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INTERACTION OF C-REACTIVE PROTEIN WITH LYMPHOCYTES AND MONOCYTES: COMPLEMENT-DEPENDENT ADHERENCE AND PHAGOCYTOSIS[1]

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The serum constituent C-reactive protein (CRP), which activates the classical complement (C) pathway when reacting with its substrates, was examined for its ability to mediate reactions of opsonic adherence and phagocytosis. Erythrocytes coated with C-polysaccharide (CPS) and reacted with CRP (E. CPS-CRP) failed to adhere to B cells and displayed only minimal adherence to monocytes. However, upon the addition of absorbed C or purified C components these cells were found to possess the cleavage products C4b and C3b, which in turn resulted in attachment of these cells to both human B lymphocytes and peripheral blood monocytes. E. CPS-CRP treated with C in the absence of antibody were readily phagocytosized by glass-adherent human monocytes. The phagocytosis of E. CPS-CRP-C was not only mediated by CRP but also required the presence of CRP on the surface of the red cells. The extent of ingestion was proportional to the amount of CRP on the red cell intermediate and was reduced by blocking monocyte receptors with aggregated human γ-globulin (HGG) at concentrations which did not impair the uptake of other particles. The mediation by CRP of reactions of opsonic adherence and phagocytosis as outlined in these studies points to a significant role for CRP in reactions of host defense and inflammation.

The trace serum constituent C-reactive protein (CRP)[5], originally defined by its calcium-dependent precipitation of a pneumococcal cell wall teichoic acid, C-polysaccharide (CPS) (1, 2), becomes greatly elevated in concentration during the acute stages of febrile illnesses and in a variety of inflammatory processes (3–5). Recent studies have indicated that CRP efficiently activates the classical complement (C) pathway upon reacting with CPS and choline phosphatides (6), as well as with synthetic polycations and naturally occurring cationic proteins (7, 8). The consumption of C initiated by CRP also results in the C-dependent passive lysis of CPS-coated erythrocytes (E. CPS) (9). These studies have demonstrated that CRP possesses at least one function in common with the F_c-region of immunoglobulin, which also initiates the C sequence upon reacting with its substrates. CRP shares several additional functions with antibody, including the ability to initiate reactions of precipitation (10), agglutination (11) and capsular swelling (12); however, it is structurally distinguishable from the immunoglobulins on the basis of its antigenicity (13) and its possession of six apparently identical subunits (14). Other distinctive characteristics of CRP are its binding specificities (8, 10, 15) and its site of synthesis (16).

The phagocytosis of particles by monocytes or macrophages is governed primarily by the presence of opsonins (IgG and/or bound C components) on the surface of the particle and by the receptors for these ligands on the membrane of mononuclear phagocytes (17). The F_c-receptor mediates both attachment and ingestion of IgG-coated particles by macrophages, whereas the receptor for the opsonic C fragments mediates attachment but not ingestion unless the macrophages are activated (18, 19). A few reports have indicated that CRP enhances the phagocytosis of a variety of bacteria by human neutrophils (20–23); however, the mechanism of enhancement by CRP in these studies was not resolved. Therefore, we have undertaken studies to investigate whether CRP can mediate the attachment and ingestion of E. CPS by human phagocytes and whether these events are dependent on C activation. In this communication, we show that CRP generates the deposition of opsonically active C-cleavage products, and both mediates and is required for the C-dependent ingestion of E. CPS by human monocytes. These findings point to an important biologic role for CRP in host defense.

