

## INTERACTIONS OF C-REACTIVE PROTEIN WITH THE COMPLEMENT SYSTEM

### III. Complement-Dependent Passive Hemolysis Initiated by CRP\*,‡

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C-reactive protein (CRP)<sup>1</sup> is a trace constituent of normal blood which increases as much as 1,000-fold during the acute phase of most inflammatory reactions (2–4). It is characterized in part by its unique calcium-dependent precipitation reaction with the C-polysaccharide (CPS) (5,6), a cell wall teichoic acid of the pneumococcus (7); this specificity is directed largely towards the phosphorylcholine moiety of the CPS (8). Previous studies have indicated that CRP is a potent activator of the classical complement (C) pathway not only when reacting with CPS and choline phosphatides (9), but also in the presence of a variety of synthetic and naturally occurring polycations (10,11). Such studies have stressed the efficient activation and consumption of C components C1–C5. The experiments described herein were undertaken in an attempt to determine whether the consumption of C initiated by CRP was reflecting a constructive activation which could result in the generation of full hemolytic activity.

#### Materials and Methods

**CPS.** A Cs-capsulated variant of pneumococcus (12) (generously provided by Dr. Gerald Schiffman, Department of Microbiology, Downstate Medical Center, Brooklyn, N. Y.) was grown in brain heart infusion broth (Difco Laboratories, Detroit, Mich.), collected by centrifugation (10,000 *g*), and washed in saline. The CPS was extracted by the method of Anderson and McCarty (13) as modified by Gotschlich and Liu (14), except that enzymic digestion was omitted. For affinity chromatography Bio-Gel A-50 m (50–100 mesh) (Bio-Rad Laboratories, Richmond, Calif.) was activated with cyanogen bromide under standard conditions (15), and CPS was attached to give a final concentration of 2–5 mg/ml settled bead volume.

**Isolation of CRP.** CRP was isolated from pleural or ascitic fluids obtained from patients undergoing therapeutic paracentesis. The fluids were centrifuged 5,000 *g* for 15 min, stored at 4°C with 0.1% sodium azide, and clarified by filtration through Celite (Hyflo Super-Cel, Johns-Manville Products Corporation, Lompoc, Calif.) immediately before fractionation. CRP was

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<sup>1</sup> *Abbreviations used in this paper:* C, complement; CPS, C-polysaccharide; CRP, C-reactive protein; E, erythrocyte; GVB, gelatin-Veronal-buffered saline with optimal divalent cations; E-CPS; sheep erythrocytes to which CPS has been attached; SDS, sodium dodecyl sulfate; T-15, IgA antiphosphorylcholine myeloma protein from the TEPC-15 myeloma of BALB/c mice.

collected from the fluids upon passage through a CPS-Bio-Gel A-50 m column (2.5 × 15 cm) at 0.3–1.0 liters/h. The column was washed with Tris-buffered saline (pH 8.0, 2 mM calcium chloride) until  $A_{280 \text{ nm}}$  was <0.05, and the CRP was selectively eluted with isotonic Tris-citrate buffer (pH 8.0) at 50–100 ml/h. Fractions containing high concentrations of CRP were pooled and further purified by filtration through a Bio-Gel A-0.5 m (Bio-Rad Laboratories) column (2.5 × 95 cm) to remove aggregates and subunits of CRP that accumulated during preparation and storage. Fractions containing intact CRP were pooled, stored at 4°C, and refractionated by gel filtration when required.

**Anti-CPS Antibodies.** Rabbits were immunized by six intravenous injections (three per wk) of a vaccine prepared by formalinization of the Cs-pneumococcus (16). Antisera were obtained 6–14 days after the final injection and contained 10–15 mg antibody/ml by quantitative precipitin analysis. These antisera were shown to be free of antiphosphorylcholine antibody by the absence of inhibition of precipitation by phosphorylcholine and to be 7S (IgG) by the resistance of passive hemagglutinating activity to 2-mercaptoethanol. Such antisera were heat inactivated (56°C, 1 h) before use.

The murine TEPC-15 IgA myeloma of BALB/c mice was generously provided by Dr. D. A. Rowley of the University of Chicago and was maintained by serial passage in female BALB/cJ mice 6–8 wk of age. The myeloma protein (T-15) was isolated from the ascites fluid of tumor-bearing mice by affinity chromatography on CPS-Bio-Gel A-50 m columns (15,17), and the dimeric IgA was isolated by gel filtration through Bio-Gel A-0.5 m as described above.

**Solid-Phase Immunoabsorbents.** Antiserum to human C1q was obtained from Behring Diagnostics, Somerville, N. J., and polyvalent antiserum to human immunoglobulins was obtained from Miles Laboratories, Kankakee, Ill. Immunoabsorbents were prepared after separation of IgG by repeated precipitation with 16% sodium sulfate and resuspension in 20% sodium sulfate; 0.1 vol glutaraldehyde (2.5%) was added, and the suspension was stirred at room temperature for 1 h. The insolubilized gamma globulin was washed repeatedly with Veronal-buffered saline and stored at 4°C until required; such insolubilized antibodies retained strong antigen-binding capacity.

