediated phagocytosis by soluble pentraxins. These results establish the antibody-like functions for pentraxins in the FcγR pathway, suggest an evolutionary overlap between the innate and adaptive immune systems, and have novel therapeutic implications for autoimmune diseases.

The pentraxin family is divided into two subclasses, the classical short chain pentraxins, CRP and SAP, and the long chain pentraxins3. Both SAP and CRP recognize various pathogenic bacteria, fungi and yeasts3, and activate the classical complement pathway through C1q4. Long pentraxins, such as PTX3 which contain an additional N-terminal domain, are produced by macrophages and myeloid dendritic cells in response to proinflammatory stimuli9, 10. Human has three classes of activating Fcγ receptors, FcγRI,

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FcγRIIa and FcγRIII, and one inhibitory receptor FcγRIIb11. In addition to activating phagocytosis through FcγR 5-8, both SAP and CRP also induce protective immune responses12, and high levels of CRP protect mice from endotoxin shock through FcγR13, 14. While pentraxins can both activate and regulate immune responses, the molecular mechanisms and the balance of these antibody-like functions remain unresolved. Here we present structural and functional evidence for the involvement of pentraxins in the activation of FcγR and suggest their potential role in modulating antibody-mediated inflammatory responses.

While immune complexes are known to activate FcγR leading to phagocytosis and cytokine secretion, it is not clear if pentraxins induce similar FcγR activation7, 8. To investigate whether FcγR recognize pathogens through pentraxin opsonization, we examined the engulfment of pentraxin-opsonized zymosan by human monocyte-derived macrophages (MDM). Texas red-labeled zymosan particles were efficiently internalized by MDM upon opsonization with human SAP, CRP or IgG compared to unopsonized particles (Fig 1a). The cup-shaped enrichment of FcγRIIa (labeled green) surrounding the SAP-and CRP-bound zymosan particles indicates the involvement of FcγR in phagocytosis. The addition of soluble IgG reduced phagocytosis of SAP-opsonized zymosan by 90% from 3.8 ± 0.5 to 0.4 ± 0.2 zymosan particles/MDM, further confirming the role of FcγR. We then investigated cytokine secretion as a result of SAP-FcγR interaction. To avoid zymosan and endotoxin-mediated activation, CD14+ monocytes were treated with purified SAP in either an aggregated or monomeric form without zymosan and in the presence of polymixin B. SAP treatment resulted in dose-dependent secretion of IL-10, IL-8 and IL-6 by monocytes (Fig. 1b), and only the aggregated but not monomeric SAP stimulated cytokines suggesting a requirement for receptor cross-linking by SAP in cytokine production (Fig. 1c). Cytokine secretion was dramatically reduced if SAP was pre-treated with bead-bound proteinase K or pre-cleared with phosphoethanolamine (PE)-conjugated Sepharose (Fig. 1d). In addition, antibodies against FcγR as well as a *Syk* inhibitor, piceatannol that blocks FcγR signaling significantly inhibited cytokine secretion, confirming the involvement of FcγR (Fig. 1e, f). To assess the contribution of potential contaminating LPS and/or peptidoglycan in the SAP sample to cytokine secretion, bone marrow-derived macrophages (BMDM) from $MyD88^{-/-}$ and $RIP2^{-/-}$ mice were treated with SAP and assayed for cytokine production. Similar or higher levels of IL-6 and CCL2 were detected in SAP-but not PBS-treated BMDM from $MyD88^{-/-}$ mice compared to wild type BMDM (Fig. 1g). TNF-α production was lower in SAP-treated $MyD88^{-/-}$ than wild type BMDM but remained 10-20 fold higher compared to PBS treatment. Similarly, comparable amounts of cytokines were released in BMDM from the $RIP2^{-/-}$ and wild type mice in response to SAP. The results show that BMDM from both $MyD88^{-/-}$ and $RIP2^{-/-}$ mice produce cytokines upon SAP stimulation independent of TLR and NOD receptor pathways. However, a partial reduction in TNF-α level from the $MyD88^{-/-}$ compared to the wildtype mice indicates a potential synergistic activation between FcγR and TLR.

To investigate the structural mechanism for pentraxin-mediated FcγR activation, we determined the crystal structure of human SAP in complex with the extracellular domain of FcγRIIa to 2.8Å resolution with the final R-factors of 20.7% and 27.9% for R_{cryst} and R_{free},

respectively (Supplementary Table 1). The refined (2Fo-Fc) density map was continuous throughout the complex except for one SAP loop, residues 140-146, which was disordered in four of the five SAP subunits. Each asymmetric unit contained one FcγRIIa molecule bound to the effector face of one SAP pentamer with the D1 and D2 domains of the receptor spanning diagonally over SAP and contacting the A and C subunits of the pentraxin, respectively (Fig 2). This diagonal spanning of FcγRIIa over the SAP pentamer ensures a 1:1 stoichiometry in SAP-FcγRIIa recognition and thus setting the need for the binding of multivalent pathogens in Fc receptor aggregation. The conformations of the receptor contacting subunits A and C of SAP do not differ significantly from the other three non-receptor contacting subunits nor from those of receptor-free SAP 15 (Fig 2d). In fact, the entire SAP pentamer of the current complex, despite the lack of bound Ca^{2+} ions and small ligands, can be superimposed onto that of receptor-free, Ca^{2+}-bound SAP with a root-mean-square (r.m.s.) deviation of 0.8 Å. Similarly, the structure of FcγRIIa in the complex is nearly identical to that of ligand-free FcγRIIa with r.m.s. differences of 0.9 Å for 171 Cα atoms16, suggesting rigid body docking between SAP and the receptor. The only conformational change in the receptor involved a ~3 Å movement in the N-terminal BC loop (residues 28-35) of the FcγRIIa toward the A subunit of SAP (Fig. 2d).

