Human C-reactive protein does not bind to fcγRIIa on phagocyti cells

Eirikur Saeland, … , Lieke A.M. Sanders, Jan G.J. van de Winkel

Letter to the Editor

C-reactive protein (CRP) is an acute phase protein in humans with an important role in innate immunity. During inflammation it can be upregulated from a concentration of less than 1 μg/ml to as high as 500 μg/ml. CRP opsonizes foreign particles (1), activates complement (2), and can directly interact with phagocytic cells (3–6). The identification of CRP-binding receptors on phagocytic cells has been tedious, but in February 2000, Stein et al. reported in the JCI that CRP binds to FcγRIIa (CD32) and, more specifically, to the R131 polymorphic form of the receptor (6). Repeating these experiments and using different CRP-detecting antibodies of the same isotype used in that study (a mouse IgG1), we have confirmed that anti-CRP reagents can detect interaction between CRP and leukocytes (Figure 1a). CRP, indeed, bound the H131 form to a much lesser extent in spite of similar levels of FcγRIIa expression on cells (data not shown). Identical results were obtained with two different anti-CRP antibodies and using a number of secondary reagents. Upon biotinylation of anti-CRP antibody, however, binding to cells was abrogated, even though biotinylated antibodies effectively bound CRP in ELISA (data not shown). We then generated F(ab′)2 fragments of the mIgG1 anti-CRP antibodies by pepsin digestion, removed the Fc portion on a protein A column, and demonstrated purity of F(ab′)2 fragments by SDS-PAGE. […]

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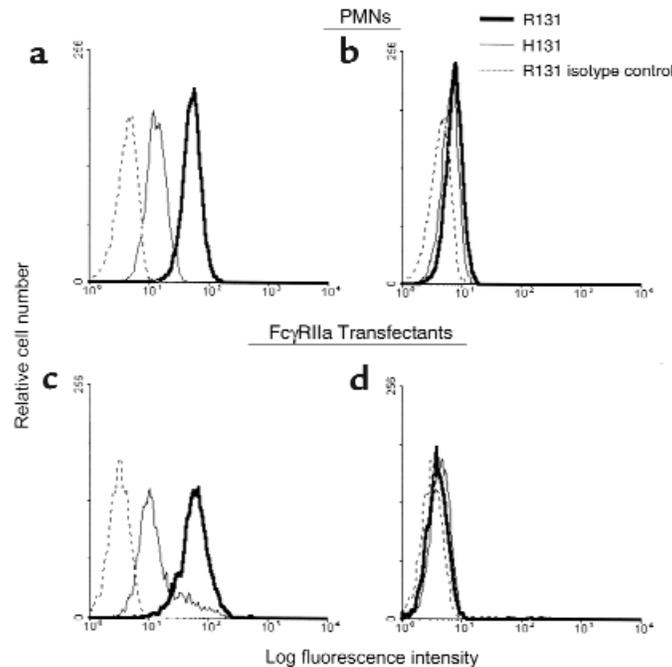## Human C-reactive protein does not bind to FcγRIIa on phagocytic cells

C-reactive protein (CRP) is an acute phase protein in humans with an important role in innate immunity. During inflammation it can be upregulated from a concentration of less than 1 µg/ml to as high as 500 µg/ml. CRP opsonizes foreign particles (1), activates complement (2), and can directly interact with phagocytic cells (3–6). The identification of CRP-binding receptors on phagocytic cells has been tedious, but in February 2000, Stein et al. reported in the *JCI* that CRP binds to FcγRIIa (CD32) and, more specifically, to the R131 polymorphic form of the receptor (6).

Repeating these experiments and using different CRP-detecting antibodies of the same isotype used in that study (a mouse IgG1), we have confirmed that anti-CRP reagents can detect interaction between CRP and leukocytes (Figure 1a). CRP, indeed, bound the H131 form to a much lesser extent in spite of similar levels of FcγRIIa expression on cells (data not shown). Identical results were obtained with two different anti-CRP antibodies and using a number of secondary reagents. Upon biotinylation of anti-CRP antibody, however, binding to cells was abrogated, even though biotinylated antibodies effectively bound CRP in ELISA (data not shown). We then generated F(ab')$_2$ fragments of the mIgG1 anti-CRP antibodies by pepsin digestion, removed the Fc portion on a protein A column, and demonstrated purity of F(ab')$_2$ frag-

ments by SDS-PAGE. No residual binding of CRP to FcγRIIa-R131 on polymorphonuclear leukocytes or FcγRIIa-transfected IIA1.6 cells could be detected using F(ab')$_2$ fragments (in a concentration range of 4–100 µg/ml) (Figure 1, b and d, and data not shown), even though the F(ab')$_2$ fragments effectively bound CRP in ELISA. An intact Fc region of anti-CRP antibodies was thus found to be crucial for binding of CRP to FcγRIIa.