**Immuno-electrophoresis.** Immuno-electrophoresis was performed on glass plates by the method of Scheidegger (18).

**Polyacrylamide Gel Electrophoresis.** CRP was examined by polyacrylamide gel electrophoresis in the presence and absence of standard proteins in sodium dodecyl sulfate (SDS) exactly according to the method of Neville (19). Gels stained with Coomassie Brilliant Blue were analyzed densitometrically with a gel scanner attachment to a Beckman Acta CV spectrophotometer (Beckman Instruments, Spinco Div., Palo Alto, Calif.).

**C Sources.** Human serum was obtained from healthy blood donors as previously described (10) and from a patient recently defined<sup>2</sup> as congenitally deficient in C6; sera were stored at –70°C until required. Fresh frozen guinea pig serum was obtained from Texas Biological Laboratories, Inc., Fort Worth, Tex., and C4-deficient guinea pig serum was prepared from C4-deficient guinea pigs (20) maintained in our laboratory. Before use all sera were absorbed by passage at 4°C through CPS-Bio-Gel A-50 m columns to remove natural antibody to CPS. 10 ml (or less) serum was absorbed per ml column bed volume, and filtration rates did not exceed 2 bed vol/h; under these conditions no significant loss of C activity was observed. Natural antibody to sheep erythrocytes (E) was removed by repeated 15–30-min absorptions (30% E by volume) at 0°C; it was found necessary to absorb human sera five times and guinea pig sera three times to remove detectable hemolytic antibodies. Total hemolytic C was measured by standard procedures (21). Certain sera were pretreated with several agents to result in total (>99%) depletion of classical hemolytic C activity: these included zymosan (4 mg/ml serum, 37°C, 1 h), immune complexes (BSA–anti-BSA complexes formed at equivalence, 100 µg/ml, 1 h, 37°C), hydrazine (18 mM, 37°C, 2 h), and heat inactivation (56°C for 1 h). Human C1q was prepared according to the method of Agnello et al. (22) and was essentially free of contaminating immunoglobulins; human C4 and C6 were purchased from Cordis Laboratories, Miami, Fla.

**Passive Hemagglutination and Hemolysis.** E were washed in saline and brought to 50% packed

<sup>2</sup> Lim, D., A Gewurz, T. F. Lint, S. Chaze, and H. Gewurz. Absence of the sixth component of complement in a patient with repeated episodes of meningococcal meningitis. Submitted for publication.

TABLE I  
Purification of CRP from Human Ascites Fluid

| Step                       | Volume    | Protein                  | CRP*      | Purification factor | Recovery |
|----------------------------|-----------|--------------------------|-----------|---------------------|----------|
|                            | <i>ml</i> | <i>A<sub>280</sub> U</i> | <i>mg</i> |                     | <i>%</i> |
| Ascites fluid‡             | 7,500     | 380,000                  | 470       | 1                   | 100      |
| 1. Affinity chromatography | 62        | 618                      | 320       | 417                 | 68       |
| 2. Gel filtration          | 97        | 390                      | 210       | 430                 | 44       |

\* CRP was determined by radial immunodiffusion.

‡ Ascites fluid was clarified and subjected to affinity chromatography exactly as described.

cell volume. These were sensitized with C polysaccharide by the chromium chloride method (23) as suggested by Cosenza et al. (24): 0.5-ml aliquots of E were mixed with a 0.5 ml CPS solution (0.1–10 mg/ml), an equal volume of CrCl<sub>3</sub> (0.5 mg/ml) was added, and the mixture was incubated with occasional mixing for 5 min at room temperature. The erythrocytes so sensitized (E-CPS) were washed three times by centrifugation with gelatin-Veronal-buffered saline containing optimal divalent cations (GVB) (21), resuspended to a final concentration of 10<sup>9</sup> E-CPS/ml in this buffer, and stored at 4°C until required; the cells were stable for 1–3 wk. Hemagglutination was performed in microtiter plates (Cooke Laboratory Products, Alexandria, Va.) and recorded as minimum concentrations required for complete agglutination after 2 h at room temperature.

To test for passive lysis of E-CPS, cell suspensions ( $2 \times 10^8$ /ml) were added to equal volumes of CRP or antibody dilution and incubated with gentle mixing for 1 h at 0°C; the cells were washed in ice-cold GVB and resuspended to 10<sup>8</sup>/ml. C dilutions were prepared in GVB (0.5 ml) and  $5 \times 10^7$  E-CPS sensitized with CRP or antibody were added. The mixtures were incubated at 37°C for 1 h, diluted with 6.5 ml GVB, and centrifuged, and the  $A_{412 \text{ nm}}$  of the supernate was determined. The degree of specific lysis was calculated based on the absorbance of an equivalent volume of cells totally lysed in water, correcting for the lytic activity and  $A_{412 \text{ nm}}$  of the C source and is expressed as a percentage.