The complex buries a total of ~1962 $Å^2$ of solvent-accessible surface area equally distributed between the A and C subunits of SAP and has a shape complementarity index of 0.5817, similar to those between TCR and MHC, between KIR and HLA (Sc=0.5-0.6) but less than those between antibodies and antigens (Sc>0.7)18, 19. Both the A and C subunits of SAP use their ridge helices (Pro 166 to Gln 174), especially Tyr 173 and Gln 174, and the C-terminal residues (Pro 200 to Pro 204) to contact the D1 and D2 domains of the FcγR (Fig. 3), thus highlighting these residues as functional hot spots on SAP. On the receptor side, both the D1 and D2 domains use their topological equivalent BC, C'E loops and the C strand to contact SAP and the binding site is away from the predicted N-linked glycosylation sites. The D1-A interface consists of a salt bridge between Asp 35 of FcγRIIa and Arg 38 of SAP, four hydrogen bonds, and *van der Waals* (VDW) interactions involving Tyr 173 and Gln 174 from the ridge helix of SAP (Fig 3a, Supplementary Table 2). The D2-C interface, in contrast, is entirely mediated by VDW interactions (Fig 3b, Supplementary Table 2). Residues at the D1-A interface are more conserved across the species than those at the D2-C interface (Supplementary Fig S1).

The critical receptor contact region of SAP involves the ridge helix and its pentameric assembly, conserved features of the pentraxin family. Likewise, the structure of FcγRIIa displaying only 1.2 Å r.m.s. deviation among the Cα atoms from FcγRIII is well conserved with significant interface sequence homology shared among FcγRs (Fig S1). This suggests the possibility of a broader recognition between pentraxins and FcγR5, 7, 8. Using BIAcore binding, we showed that SAP, CRP and PTX3 all recognized FcγRs with affinities ranging from 10^{-5} to 10^{-7} M, similar to those between isoforms of IgG and FcγR20 (Supplementary Table 3, Fig. S2). SAP bound tightest to FcγRI with a dissociation constant (K_D) of 0.48 μM, weaker to FcγRIIa, FcγRIIb and FcγRIII (1-3 μM in K_D). CRP displayed similar affinities of 2-4 μM to all four FcγR isoforms. PTX3 only recognized FcγRIII. Notably, SAP

and CRP but not PTX3 recognized the inhibitory Fc receptor, FcγRIIb, suggesting a potential regulatory function for these pentraxins.

The structural conservation and broad pentraxin-FcγR recognition suggest the complex structure to be a prototypic model for pentraxin recognition by FcγR. To test this, we modeled the CRP structure into the current SAP-FcγRIIa complex. Although SAP and CRP differ in their relative monomer orientations with respect to the pentamer by ~25° 21, 22, both use common residues at the pentameric interface, and CRP can be placed at the FcγRIIa interface in the model without serious steric hindrance (Fig. S3). To verify the CRP-FcγRIIa model, three putative interface residues of CRP, including the salt bridge forming residue His 38 and two ridge helix residues, Tyr 175 and Leu 176, were mutated and the binding of mutant CRP to FcγR was measured by BIAcore. While wildtype CRP binds FcγRIIa with 1.9 μM affinity, H38A, Y175L and L176A mutations resulted in a two to eight fold decrease in the solution binding affinity (Supplementary Table 3). These mutational results, together with the earlier mutagenesis studies 23, indicate that FcγR recognition is conserved between SAP and CRP. A similar decrease in affinity was also observed between the CRP mutants and FcγRIII, indicating a further structural preservation in FcγRIII recognition. Interestingly, the mutations did not affect CRP recognition of FcγRI, implying a variation in CRP-FcγRI interaction potentially attributed to the presence of an additional domain in FcγRI.

Polymorphism at residue 131 of FcγRIIa affects its recognition of immunoglobulins24, 25 26. This polymorphism also affected CRP binding27. By isothermal titration calorimetry (ITC), CRP bound the arginine and histidine isoforms of FcγRIIa with ~4 and <10 μM affinities, respectively (Fig S4). The side chain of Arg but not His 131 could form hydrogen bonds with the main chains of Gly 178 and Pro 179 from CRP.