MATERIALS AND METHODS

Purification of CRP and CPS. CRP was purified from human ascites or pleural fluids by affinity chromatography. The basis for this purification is the calcium-dependent binding of CRP to CPS and the method has been described in detail by this laboratory previously (9). Analysis of these preparations established that CRP was free of immunoglobulin and C components, and appeared as a single band by both immunoelectrophoresis and polyacrylamide gel electrophoresis (9). Purified CRP was dialyzed against 0.85% saline, sterilized by Millipore

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[5] Abbreviations used in this paper: A, antibody; BDB, bis-diazobenzidine; CPS, C-polysaccharide; CRL, complement receptor lymphocytes; CRP, C-reactive protein; E, sheep erythrocytes; E. CPS, CPS-coated sheep erythrocytes; E. CPS-CRP and E. CPS-A, E. CPS sensitized with CRP or IgG antibody; FCS, fetal calf serum; GVB, gelatin-Veronal-buffered saline containing Ca^{++} (0.15 mM) and Mg^{++} (1.0 mM); HGG, human γ-globulin; and PBL, peripheral blood lymphocytes.

filtration, and stored at 4°C for not longer than 1 month before use. CRP concentrations were measured by radial immunodiffusion against a monospecific antiserum raised in goats. CPS was extracted from a Cs-capsulated variant of pneumococcus by the method of Gotschlich and Liu (24), except that the final enzymatic digestion step was omitted. The CPS was stored in a lyophilized form and dissolved in normal saline at 5.0 mg/ml just before use.

Isolation of monocytes and lymphocytes. Mononuclear cells were obtained from the blood of healthy human donors by separation on Ficoll-Hypaque, washed, and resuspended in RPMI-1640 containing 10% heat-inactivated fetal calf serum (FCS) (International Scientific Industries, Cary, Ill.). B lymphocytes were purified by removing the T cells as E-rossettes on Ficoll-Hypaque (25). T lymphocytes were enriched from peripheral blood lymphocytes (PBL) by removing B cells by filtration through nylon wool (26). The lymphoblastoid cell line, Raji, was maintained by twice weekly passage in T-flasks.

Human monocytes were isolated from suspensions of mononuclear cells on a column containing siliconized glass-beads of 100-μ diameter (Potters Industries, Carlstadt, N. J.) by the method described by Rabinowitz (27) with slight modifications. The glass-bead column (1 x 20 cm) was washed and preincubated with autologous plasma before the cells (2 to 4 $\times$ 10^7) in RPMI-1640 plus 25% autologous plasma were applied to the column and incubated for 60 min at 37°C. Nonadherent cells were removed by washing with 5 bed volumes of RPMI-1640 + 10% FCS; the adherent cells were eluted with 0.1 M sodium phosphate buffer, pH 7.4 (28). Elutions were performed at 1 drop/sec under positive pressure. The proportion of monocytes among the eluted adherent cells was assessed by the ingestion of opsonized yeast (*Candida albicans*) and was found to be in the range of 65 to 95% (mean 82%). These monocyte suspensions were used only to assess attachment of red cell intermediates.

Source of antisera and C. Anti-CPS antibody was prepared by immunizing rabbits i.v. with a formalinized Cs-pneumococcus vaccine (9). Sera collected 10 days after the final injection contained 15 mg antibody/ml which was judged to be 7S (IgG) by the 2-mercaptoethanol resistance of the hemagglutination of E coated with CPS (see next section). The IgG and IgM fractions of rabbit anti-sheep E were obtained from Cappel Laboratories, Downington, Pa. and from Cordis Laboratories, Miami, Fla., respectively; the IgM was further purified by gel filtration on Bio-Gel A-0.5m (Bio-Rad Laboratories, Richmond, Calif.). Human serum used as a source of C was absorbed by passage through a CPS-Bio-Gel A-50m column to remove trace amounts of CRP and natural antibodies to CPS. This serum was also absorbed five times with sheep E (30% v/v) at 0°C to remove detectable hemolytic antibodies. The absorption resulted in a reduction of total hemolytic C activity from 23 CH_{50} units/ml to 18 CH_{50} units/ml, as determined by a standard assay procedure (29).