## Results

*Purification and Characterization of CRP.* A simple affinity chromatography procedure was applied for the isolation of CRP in order to circumvent the hazards associated with exposure to organic solvents and high salt concentrations (Table I). All steps were performed at 0–4°C, and no change in ionic strength or pH was necessary. Serous fluids were clarified by centrifugation and filtration and passaged through CPS-agarose columns on which CRP was quantitatively retained. After thorough washing with calcium-containing buffers, the CRP was eluted with citrate-buffered saline. This resulted in a purification greater than 400-fold and a recovery of 68%. An aliquot of this CRP preparation was further purified by passage through Bio-Gel A-0.5 m; although this did not provide a dramatic increase in purity (Fig. 1, Table I), it did serve to remove aggregates and subunits of CRP that accumulated during preparation and storage. This step also took advantage of a weak association between agarose and CRP which served to reduce potential contamination with IgG, demonstrated by the marked asymmetry of the peak and its elution in a region of lower molecular weight than that anticipated for CRP.

CRP, so prepared, failed to react even at high concentrations with antisera to normal human serum, and on immunoelectrophoresis developed with antisera

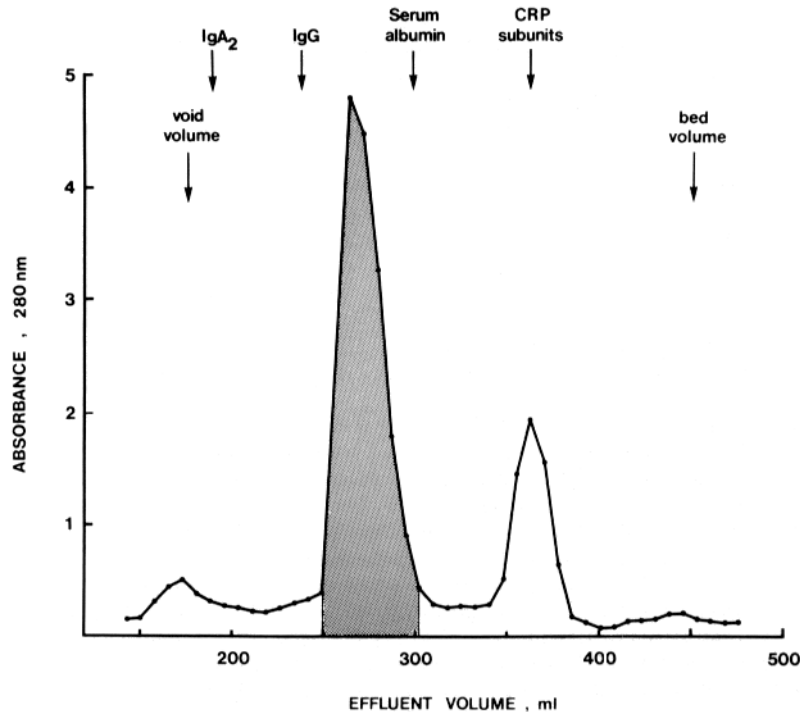


FIG. 1. Passage of CRP (shaded area) prepared by affinity chromatography through Bio-Gel A-0.5 m (column:  $2.5 \times 95$  cm) in Tris-buffered saline, pH 8.0. Elution characteristics of the column were established by passage of a mixture of standard proteins as indicated; CRP subunits were identified by their electrophoretic pattern in SDS-polyacrylamide gel and reappearance upon rechromatography of the main peak.

to CRP appeared as a single band in the region of fast migrating gamma globulins (Fig. 2). When electrophoresed in polyacrylamide gels in the presence of SDS (Fig. 3), all CRP preparations examined showed a major component of an estimated  $23,300 \pm 500$  daltons and a minor component which migrated slightly faster. The analogous rabbit protein could be prepared in an identical way and resulted in a similar electrophoretic pattern.

*CRP-Mediated Agglutination of CPS-Coated Erythrocytes and Lysis by Human C.* Sensitization of washed sheep E with various concentrations of CPS (0.1–10 mg/ml) resulted in cells (E-CPS) that were readily agglutinated upon the addition of small amounts (0.4–3.2  $\mu\text{g}/\text{ml}$ ) of CRP, rabbit anti-CPS, or T-15 myeloma protein, indicating adequate sensitization with the pneumococcal C-polysaccharide. It was of interest that CRP was as efficient in bringing about this hemagglutination as were the two antibody preparations (Table II).

The E-CPS were sensitized for lysis by incubation at  $0^\circ\text{C}$  for 1 h with various concentrations of antibody or CRP and tested in dilutions of human or guinea pig sera which had been extensively absorbed with both CPS and sheep E to remove natural antibodies. The dose-response curve for lysis of such cells by human C ( $2 \text{ CH}_{50}$ ) after sensitization with 10  $\mu\text{g}/\text{ml}$  CRP is shown in Table II and Fig. 4. It is clear that efficient lysis, comparable to that seen with rabbit anti-

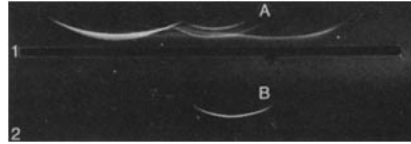


FIG. 2. Immunoelectrophoresis of (A) normal human serum and (B) CRP (2 mg/ml) purified as described, developed with (1) goat antinormal human serum, and (2) rabbit antihuman CRP; the anode is at the left.