As FcγRIIa recognized both SAP and IgG, we then examined whether the receptor binding to SAP affected its binding to immunoglobulins. Unexpectedly, the overlay of the SAP-FcγRIIa on the Fc-FcγRIII structure showed that the IgG binding site on the Fc receptor partially overlapped with that for SAP 28(Fig. 4). Both SAP and IgG interact with the BC and FG loops as well as the C and C' strands of the FcγR D2 domain, creating a steric clash between the CH_2 domain of IgG and the C and D subunits of SAP. The shared recognition predicts a competition between IgG and SAP for FcγR binding. Indeed, using a BIAcore binding assay, the association between SAP and FcγRIIa was effectively competed off by increasing concentrations of IgG_1 (Fig. 4c). Further, soluble SAP blocks, in a dose dependent manner, the phagocytosis of IgG-opsonized sheep red blood cells (EIg) by human MDM (Fig 4d). Similarly, CRP binding to Fc receptors (FcγRIIa, FcγRIIb and FcγRIII) was also inhibited by IgG_1 in a dose dependent manner (Fig 4e), and CRP inhibited IgG-mediated phagocytosis of EIg (Fig. 4f). The similarity between CRP and SAP in competing with IgG binding to FcγR further supports their conserved receptor recognition structure. As the plasma concentration of CRP but not SAP may reach greater than 200 μg/ml during the acute phase2, the inhibition of IgG-mediated phagocytosis by CRP suggests a potential downregulation of antibody-mediated FcγR activation by CRP in the late stage of the acute phase when an excess amount of soluble CRP is available.

In summary, we showed through structural and functional studies that pentraxins directly recognize FcγR, activate phagocytosis and induce cytokine secretion. While pentraxins recognized FcγR with variable affinity and specificity, they likely share a conserved receptor recognition. The overlapping SAP and IgG binding sites on FcγR blocked antibody-mediated phagocytosis by soluble pentraxins. Taken together, these results provide structural and functional evidence for the involvement of pentraxins in FcγR-mediated immune functions. Thus, pentraxins possess similar functions as antibodies that activate both the complement and Fc receptor pathways. This parallel between pentraxins and antibodies suggests that pentraxins were ancient antibodies in evolution, and that they function as antibodies in more primitive organisms. The competition in FcγR binding between IgG and pentraxins suggests potential novel strategies for treating autoimmune diseases based on soluble pentraxins rather than IVIg.

Method Summary

Recombinant ectodomains of FcγRIIa and IIb1 (1-171) were expressed in *E.coli* and refolded using a pET30a vector with a C-terminal His_6-tag. SAP and CRP were purified from human plasma or pleural fluid. CRP mutants were expressed in baculovirus system. For confocal and fluorescence microscopy, human MDM were incubated with SAP, CRP, rabbit anti-zymosan IgG or PBS opsonized zymosan (conjugated with Texas red) at 37° C for 30 min. The samples were stained with an anti-CD32 mAb and an Alexafluor 488 conjugated goat anti-mouse $F(ab')_2$. For inhibition, soluble IgG was added during the phagocytosis. For phagocytosis of sRBC, fresh sRBC were opsonized with a rabbit anti-sRBC IgG and mixed with MDM by centrifugation at 20:1 and incubating at 37°C for 2 h in the presence or absence of SAP, CRP, anti-CD32 or anti-CD64. For Cytokine release experiments, purified CD14+ monocytes were incubated with 50 μg/ml or otherwise indicated concentrations of aggregated SAP (Supplementary Fig S5) for 24 h and cytokines were measured by ELISA. Monomeric SAP was isolated on a Superdex 200 column in the presence of 10mM Methyl-β-D-galactopyranoside. SAP was degraded by a bead-bound proteinase K or depleted by phosphoethanolamine (PE)-conjugated Sepharose. Anti- FcγR blocking antibodies or isotype controls were added at 5μg/ml before adding SAP. Bone marrow cells from $MyD88^{-/-}$, $RIP2^{-/-}$ and wildtype mice were differentiated to BMDM and then incubated with 50 μg/ml of aggregated SAP for cytokine release. The complex crystals were grown from 2.0 M $(NH_4)_2SO_4$, 5% isopropanol by hanging drop vapor diffusion, X-ray data were collected to 2.8 Å resolution at SER-CAT beamlines and processed with HKL2000. The complex structure was solved by molecular replacement method using Phenix package. Model building and refinement were carried out using O and CNS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Reference List