Sensitization of E and E. CPS with Antibody and CRP. Sheep E were coupled with CPS by the $CrCl_3$ method as described previously (9). Throughout these studies 0.5 ml of 50% E was mixed with equal volumes of CPS (5 mg/ml) and $CrCl_3$ (0.5 mg/ml) for the coupling reaction. These cells (E. CPS) were washed and stored in gelatin-Veronal-buffered saline (GVB) with divalent cations (Mg^{++}, 1.0 mM; Ca^{++}, 0.15 mM) at 4°C. To sensitize with anti-CPS or CRP, a 1% solution of E. CPS was incubated with subagglutinating concentrations of either the antibody or CRP at 0°C. These concentrations were usually 0.5 to 2 μg/ml of CRP and 0.2 to 1.0 μg/ml of anti-CPS and were equivalent to the concentration in the first well not showing hemagglutination in a microtiter system. Both EA(IgG) and EA(IgM) were prepared with subagglutinating doses of antibodies mixed with E at 0°C for 1 hr in GVB.

Preparation of C-coated indicator red cells. E. CPS sensitized with CRP (E. CPS-CRS), and antibody (E. CPS-A) were incubated with 1 CH_{50} unit of the absorbed human C source for 60 min at 37°C. EA(IgM) were treated in a similar manner with the same C source. Unlysed red cells were washed three times in cold GVB and resuspended to 1% (2×10^8/ml) in RPMI-1640 buffered with 0.01 M HEPES. Erythrocytes with either C4b or C3b on their surface were prepared by the sequential addition of purified human C1, C4, C2, and C3. Briefly, E.CPS-CRP and EA(IgM) at 5×10^9/ml were incubated with optimal amounts of C1 for 20 min at 30 C in GVB, followed by C4 for 30 min at 37°C to generate cells containing C14; these intermediates were also used to prepare cells containing C1-3 by reaction with 200 units each of purified C2 and C3 for 30 min at 32°C. These cells were washed (three times) in media buffered with HEPES and adjusted to 2×10^8/ml. Purified C1 was prepared according to Nelson *et al.* (30); purified C4, C2, and C3 were obtained from Cordis Laboratories. C4b and C3b were detected on these intermediate red cells by agglutination with antisera specific for human C4 and C3, respectively.

Assays for rosette formation. Peripheral blood lymphocytes (PBL), T or B-enriched fractions of PBL, Raji cells, or purified monocytes (5×10^5) were mixed with 5×10^7 of the various intermediate red cells in a total volume of 0.5 ml in RPMI buffered with 0.01 M HEPES. The mixtures were incubated for 30 min at 37°C and the lymphoid cells were examined microscopically for rosette formation ($\geq$ 3 erythrocytes/cell) by counting 200 cells in a hemacytometer under phase contrast illumination.

Phagocytic assays. Monocyte monolayers were formed by allowing 2.0×10^6 human peripheral mononuclear cells to attach to 10.5 x 22 mm cover slips in Leighton tubes on a rocking platform for 60 min at 37°C in a CO_2-incubator. The monolayers were washed four times with warm media to remove lymphocytes and incubated with RPMI containing 5% FCS which had been absorbed on a CPS-Bio-Gel column; 98% or more of the attached cells were morphologically identifiable as monocytes. The various erythrocyte intermediates (0.5 ml of a 2.0% suspension) were added to the monocyte monolayers and incubated for 60 min with rocking. The monolayers were rinsed thoroughly with warm media, then exposed to a warm solution of 0.83% NH_4Cl in 0.01 M TRIS buffer (pH 7.5) for 30 sec to lyse uningested (attached) red cells, fixed in methanol, and stained sequentially with Wright's stain and Giemsa stain. Morphologically distinguishable monocytes (at least 200/cover slip) were scored for at least one clearly ingested erythrocyte. Inhibition experiments were conducted with heat-aggregated (63°C, 20 min) human γ-globulin (HGG) that had been centrifuged at 100,000 $\times$ G for 90 min to obtain a pellet of aggregates of a size greater than 15×10^6 daltons. These aggregates were resuspended in saline at 10 mg/ml. The aggregated HGG at appropriate dilutions in media was added to the monolayers 15 min prior to the addition of the various indicator red cells to permit binding to the monocyte F_c receptors.