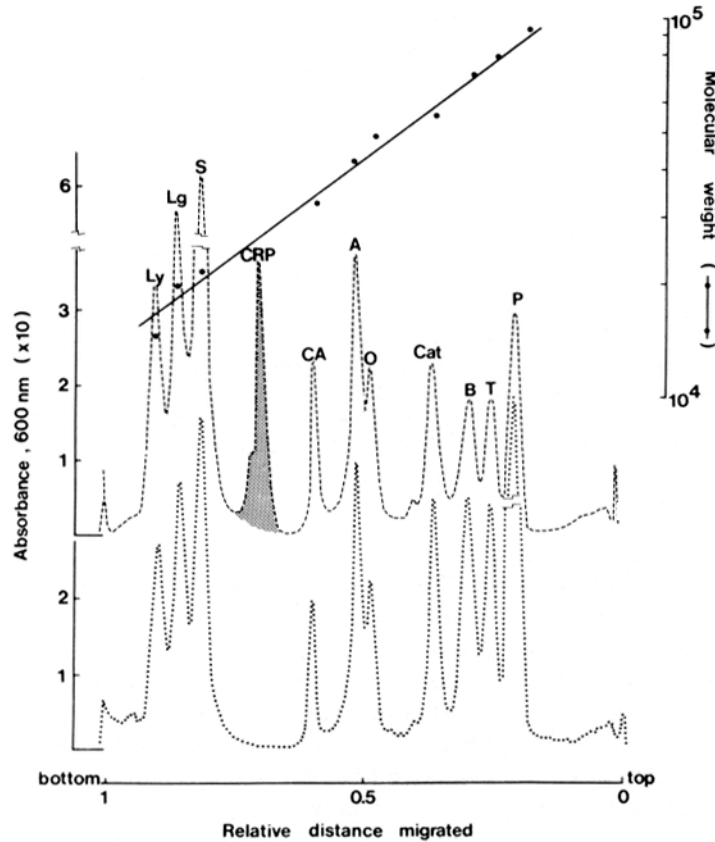


FIG. 3. Densitometric analysis of SDS-polyacrylamide gels (19) after electrophoresis of a mixture of standard proteins in the presence (---) and absence (···) of CRP (shaded area). All preparations of CRP revealed the presence of a major component ( $23,300 \pm 500$  daltons) and a slightly faster migrating minor component. The molecular weight of the major component was calculated by regression analysis based on the migration of the following standard proteins: Ly, lysozyme; Lg,  $\beta$ -lactoglobulin; S, soybean trypsin inhibitor; CA, carbonic anhydrase; A, rabbit muscle aldolase; O, ovalbumin; Cat, bovine hepatic catalase; B, bovine serum albumin; T, human transferrin; and P, phosphorylase-a.

CPS, was initiated by CRP. Increased lysis was observed as the amount of CPS used for sensitization was increased, and 5 mg/ml was used for most of the subsequent experiments. No lysis was observed after sensitization with the T-15 IgA antibody.

TABLE II  
Sensitization of E with CPS for Agglutination and Lysis

| Experi-<br>ment* | CPS          | Hemagglutination‡ |              |      | Specific hemolysis§ |     |          |     |          |     |
|------------------|--------------|-------------------|--------------|------|---------------------|-----|----------|-----|----------|-----|
|                  |              | CRP               | Anti-<br>CPS | T-15 | CRP                 |     | Anti-CPS |     | T-15     |     |
|                  |              |                   |              |      | HuC                 | GPC | HuC      | GPC | HuC      | GPC |
|                  | <i>mg/ml</i> |                   | <i>µg/ml</i> |      | <i>%</i>            |     | <i>%</i> |     | <i>%</i> |     |
| I                | —            | >100              | >100         | >100 | <5                  | <5  | <5       | <5  | <5       | <5  |
|                  | 0.1          | 1.6               | 0.4          | 0.8  | 11                  | <5  | 19       | 55  | <5       | <5  |
|                  | 0.3          | 0.8               | 0.4          | 1.6  | 32                  | <5  | 39       | 81  | <5       | <5  |
|                  | 1.0          | 0.8               | 0.4          | 1.6  | 59                  | 5   | 58       | 89  | <5       | <5  |
|                  | 3.0          | 0.8               | 0.8          | 3.2  | 85                  | 12  | 59       | 90  | <5       | <5  |
|                  | 10.0         | 0.8               | 0.8          | 3.2  | 97                  | 44  | 86       | 81  | <5       | 7   |
| II               | 5.0          | 0.4               | 0.6          | 2.4  | 94                  | 69  | 100      | 100 | ND       | ND  |
| III              | 5.0          | ND                | ND           | ND   | 93                  | 65  | 100      | 91  | ND       | ND  |
| IV               | 5.0          | 0.8               | 0.2          | ND   | 100                 | 56  | 98       | 100 | ND       | ND  |

\* Results of four separate experiments using different preparations of CRP and sensitized E.