1. Steel DM, Whitehead AS. The major acute phase reactants: C-reactive protein, serum amyloid P component and serum amyloid A protein. Immunol Today. 1994; 15:81–88. [PubMed: 8155266]
2. Pepys MB, et al. Comparative clinical study of protein SAP (amyloid P component) and C-reactive protein in serum. Clin Exp Immunol. 1978; 32:119–124. [PubMed: 668189]
3. Garlanda C, Bottazzi B, Bastone A, Mantovani A. Pentraxins at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. Annu Rev Immunol. 2005; 23:337–366. [PubMed: 15771574]
4. Kaplan MH, Volanakis JE. Interaction of C-reactive protein complexes with the complement system. I. Consumption of human complement associated with the reaction of C-reactive protein with pneumococcal C-polysaccharide and with the choline phosphatides, lecithin and sphingomyelin. J Immunol. 1974; 112:2135–2147. [PubMed: 4151108]
5. Bharadwaj D, Stein MP, Volzer M, Mold C, Du Clos TW. The major receptor for C-reactive protein on leukocytes is fcgamma receptor II. J Exp Med. 1999; 190:585–590. [PubMed: 10449529]
6. Marnell LL, Mold C, Volzer MA, Burlingame RW, Du Clos TW. C-reactive protein binds to Fc gamma RI in transfected COS cells. J Immunol. 1995; 155:2185–2193. [PubMed: 7636267]
7. Bodman-Smith KB, et al. C-reactive protein-mediated phagocytosis and phospholipase D signalling through the high-affinity receptor for immunoglobulin G (FcgammaRI). Immunology. 2002; 107:252–260. [PubMed: 12383205]
8. Bharadwaj D, Mold C, Markham E, Du Clos TW. Serum amyloid P component binds to Fc gamma receptors and opsonizes particles for phagocytosis. J Immunol. 2001; 166:6735–6741. [PubMed: 11359830]
9. Alles VV, et al. Inducible expression of PTX3, a new member of the pentraxin family, in human mononuclear phagocytes. Blood. 1994; 84:3483–3493. [PubMed: 7949102]
10. Han B, et al. TNFalpha-induced long pentraxin PTX3 expression in human lung epithelial cells via JNK. J Immunol. 2005; 175:8303–8311. [PubMed: 16339571]
11. Ravetch JV, Bolland S. IgG Fc receptors. Annu Rev Immunol. 2001; 19:275–290. [PubMed: 11244038]
12. Bickerstaff MC, et al. Serum amyloid P component controls chromatin degradation and prevents antinuclear autoimmunity. Nat Med. 1999; 5:694–697. [PubMed: 10371509]
13. Xia D, Samols D. Transgenic mice expressing rabbit C-reactive protein are resistant to endotoxemia. Proc Natl Acad Sci U S A. 1997; 94:2575–2580. [PubMed: 9122237]
14. Mold C, Rodriguez W, Rodic-Polic B, Du Clos TW. C-reactive protein mediates protection from lipopolysaccharide through interactions with Fc gamma R. J Immunol. 2002; 169:7019–7025. [PubMed: 12471137]
15. Emsley J, et al. Structure of pentameric human serum amyloid P component. Nature. 1994; 367:338–345. [PubMed: 8114934]
16. Maxwell KF, et al. Crystal structure of the human leukocyte Fc receptor, Fc gammaRIIa. Nat Struct Biol. 1999; 6:437–442. [PubMed: 10331870]
17. Lawrence MC, Colman PM. Shape complementarity at protein/protein interfaces. J Mol Biol. Dec 20.1993 234:946–950. [PubMed: 8263940]
18. Boyington JC, Motyka SA, Schuck P, Brooks AG, Sun PD. Crystal structure of an NK cell immunoglobulin-like receptor in complex with its class I MHC ligand. Nature. 2000; 405:537–543. [PubMed: 10850706]
19. Ysern X, Li H, Mariuzza RA. Imperfect interfaces. Nat Struct Biol. 1998; 5:412–414. [PubMed: 9628472]

20. Hulett MD, Hogarth PM. Molecular basis of Fc receptor function. Adv Immunol. 1994; 57:1–127. [PubMed: 7872156]
21. Shrive AK, et al. Three dimensional structure of human C-reactive protein. Nat Struct Biol. 1996; 3:346–354. [PubMed: 8599761]
22. Thompson D, Pepys MB, Wood SP. The physiological structure of human C-reactive protein and its complex with phosphocholine. Structure. 1999; 7:169–177. [PubMed: 10368284]
23. Bang R, et al. Analysis of binding sites in human C-reactive protein for Fc{gamma}RI, Fc{gamma}RIIA, and C1q by site-directed mutagenesis. J Biol Chem. 2005; 280:25095–25102. [PubMed: 15878871]
24. Tax WJ, Willems HW, Reekers PP, Capel PJ, Koene RA. Polymorphism in mitogenic effect of IgG1 monoclonal antibodies against T3 antigen on human T cells. Nature. 1983; 304:445–447. [PubMed: 6224091]
25. Clark MR, Stuart SG, Kimberly RP, Ory PA, Goldstein IM. A single amino acid distinguishes the high-responder from the low-responder form of Fc receptor II on human monocytes. Eur J Immunol. 1991; 21:1911–1916. [PubMed: 1831131]
26. Parren PW, et al. On the interaction of IgG subclasses with the low affinity Fc gamma RIIa (CD32) on human monocytes, neutrophils, and platelets. Analysis of a functional polymorphism to human IgG2. J Clin Invest. 1992; 90:1537–1546. [PubMed: 1401085]
27. Stein MP, et al. C-reactive protein binding to FcgammaRIIa on human monocytes and neutrophils is allele-specific. J Clin Invest. 2000; 105:369–376. [PubMed: 10675363]
28. Radaev S, Motyka S, Fridman WH, Sautes-Fridman C, Sun PD. The structure of a human type III Fcgamma receptor in complex with Fc. J Biol Chem. 2001; 276:16469–16477. [PubMed: 11297532]

