The Major Receptor for C-Reactive Protein on Leukocytes Is Fcγ Receptor II

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Summary

C-reactive protein (CRP) is an acute phase serum protein that shares several functions with immunoglobulin (Ig)G including complement activation and binding to receptors on monocytes and neutrophils. The identity of the receptor for CRP has been the target of extensive research. We previously determined that CRP binds to the high affinity receptor for IgG, FcγRI (CD64). However, this interaction could not account for the majority of binding of CRP to neutrophils or monocytes. We now determine that CRP also interacts with FcγRIIa (CD32), the low affinity receptor for IgG on monocytes and neutrophils. COS-7 cells were transfected with a construct containing the human FcγRIIA cDNA. CRP binding and the presence of CD32 were detected by mAb and analyzed by two-color flow cytometry. Cells expressing CD32 bound CRP in a dose-dependent and saturable manner consistent with receptor binding. CRP bound to transfectants and K-562 cells with similar kinetics, and in both cases binding was completely inhibited by aggregated IgG. On monocytic cell lines, treatment with Bt2cAMP increased FcγRI expression and enhanced CRP binding. CRP also specifically precipitated FcγRI and FcγRII from the monocytic cell line, THP-1. It is suggested that the major receptor for CRP on phagocytic cells is FcγRII.

Key words: C-reactive protein • Fc receptors • FcγRII • receptors • immunoglobulin G

Materials and Methods

Preparation of CRP. Human CRP was purified from human pleural fluids exactly as previously described (12). The purity of the preparation was determined by 12% SDS-PAGE. No contaminating proteins were detected on overloaded gels.

Reagents and Antibodies. The monoclonal anti-CRP antibody, 2C10, was the gift of Dr. Larry Potempa (ImmTech, Evanston, on November 3, 2018 jem.rupress.org Downloaded from http://doi.org/10.1084/jem.190.4.585 Published Online: 16 August, 1999 | Supp Info:
Flow Cytometry. Cells were analyzed using a Becton Dickinson FACSCalibur® flow cytometer equipped with CellQuest software (Becton Dickinson). The population analyzed was gated by forward and side scatter to exclude dead cells. A minimum of 30,000 cells was collected. For all measurements of CRP binding, the binding of 2C10 and the anti-mouse secondary antibody were subtracted. Results are presented as the geometric mean channel fluorescence (gMCF).

Precipitation of Cell Surface Receptors. THP-1 cells were treated with 50 μg/ml prouase (Calbiochem) for 30 min at 37°C, washed, and radiolabeled with 125I using lactoperoxidase (14). Membrane extracts were prepared at 5 × 10^6 cells/ml using 1% NP-40 in PBS with 1 mM AEBSF, 1 μM pepstatin A, 10 μM E-64, 10 μM bestatin, 100 μM leupeptin, and 2 μg/ml aprotinin (lys-buffer). CRP beads were prepared by coupling 18 mg CRP/ml Affigel 15 according to the manufacturer's protocol. Antibody beads were prepared by incubation of 5 μg anti-CD32 mAb (IV.3 or AT10) or anti-CD64 mAb 197 with 15 μg/ml Affigel 15 according to the manufacturer's protocol. CRP beads, anti-CD32 Sepharose, or anti-CD64 Sepharose in

Figure 1. CRP binds to COS-7 cells transfected with the FcγRIIa cDNA, and to K-562 cells with similar kinetics. Cells were incubated with increasing doses of CRP and binding was detected with 2C10 and PE-GAM, as described in Materials and Methods. Results are expressed as the geometric mean channel fluorescence (gMCF).
lysis buffer for 4 h at 4°C. The beads were then washed four times with lysis buffer and once with 0.1% NP-40 in the same buffer. The samples were boiled for 3 min in Laemmli's sample buffer and run on 4–20% gradient SDS-PAGE gels (Novex). The gels were dried and autoradiography was performed using a Storm imaging system (Molecular Dynamics).

Data Analysis. The binding kinetics of CRP to cells were analyzed using GraphPad PRISM™ software (GraphPad Software, Inc.). ImageQuant software (Molecular Dynamics) was used for quantitation of radioactive bands. Experiments were repeated at least twice.