‡ Minimum concentration required for complete hemagglutination.

§ Maximum percent lysis of CPS-coated E, presensitized with 10 µg/ml CRP, rabbit anti-CPS, or T-15 upon addition of 2 (Expt. I) and 3 (Expt. II-IV) CH<sub>50</sub> human C or 3 (Expt. I) and 10 (Expt. II-IV) CH<sub>50</sub> guinea pig C.

|| ND, not determined.

The dependence of this reaction on both CRP and C levels was further characterized. It can be seen in Fig. 5 that lysis of a given cell population was greater as larger amounts of CRP were used for sensitization; 5–10 µg/ml was optimal for lysis of cells sensitized with 0.1–10.0 mg/ml CPS in the presence of 2 CH<sub>50</sub>/ml. It can also be seen that both CRP and rabbit anti-CPS at 1 and 10 µg/ml were quantitatively and qualitatively similar in sensitizing E-CPS for lysis by human C (Fig. 6; Table II). Even cells suboptimally sensitized could be lysed when sufficient C was available (Fig. 6). Absorption of CRP solutions (1–100 µg/ml) with sufficient insolubilized polyvalent antihuman immunoglobulin to bind >50 µg human IgG failed to reduce their capacity to sensitize for hemolysis.

To determine whether the lysis observed indeed was C dependent, sera depleted of or deficient in C were tested for their ability to support lysis (Table III). C inactivation by zymosan, immune complexes, hydrazine, or heating resulted in loss of ability to support CRP-mediated hemolysis of E-CPS; the minimal residual lysis seen selectively with hydrazine-treated serum is discussed below. Serum from an individual<sup>2</sup> congenitally deficient in C6 was unable to lyse E-CPS sensitized with CRP or antibody, and this activity could be partially reconstituted by the addition of purified human C6.

*CRP-Mediated Lysis of E-CPS by Guinea Pig C.* Suitably absorbed guinea pig C also could induce lysis of CRP-sensitized E-CPS, although much less efficiently than human C, and larger amounts of CPS were required to sensitize

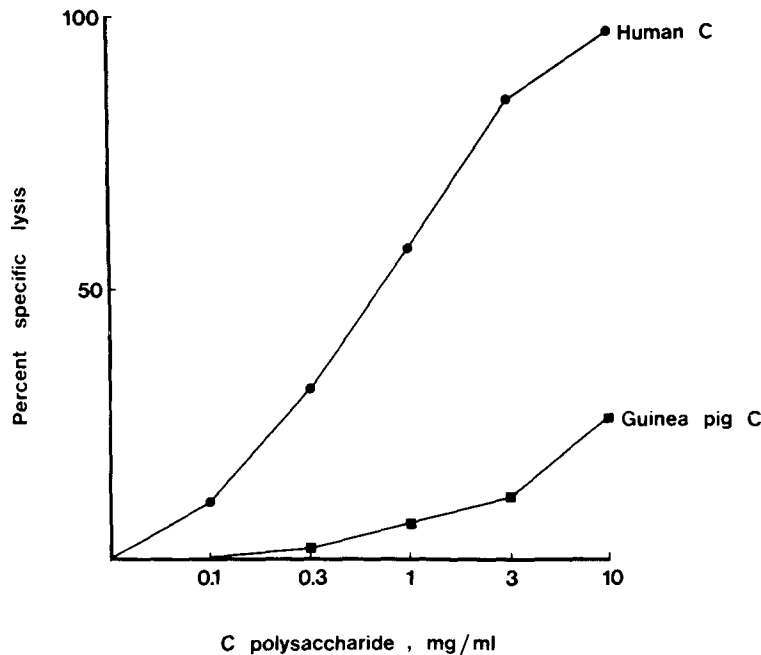


FIG. 4. Lysis of E-CPS-CRP ( $5 \times 10^7$ ), coated with 0.1-10.0 mg CPS/ml and presensitized with  $10 \mu\text{g}$  CRP/ml, by human ( $2 \text{ CH}_{50}$ ) and guinea pig ( $3 \text{ CH}_{50}$ ) sera in a total volume of 1.0 ml during 1-h incubation at  $37^\circ\text{C}$ .

E even for partial lysis (Fig. 4). Distinct though incomplete lysis was observed using cells sensitized with 1-10 mg CPS/ml and 1-10  $\mu\text{g}$  CRP/ml when 3  $\text{CH}_{50}$  guinea pig C/ml were added (Fig. 5); higher concentrations of CPS might have enabled the preparation of cells that could be more extensively lysed by guinea pig C, but the limited availability of CPS precluded such experiments. It may be noted (Table II) that whereas antibody-sensitized E-CPS were much more efficiently lysed by guinea pig than by human C, guinea pig C was much less effective in the lysis of CRP-sensitized cells. The profound differences observed when guinea pig C was reacted with E-CPS sensitized with increments of antibody and CRP, respectively, also is shown in Fig. 6. Again, whereas antibody at both 1 and 10  $\mu\text{g}/\text{ml}$  resulted in greater lysis in the presence of guinea pig than human C, CRP induced much less lysis in the presence of guinea pig C; 10-fold more CRP and 3-5-fold more C were required.