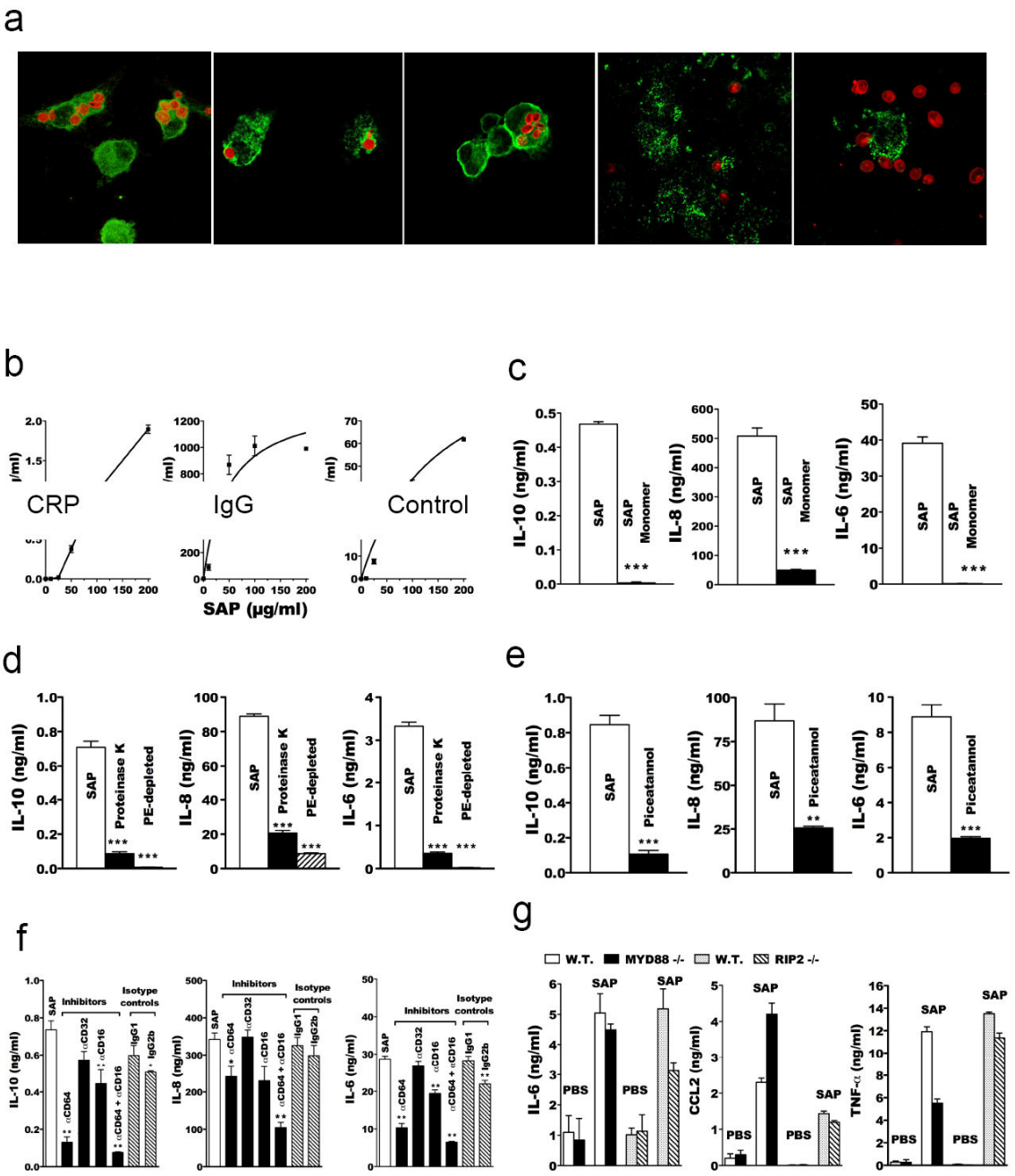


Figure 1.

Pentraxin activation of FcγR results in opsonization and cytokine release. a) SAP- and CRP-opsonized zymosan are phagocytosed by human macrophages through FcγRIIa. MDM incubated with zymosan (red) opsonized with (from left to right) SAP, CRP, IgG, PBS control, or SAP in the presence of 10 mg/ml IVIg. b-f) The production of IL-10, IL-8 and IL-6 by CD14+ monocytes in response to b) different concentrations of aggregated SAP; c) 50 μg/ml aggregated or monomeric SAP; d) SAP pre-treated with proteinase K or pre-cleared with PE-Sepharose; and e) SAP in the presence of 25 μg/ml piceatannol; f) SAP

treatment in the presence of FcγR specific antibodies or control Ig. g) Cytokine release by BMDM from wild type, MyD88$^{-/-}$ and RIP2$^{-/-}$ mice stimulated with SAP.