The phagocytosis of opsonized yeast (5×10^8 *Candida albicans* incubated with 50% serum for 15 min at room temp) was performed by adding 10^8 yeast particles to similarly prepared monocyte monolayers. Latex beads of 2.0 μ diameter

(Dow Chemical Co., Midland, Mich.) were also used as ingestible particles by adding 0.1 ml of a 1% suspension in media to each Leighton tube. Uptake was assessed by visually scoring as positive cells those with two or more ingested particles.

Phagocytosis was also evaluated by exposing the monolayers to equal numbers of the various E intermediates described above that had been labeled with ^{51}Cr (2×10^8 cells plus 100 μCi of ^{52}Cr as sodium chromate in saline, New England Nuclear, Boston, Mass.) after the erythrocytes had been prepared with all the reactants. For the uptake of ^{51}Cr-labeled indicator red cells, 4×10^6 mononuclear cells were used to prepare each monocyte monolayer. After a 60-min exposure to the ^{51}Cr-labeled red ce lls, the coverslips were rinsed with medium and subjected to lysis with warm (37°C) NH_4 Cl-TRIS buffer before counting on a gamma spectrometer (G. D. Searle Co., Chicago, Ill.). The CPM of ^{51}Cr on the coverslips after lysis of attached red cells represented the extent of ingestion by the monocytes. Photographs of monocyte phagocytosis were taken under phase contrast or brightfield illumination on a Leitz Orthoplan microscope with Kodak Plus-x film.

RESULTS

Formation of rosettes with lymphocytes by CRP-sensitized E. CPS reated with C. Since peripheral human B cells possess surface receptors for the F_c portion of IgG (31) and the cleaved C components C4b (32, 33) and C3b (34), we examined the ability of various human lymphoid cells to bind to E. CPS sensitized with CRP alone, with CRP plus serum C, or with CRP plus purified human C components. Rosette formation with PBL occurred only when E. CPS-CRP were reacted with serum C (Table I). The serum C was absorbed extensively to remove any naturally occurring antibodies to CPS or E. The percentage of lymphocytes forming rosettes was proportional to the number of B cells present in PBL from various donors; it was increased in a B cell enriched fraction of the PBL, and greatly reduced in T cell enriched preparations (Table I). Rosette formation with EA(IgM) treated with the same C source was equivalent to that measured with E. CPS-CRP-C. Cells of the lymphoblastoid B cell line, Raji, readily formed rosettes with the C -treated erythrocytes.

TABLE I

Rosette formation of human peripheral blood lymphocytes (PBL) with E. CPS sensitized with CRP and reacted with C or purified C components

Lymphoid Cell Source	Ig POS[b]	% of Lymphocytes Forming Rosettes with (Range)[a]				
		EAC	E. CPS-CRP	E. CPS-CRP-C	E. CPS-CRP-C14	E. CPS-CRP-C1-3
	%					
PBL	25 (19-30)	20 (14-26)	1 (0-2)	17 (13-22)	9 (4-14)	15 (12-21)
T-enriched[c] (Nylon wool)	2 (1-4)	3 (2-4)	1	3 (1-6)	0	0
B-enriched[d] (E-rosettes)	57 (51-59)	51 (48-54)	0	40 (39-42)	23 (17-29)	38 (31-44)
Raji cells		90 (85-97)	0	70 (63-76)	4 (1-7)	69 (60-75)

[a] Percentages of rosette-forming cells determined with PBL from each of eight healthy donors.

[b] Cells stained with polyvalent anti-GAM fluorescent antiserum.

[c] Effluent lymphocytes from a nylon wool column.

[d] Cells remaining after removal of E-rosette-forming cells on Ficoll-Hypaque.