In earlier studies on the consumption of C by CPS-CRP complexes, Volanakis and Kaplan had reported the requirement of human C1q for consumption of guinea pig C by CRP (25). It therefore was of interest to test whether CRP-mediated hemolysis by guinea pig C could be potentiated by the addition of human C1q (Fig. 7). The addition of increasing amounts of C1q to various concentrations of guinea pig C enhanced lysis more effectively after sensitization at low concentrations than at high concentrations of CRP; a similar maximal extent of lysis at the intermediate and high levels indicated that optimal conditions had been established. The addition of human C1q to guinea pig C failed to affect the extent of lysis of antibody-sensitized E-CPS. Immunoab-

## C-DEPENDENT PASSIVE HEMOLYSIS INITIATED BY CRP

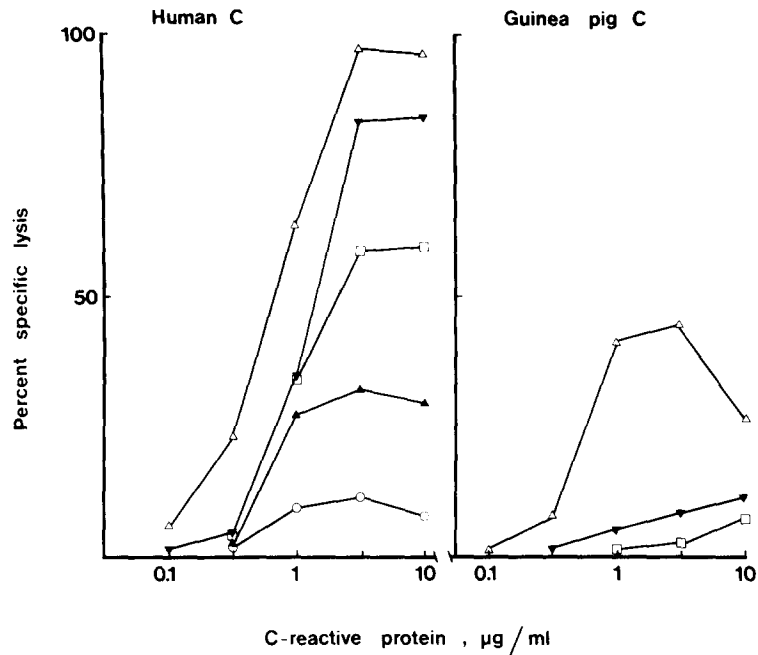


FIG. 5. Sheep E were coated with 10.0 ( $\Delta$ - $\Delta$ ), 3.0 ( $\nabla$ - $\nabla$ ), 1.0 ( $\square$ - $\square$ ), 0.3 ( $\blacktriangle$ - $\blacktriangle$ ), and 0.1 ( $\circ$ - $\circ$ ) mg CPS/ml, sensitized with 0.1-10.0  $\mu$ g CRP/ml, and lysed with human (2  $CH_{50}$ ) or guinea pig (3  $CH_{50}$ ) C.

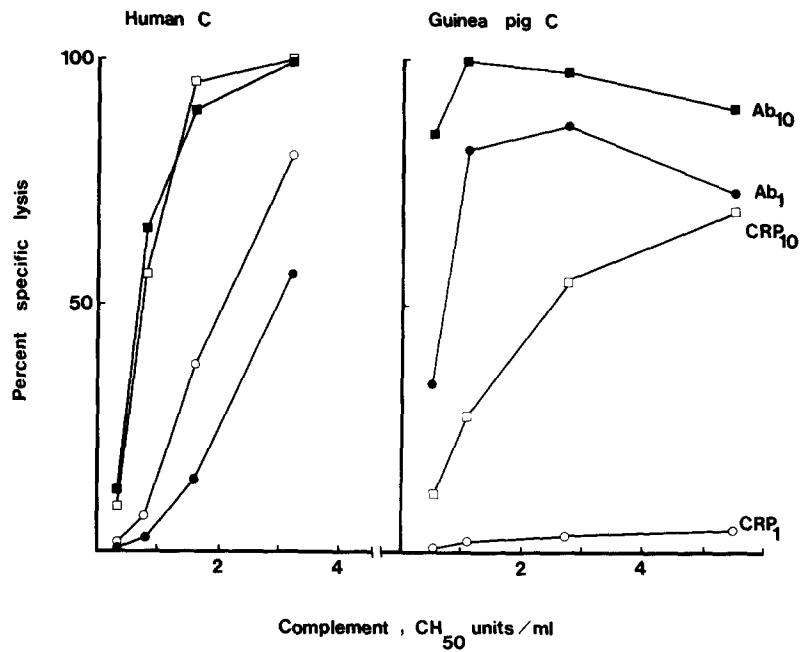


FIG. 6. Sheep E coated with 5 mg/ml CPS (E-CPS) were sensitized with human CRP (10  $\mu$ g/ml,  $\square$ - $\square$ ; 1  $\mu$ g/ml,  $\circ$ - $\circ$ ) or rabbit anti-CRP (10  $\mu$ g/ml,  $\blacksquare$ - $\blacksquare$ ; 1  $\mu$ g/ml,  $\bullet$ - $\bullet$ ) and lysed with varying amounts of human (left) or guinea pig (right) C.