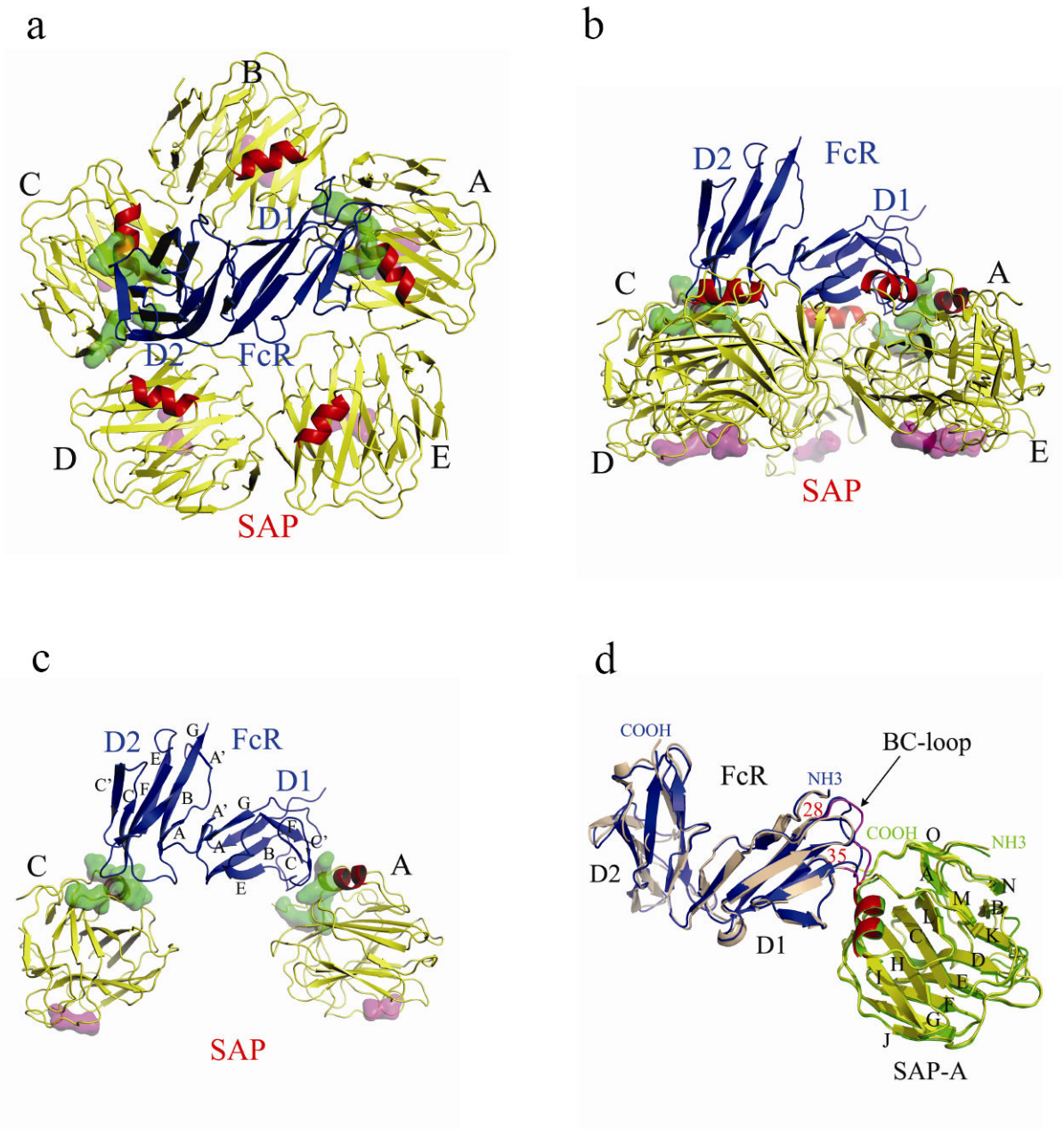


Figure 2.

Crystal structure of SAP-FcγRIIa complex. The view is from the face (a) and side (b and c) of SAP, with panel c highlighting only the receptor contact A and C subunits. The five SAP subunits are shown in yellow with ridge helices in red, and FcγRIIa is colored in blue. The interface is represented by molecular surface in green. The calcium and ligand binding sites on SAP are highlighted in magenta. d) Comparison between the free (green) and receptor-bound (yellow) SAP, and between the free (wheat) and SAP-bound (blue) FcγRIIa structures. For clarity, only the A subunit is shown from the superposition of SAP pentamer. The BC loop (residues 28-35) of D1 domain is indicated.