Our data are in excellent agreement with earlier work, where it has been documented that mIgG1 binds preferentially to the R131 form of the receptor (7, 8). This observation is, furthermore, consistent with other work documenting that CRP binding to phagocytic cells does not require Fc receptors (3, 4). Our present data indicate that FcγRIIa cannot be considered a phagocytic CRP-binding molecule, although they do not exclude

Figure 1

Detection of CRP binding to FcγRIIa depends on Fc region of anti-CRP antibodies. Polymorphonuclear leukocytes (PMNs) were isolated from donors genotyped for FcγRIIa-R131 or H131 polymorphic forms. CRP was isolated from peritoneal fluid (kindly provided by C.E. Hack, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands), and 100 µg/ml CRP was incubated with 3×10^5 PMNs (**a** and **b**) or FcγRIIa-transfected IIA1.6 cells (**c** and **d**) in PBS with 10% BSA (Roche Nederland BV, Mijdrecht, The Netherlands) and 0.05% sodium azide for 1 hour at 4°C. Cells were washed and incubated with 50 µg/ml of a whole mouse IgG1 anti-CRP antibody (Clone CRP 8; Sigma Chemical Co., St. Louis, Missouri, USA) (**a** and **c**) or F(ab')$_2$ fragments of anti-CRP (**b** and **d**) for 30 minutes, washed again, and further incubated with an FITC-labeled goat F(ab')$_2$ anti-mouse κ light chain antiserum (Jackson ImmunoResearch Laboratories Inc., West Grove, Pennsylvania, USA) (**a**, **b**, and **d**) or FITC-labeled goat F(ab')$_2$ fragments of anti-mIgG1 (Southern Biotechnology Associates, Birmingham, Alabama, USA) (**c**) for 30 minutes. An FITC-labeled mIgG1 isotype control (DAKO A/S, Glostrup, Denmark) was included in all experiments, and cells were analyzed by flow cytometry. Data are representative of more than five individual experiments yielding almost identical results.

the possibility that CRP interacts with other receptors on these cells. We therefore wish to alert other investigators to the dangers of using whole antibodies for detection of CRP binding. Because of interaction with Fc receptors, this approach may significantly affect the outcome of in vitro analyses.

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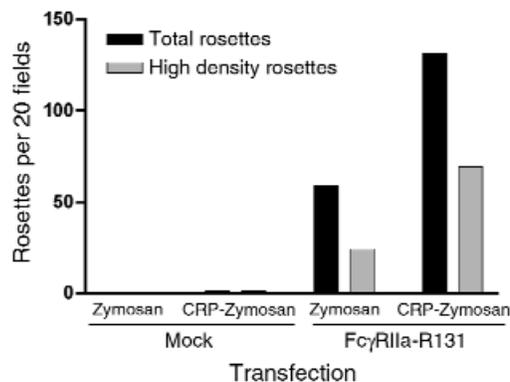
Reply to "Human C-reactive protein does not bind to FcγRIIa on phagocytic cells"

We welcome the opportunity to comment on the concerns of Dr. van de Winkel and colleagues about our observations that human FcγRIIa is a receptor for C-reactive protein (CRP).

The original observation that human FcγRIIa is the major receptor for human CRP, reported in the *Journal of Experimental Medicine* (1), was based on the specific and saturable binding of CRP to COS cells transfected with human FcγRIIa. That observation was supported by the direct immunoprecipitation by immobilized CRP of FcγRIIa from THP-1 cells (see Figure 5 in ref. 1), independent of any antibody-mediated interactions. Further evidence for the role of FcγRIIa as the major receptor for CRP was provided in our paper in the *JCI* (2). In this work, we demonstrated that, independent of antibody, CRP directly elicits a rise in intracellular Ca^{2+} levels in polymorphonuclear leukocytes (PMNs) from donors homozygous for the R131 allele of FcγRIIa but not in donors homozygous for the H131 allele (see Figure 4 in ref. 2). Additional evidence that CRP does indeed bind to human FcγRIIa is shown by our recent studies documenting rosetting of CRP-opsonized zymosan by FcγRIIa-transfected COS cells (Figure 1 in this letter). In these studies, we have focused on transfection of the R131 allele of FcγRIIa because of the preferential binding of CRP to that allele compared with the H131 allele (2).

The concern, expressed by van de Winkel and colleagues, about the use of mAb 2C10 in the detection of CRP binding does raise an appropriate technical caution about murine IgG subclasses and their interaction with the human FcγRIIa H131/R131 polymorphism. We would also raise a second technical caution. In the data provided by van de Winkel and colleagues, there is a striking lack of binding of CRP to human PMNs. Numerous groups over the past 15 years have shown specific, saturable, and antibody-independent binding of CRP to neutrophils (3–8). These binding studies have been supported by functional studies including the demonstration of phagocytosis of CRP-opsonized erythrocytes by neutrophils and inhibition of this activity by modulation of FcγR (9). The inability to detect CRP binding to human PMN with the detection strategy employed by van de Winkel makes interpretation of their data regarding FcγRIIa-transfected COS cells ambiguous.

Multiple lines of evidence indicate that CRP does bind to human FcγRIIa independently of the anti-CRP mAb 2C10. The data provided by van de Winkel do not refute our observations, but they do underscore the need for careful attention to technical details in working with CRP. We would also emphasize that CRP binding is not limited to human FcγRs.

Murine FcγR have the same capacity (10–12). Binding studies using various FcγR-deficient mouse strains identified FcγRI and FcγRII as CRP receptors in the mouse and showed that cells from mice lacking all FcγR do not bind CRP (12). These results were confirmed by phagocytosis assays using CRP-opsonized zymosan (13).

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Figure 1
COS cells were transfected with FcγRIIa. After 48 hours, cells were washed and incubated with zymosan or CRP-zymosan. After washing, the cells were examined for attached FITC-zymosan. Twenty low-power fields were counted. Results are expressed as the total number of rosettes counted and the number of rosettes with greater than 20 zymosan particles attached per cell.