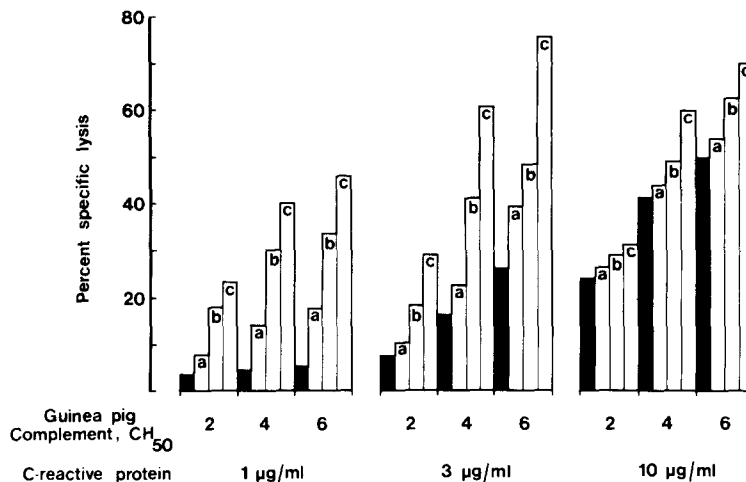


FIG. 7. Sheep E were coated with 5 mg/ml CPS, presensitized with 1, 3, and 10  $\mu$ g/ml human CRP, respectively, and lysed by 2, 4, and 6  $\text{CH}_{50}$  GPC in the presence of 1 (a), 3 (b), and 10 (c)  $\mu$ g human C1q/ml; the solid bars denote lysis in the absence of human C1q. While human C1q potentiated CRP-mediated lysis, particularly at the lower concentrations of CRP, extensive lysis by GPC was observed in the absence of added C1q.

sorption of a CRP solution with sufficient amounts of insolubilized anti-C1q antibody to bind an equivalent weight of C1q did not reduce the extent of hemolysis by guinea pig serum. Thus, in contrast to the studies cited above, an absolute requirement for human C1q in human CRP-guinea pig C interactions was not observed.

C-depleted and C4-deficient guinea pig sera were tested for their ability to support lysis (Table III). Inactivation by zymosan, immune complexes, hydrazine, and heat resulted in loss of specific lysis activity. However, this activity was only partially reduced in C4-deficient guinea pig serum, and an increment of activity was restored upon addition of purified human C4. E-CPS sensitized with anti-CPS also were lysed by C4-deficient sera. These observations suggest that CRP can activate an alternative C pathway, perhaps in an entirely analogous manner to activation by antibody of the C1-dependent bypass (26,27).

### Discussion

CRP has been found to be a potent activator of the C system when reacting with several of its substrates (9-11). This and other similarities of CRP with the immunoglobulins (reviewed in reference 11) led us to explore whether CRP could initiate and support the full hemolytic C sequence at the surface of an appropriately sensitized cell.

A new mode of preparation of CRP, involving affinity chromatography on Bio-Gel beads to which CPS had been cross-linked (15), was adapted for this purpose. This allowed purification of CRP from larger volumes of human serous fluids and resulted in higher yields and purity than were achieved by earlier methods of affinity chromatography (28,29). The purity of CRP so prepared was established by gel filtration chromatography, immunoelectrophoresis, and electrophoresis in SDS-polyacrylamide gels. The migration of CRP in SDS-polyacrylamide

TABLE III  
Complement Dependence of Passive Lysis of E-CPS by CRP and Antibody

| C source†<br>Treatment/Deficiency    | Specific lysis* |          |            |          |
|--------------------------------------|-----------------|----------|------------|----------|
|                                      | Human           |          | Guinea pig |          |
|                                      | CRP             | Anti-CPS | CRP        | Anti-CPS |
|                                      | %               | %        | %          | %        |
| Zymosan; 37°C, 60 min                | 93              | 100      | 65         | 91       |
| Antigen-antibody; 37°C, 60 min       | <5              | 7        | <5         | 21       |
| Hydrazine (0.02 M); 37°C, 120 min    | <5              | 8        | <5         | 9        |
| Heat; 56°C, 60 min                   | 20              | 17       | <5         | 12       |
|                                      | <5              | 8        | <5         | 8        |
| C6 deficient                         | <5              | <5       |            |          |
| C6 deficient + C6 (1,000 U/ml serum) | 38              | 32       |            |          |
| C4 deficient                         |                 |          | 49         | 94       |
| C4 deficient + C4 (4,000 U/ml serum) |                 |          | 75         | 100      |

\* Maximum percent lysis of E-CPS presensitized at a concentration of 10  $\mu$ g/ml CRP or rabbit anti-CPS upon incubation in human or guinea pig C.