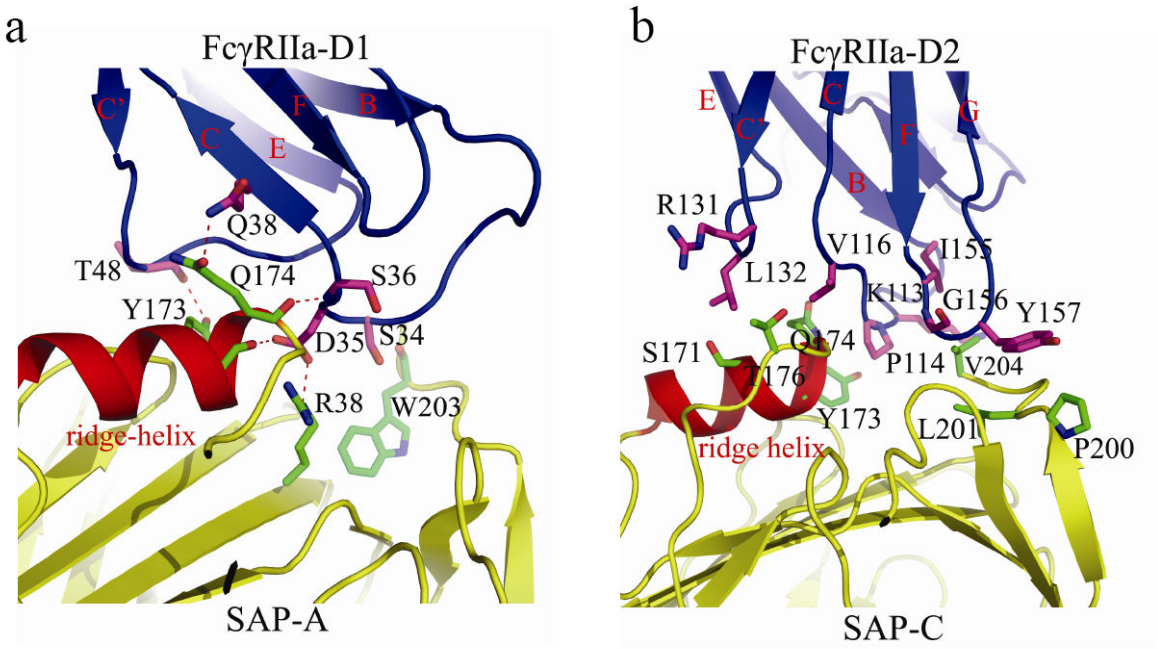
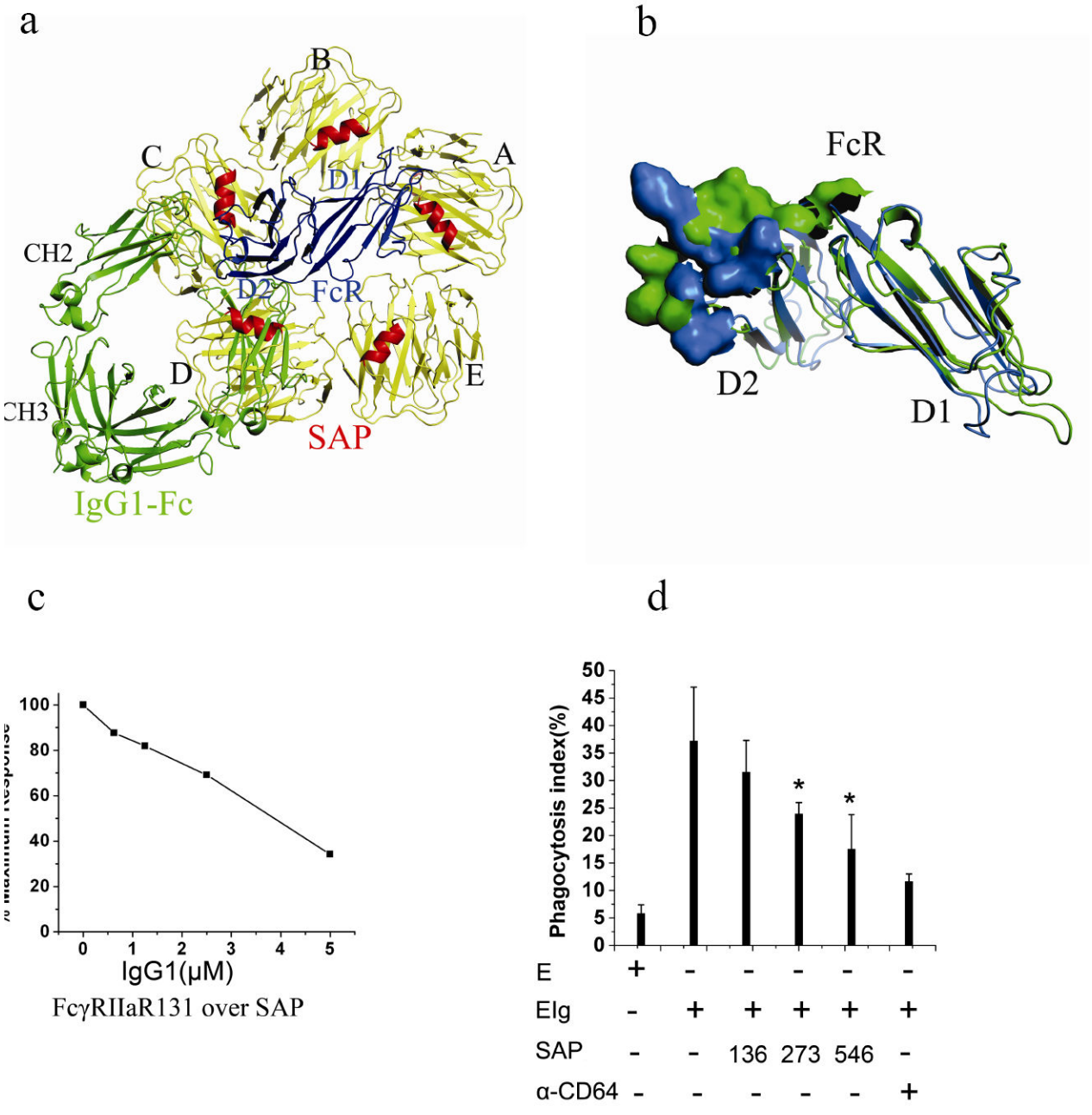