Results

Binding of CRP to Transfected COS-7 Cells and to K-562 Cells. Transfection of COS-7 cells with pcDSR α296 containing the cDNA for CD32 resulted in 70–85% of cells expressing the CD32 marker. When these cells were incubated with CRP, a dose-dependent and saturable binding of CRP was seen (Fig. 1A). The binding of CRP was fitted to a single site model using GraphPad Prism™ software. Saturation was predicted at 100 μg/ml with an apparent equilibrium binding constant (K_D) of 6.6 × 10^-8 M (7.6 μg/ml). This binding curve is similar to the binding of CRP to K-562 cells, which express FcyRII as the only FcR (Fig. 1B). The apparent K_D is 9.7 × 10^-8 M (11.2 μg/ml) with saturation at ~100 μg/ml. Background binding to mock-transfected cells was not above baseline binding of secondary antibody. The apparent affinity of CRP for transfected and K-562 cells was significantly higher than the reported values for IgG binding to FcyRII (K_D > 10^-7 M) (15) and similar to the previously reported figure for CRP binding to K-562 cells (3.8 × 10^-8 M) (6). In separate experiments using 125I-labeled aggIgG, an apparent K_D of 23 μg/ml was measured (data not shown).

Binding of CRP to transfected cells was also examined by two-color analysis to confirm that CRP bound to cells...

Figure 2. Analysis of CRP binding to transfected COS-7 cells by two-color flow cytometry. COS-7 cells were transfected with the FcγRII plasmid, pcDSR α296. Mock-transfected cells received no DNA. Cells were incubated with 200 μg/ml of CRP and binding was detected with 2C10 and PE-GAM as described in Materials and Methods. Levels of FcγRII were determined by binding of FITC-AT10. A, B, C, and D, transfected; E, F, G, and H, mock-transfected. A and E, staining with 2C10 and PE-GAM; B and F, staining for CD-32 only; C and G, staining for CRP only; D and H, staining for CRP and CD32.

Figure 3. CRP binding to FcγRII is inhibited by aggIgG. K-562 cells or COS-7 cells transfected with the FcγRII plasmid, pcDSR α296, were incubated with 25 μg/ml of CRP in the presence of increasing concentrations of aggIgG. CRP binding was detected with 2C10 and PE-GAM as described in Materials and Methods. Results are expressed as the increase in MCF compared with the secondary antibody controls. The solid line represents binding of CRP to K-562 cells and the dotted line represents the binding of CRP to transfected COS cells in the absence of aggIgG.
CRP Binds to Human FcγRIIα

expressing CD32 (Fig. 2). Single color staining with anti-CD32 (Fig. 2 B) or CRP (Fig. 2 C) showed staining of 72% (with a background binding of 6% to mock-transfected) and 63% (with a background binding of <1% of mock-transfected) of transfected cells, respectively. In the two-color analysis, CRP binding to CD32-transfected COS cells directly correlated with anti-CD32 binding with 52% of the cells stained for both markers (Fig. 2 D).

Binding of CRP to CD32-transfected COS-7 cells and to K-562 cells is blocked by IgG. To determine if CRP was binding to the same site on FcγRII as IgG, the effect of aggIgG on CRP binding was examined. As shown in Fig. 3, aggIgG inhibited CRP binding to K-562 cells in a dose-dependent manner with 50% inhibition at ~80 μg/ml of aggIgG. This finding is consistent with previous reports by us (10, 11) and others (5–7) that aggIgG effectively blocks CRP binding to its receptor. A nearly identical inhibition profile was obtained using aggIgG to inhibit CRP binding to CD32-transfected COS cells (Fig. 3). For both cell types, aggIgG did not affect the background binding of the secondary antibodies measured in the absence of CRP.