† Absorbed human (1:5) and guinea pig (1:10) sera were pretreated as described or congenitally deficient and lacked hemolytic activity in standard C assays.

gels also was consistent with its reported composition of six noncovalently-linked subunits which were described as probably identical (30). In the present study two distinct components were invariably observed: a major subunit of approximately 23,300 $\pm$ 500 daltons and a minor more rapidly migrating component. The isolation and characterization of this minor component are in progress and should reveal whether it is an additional subunit of CRP.

The ability of CRP to sensitize for C-dependent hemolysis was tested using E-CPS. CRP was as effective on a weight basis in hemagglutination tests using these cells, as were rabbit anti-CPS and the mouse antiphosphorylcholine T-15 myeloma protein, confirming a previous report of CRP possessing such activity (31). When sensitized with CRP, E-CPS were lysed by human C as efficiently as were cells sensitized with rabbit anti-CPS; the number of CRP molecules required for such lysis has not yet been determined. Experiments both with sera in which classical C hemolytic activity was inactivated by a variety of procedures and with C6-deficient serum established this lysis as C dependent. These results show that CRP can initiate a fully constructive C sequence to result in assembly of the C attack mechanism and lysis of cells to which it is attached. Since CRP also can induce extensive consumption of C components C1-C5 (11) and sufficient C4 and C3 coating of E-CPS to mediate the adherence and phagocytic reactions dependent upon these components,<sup>3</sup> it would seem that

<sup>3</sup> Mortensen, R. F., T. F. Lint, A. P. Osmand, and H. Gewurz. 1975. Interaction of C-reactive protein (CRP) with lymphocytes and monocytes: complement dependent opsonic adherence and phagocytosis. Proceedings of the 10th Leukemia Culture Conference. V. P. Eijssvoegel, editor. Academic Press, Inc., New York. In press.

CRP is capable of activating the C system to generate many if not all of its associated functions. Whether CRP can react similarly with biologically altered cells to result in C-dependent lysis and opsonization and, if so, the nature and mode of exposure of the cellular sites binding CRP, are yet to be established.

Human CRP also could sensitize E-CPS for C-dependent lysis by guinea pig serum, but much less (approximately 10-fold) efficiently than antibody. It was previously reported (25) that human C1q is required for CRP-CPS reactions to initiate consumption of guinea pig C. Although human C1q did indeed potentiate hemolysis by guinea pig C, no absolute requirement for human C1q was observed. The mechanism underlying the potentiation of lysis by human C1q (which was not seen during C-dependent lysis of antibody-sensitized E-CPS) and the dynamics of its interactions with the subcomponents of guinea pig C1 are not yet clear. It was of interest that lysis of CRP-sensitized E-CPS was observed in C4-deficient as well as in normal guinea pig sera. This may explain the residual lysis of E-CPS by human sera inactivated by hydrazine, which had resulted in complete depletion of C4 but only partial depletion of C3. It has been reported (26,27) that sheep E heavily sensitized with antibody could be lysed by a mechanism independent of C4 or C2 which required components of the alternative C pathway and a protein similar or identical to C1. Perhaps CRP also shares with antibody the ability to activate this C1-dependent bypass. The hemolytic systems described herein involving E-CPS sensitization with either CRP or rabbit anti-CPS resulted in more extensive hemolysis with lesser amounts of C than did hemolysin-sensitized sheep cells in the studies just cited (26); perhaps polymeric antigens such as CPS favor this mode of C activation, particularly when reacting as complexes preformed with antibody or CRP, and will provide a model more suitable for analysis of the mechanism underlying the C1-dependent bypass.

The results presented herein raise the possibility that a structural similarity of CRP to antibody underlies its propensity to activate C; studies to define the amino acid sequence of CRP directed to detecting such a homology are in progress. The ability of CRP to activate the C system and sensitize for C-dependent hemolysis extends its functional analogies with antibody which already include the capacity to induce precipitation (5,6), agglutination (31), and opsonic (32) reactions and to react with lymphocytes (33) and platelets (34). Taken together with the appearance during inflammatory reactions of CRP and the substances with which it interacts, these considerations suggest that like the immunoglobulins, CRP has an important role in initiating and modulating reactions of host defense and inflammation.

### Summary

Interactions of CRP with various substrates in the presence of human serum have been shown to result in efficient activation of C components C1-C5. We now report the ability of CRP to initiate C-dependent hemolysis. For this purpose CRP was isolated by affinity chromatography using pneumococcal CPS and gel filtration; its purity was established by several criteria. Erythrocytes were coated with CPS (E-CPS) and passively sensitized with CRP. C-dependent lysis of these cells was observed upon the addition of suitably absorbed human

serum, and the efficiency of hemolysis compared favorably with that initiated by rabbit IgG anti-CPS antibody. CRP also sensitized E-CPS for lysis by guinea pig C; partial lysis was seen when C4-deficient guinea pig serum was used, suggesting that CRP also shares with antibody the ability to activate an alternative C pathway. These observations demonstrate the ability of CRP to fully activate the C system and provide further evidence for a role for CRP similar to that of antibody in the initiation and modulation of inflammatory reactions via the complement system.

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