Figure 3.

The binding interfaces between SAP and FcγRIIa. a) The interface between the D1 domain of FcγRIIa (blue and magenta) and the A subunit of SAP (yellow and green) is shown with participating side residues shown in sticks. The hydrogen bond interactions are represented by red dashed lines. b) The interface between the D2 domain of FcγRIIa and the C subunit of SAP.



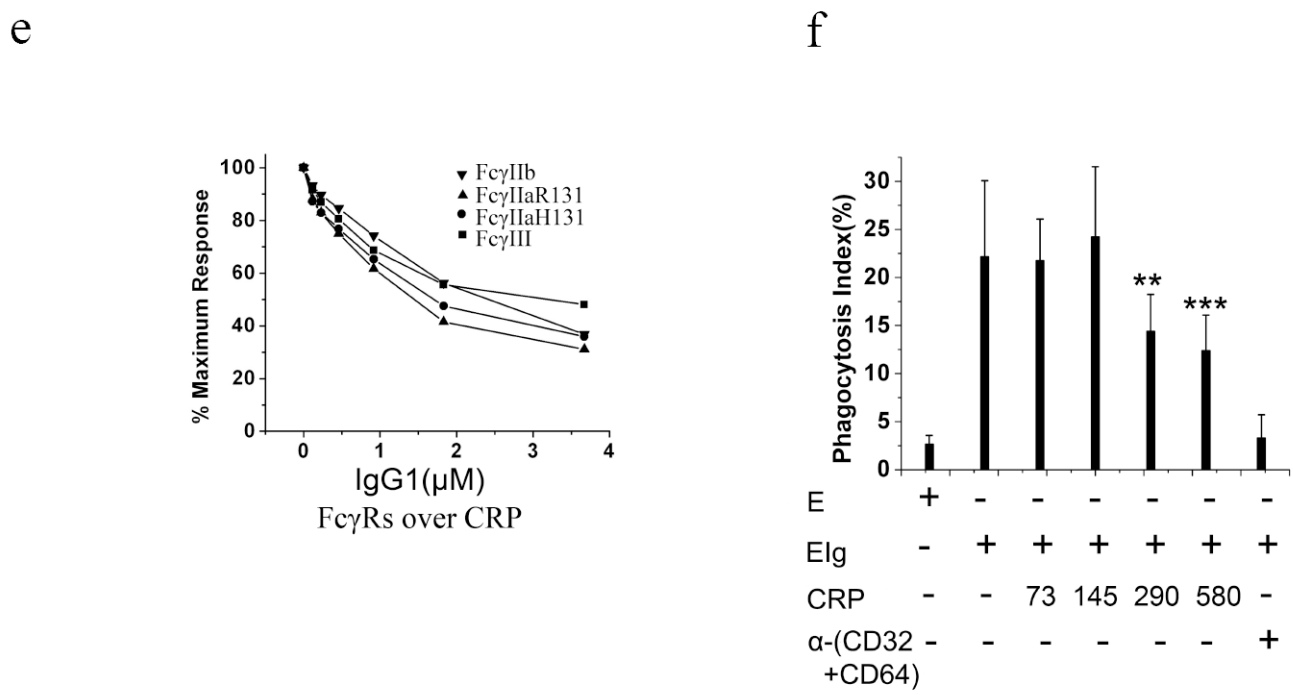


Figure 4.

Competition between human IgG_1 and SAP or CRP for binding to Fcγ receptors. a) The superposition of FcγR between SAP-FcγRIIa and Fc-FcγRIII complexes with FcγRIIa, Fc portion of IgG_1 and SAP shown in blue, green and yellow, respectively. b) The interface residues of SAP-FcγRIIa and Fc-FcγRIII complexes are depicted by molecular surface representations in blue and green on FcγRIIa (blue) and FcγRIII (green), respectively. c and e) Competition binding between SAP (c), CRP (e) and human IgG_1 using SAP or CRP immobilized CM5 sensorchips. The analytes consisted of a mixture of (c) 5 μM and (e) 2 μM of stated FcγR with various concentrations of $hIgG_1$. d) SAP and f) CRP inhibit IgG-mediated phagocytosis. Human MDM incubated with E or EIg, in the presence of various concentrations (μg/ml) of CRP, SAP, or blocking antibodies against FcγRI (25 μg/ml) and FcγRII (25μg/ml).