Enhanced Binding of CRP to Bt2cAMP-treated U-937 cells. Agents that affect the differentiation state may alter the expression of FcR on monocytic cell lines. The U-937 cell line expresses FcγRI and FcγRII. Exposure of U-937 cells to Bt2cAMP has been reported to decrease the expression of FcγRI while increasing the expression of FcγRII (17). We tested the effect of exposure of U-937 cells to Bt2cAMP on the amount of binding of CRP. Since both FcγRI and FcγRII bind CRP, a change in the level of CRP binding might suggest that one or the other receptor is more important in CRP binding. As shown in Fig. 4, Bt2cAMP decreased the expression of FcγRII and increased the expression of FcγRII as previously reported. Associated with this increase in FcγRII, a marked increase in CRP binding was seen (Fig. 4). Thus, the binding of CRP to FcγRII may be quantitatively more important than the binding to FcγRI.

Precipitation of FcR by CRP. In an attempt to identify proteins reacting with CRP on FcR-bearing cells, receptor precipitation studies were performed. THP-1 cells express both FcγRI and FcγRII. Attempts to immunoprecipitate surface-labeled proteins with immobilized CRP showed weak and nonspecific bands. However, it had previously been determined that the interaction of IgG with FcγRII could be enhanced by pronase treatment of cells (18). We recently determined that pronase treatment of FcγRII-bearing cells also markedly increases the binding of CRP (Stein, M.-P., J.C. Edberg, R.P. Kimberly, E.K. Mangan, D. Bharadwaj, C. Mold, and T.W. Du Clos, manuscript submitted for publication). When pronase-treated cells were radiolabeled and precipitated with CRP, distinct binding of two major bands was seen. These bands with M, of 70 and 42 kD corresponded to the bands precipitated by mAb to CD64 and CD32 (Fig. 5).

Discussion

The binding of CRP to FcγRII was demonstrated in cells transfected with the gene for human FcγRIIα. Binding was dose dependent and resembled the binding of CRP to the erythroblastic cell line, K-562, which expresses FcγRI as the only FcR. Because accessory molecules are not necessary for the expression of FcγRII, it is probable that CRP binds directly to FcγRII and not to associated molecules. This finding was also confirmed by the complete inhibition of CRP binding to K-562 and CD32-transfected COS cells by aggIgG. The binding and inhibi-
tion curves for the two cell types were similar. These studies suggest that not only is CRP capable of binding to FcγRI, there is reason to believe it is the only receptor on K-562 cells which express only the low affinity IgG receptor.

Precipitation of surface-labeled proteins from the monocytic cell line THP-1 with immobilized CRP demonstrated bands with the Mr of FcγRI (70 kD) and FcγRII (42 kD). The 42 kD band could be precleared from the lysate by adsorption with immobilized anti-CD32 mAb, confirming its identity as FcγRII (data not shown).

Our previous studies demonstrated that CRP also binds to the high affinity receptor for IgG, FcγRI (10, 11). These studies revealed that mononuclear cells were capable of binding CRP through FcγRI, but FcγRI could not account for all of the binding. Mononuclear cells and monocytic cell lines express FcγRII as well as FcγRI. Our current findings demonstrate the precipitation of both FcγRI and FcγRII from the THP-1 cell line, after pronase activation of FcγRII. However, treatment of U-937 cells with Bt2-cAMP, which increased FcγRII expression, also increased CRP binding, whereas treatment of U-937 cells with rIFN-γ to increase FcγRI did not increase CRP binding (our unpublished results). In our previous studies, binding to U-937 cells was completely inhibited by aggIgG but only 20% inhibited by monomeric IgG (10). These findings indicate that CRP binding to FcγRII accounts for most of the binding to these monocytic cell lines.

Taken together, our results support the hypothesis that CRP binding to phagocytic cells is mediated by FcRs. This is further supported by studies in a mouse model in which knockout mice lacking all three FcRs fail to bind CRP (Stein, M.-P., C. Mold, and T.W. Du Clos, manuscript in preparation). These findings thus suggest that there is no unique receptor for CRP, as was postulated previously, and that the major receptor for CRP on monocytic cells is FcγRIIa.

In conclusion, the acute phase protein CRP, an ancient mediator of innate immunity, binds to FcγRII, considered a receptor for the acquired immune response, with an affinity comparable to that of IgG. It is attractive to speculate that during the acute phase response, high levels of circulating CRP may influence signaling by IgG complexes through FcγRII. It remains to be determined whether CRP will have a positive or negative influence. Future studies will examine the direct effect of CRP on receptor activation and the effect of CRP on IgG-mediated signaling.

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