To demonstrate that attachment was a function of receptors for both C4b and C3b on B cells, E. CPS-CRP were sequentially reacted with purified human C components and assayed for rosette formation. The results in Table I show that the fraction of rosetting cells correlated with the fraction of complement receptor lymphocytes (CRL) as determined by the percentage of EAC-rosette-forming cells present in the lymphocyte populations. The number of C4b-dependent rosettes was similar to that seen in assays with EAC14 (4 to 10%) run simultaneously and was consistently less than the fraction of C3b-dependent rosettes. Raji cells formed rosettes with C3b-coated E. CPS-CRP but not C4b-coated cells, a finding previously noted ty others using EA(IgM) with C4b or C3b on the surface (32). These results clearly show that CRP is capable of initiating the C sequence independent of antibody and effectively enough to deposit sufficient cleaved C components on a cell surface to permit attachment to CRL.

TABLE II

The attachment of E. CPS sensitized with CRP and complement to human monocytes separated on glass bead columns[a]

Indicator Red Cells	% of Cells with Attached Indicator Cells (Range)[b]
EA(IgG)	65 (61-73)
E. CPS	3 (0-4)
E. CPS-A(IgG)	84 (62-93)
E. CPS-CRP	11 (4-24)
E. CPS-CRP-C	79 (70-85)
EA(IgM)	1 (0-2)
EA(IgM)C	80 (70-89)
E. CPS-CRP-C14	32 (25-40)
E. CPS-CRP-C1-3	62 (45-78)
EA(IgM)C14	29 (22-36)
EA(IgM)C1-3	69 (50-74)

[a] Percentage of the cells eluted from the glass-bead column that ingested opsonized yeast particles was 82% (range 66-94%).

[b] Mean percentage of the mononuclear cells forming rosettes. Studies were performed with seven different human monocyte preparations.

Opsonic adherence of E. CPS-CRP reacted with C to human monocytes. Monocyte membranes have distinguishable F_c and C3b-receptors (35, 36) that mediate the attachment of opsonized particles. Since monocyte F_c receptors can be demonstrated by rosette formation with EA(IgG), we wished to determine if CRP when combined with one of its substrates (E. CPS) might also mediate attachment to monocytes in a manner analogous to IgG. Human blood monocytes obtained from glass bead columns were assayed in cell suspensions for the adherence of the various indicator red cells listed in Table II. Phagocytic monocytes represented the majority of the adherent cell fraction as shown by the high percentage of the cells ingesting opsonized yeast (82%) and by the fraction of cells with attached EA(IgG) and E. CPS-A(IgG) (Table II). A small but significant fraction of the monocytes formed rosettes with E. CPS having only CRP on its surface, but upon the addition of absorbed human serum C to form E. CPS-CRP-C the percentage of rosette-forming cells reached values similar to those seen when monocytes were reacted with EA(IgG),

E-CPS-A(IgG) and EA(IgM)C under the same conditions (Table II). Both C4b-coated (32%) and C3b-coated (62%) E. CPS-CRP prepared with purified C components attached to monocytes, the latter more efficiently. The lack of adherence of E. CPS and E. CPS plus C indicated the absence of both cytophilic antibody to CPS on the monocytes and naturally occurring anti-CPS and anti-E in the assay mixtures.

Phagocytosis of C treated E. CPS-CRP by monocyte monolayers. In a series of separate experiments we examined the ability of monocytes attached to coverslips to ingest the same red cell intermediates used for rosette formation. Extensive phagocytosis of E. CPS-CRP was observed only when these cells were first reacted with serum C (Fig. 2*A*, Table III), scoring as positive those monocytes with at least one clearly internalized E after lysis of extracellular attached erythrocytes. Red cells opsonized with IgG antibody (EA and E. CPS-A)were ingested by a similar proportion of the monocytes as ingested E. CPS-CRP treated with C. EA(IgM) neither adhered to nor were ingested by the monocytes, in dicating the absence of IgG from the IgM preparations, whereas EA(IgM)C adhered extensively to the monocytes without being internalized. E. CPS-CRP treated with purified C components to prepare intermediates with either C4b or C3b on their surface were ingested by monocytes, although the uptake of C4b-coated cells was significantly less than the uptake of cells coated with both C4b and C3b (Table III). Assays with C4b- and C3b-coated intermediates prepared with EA(IgG) resulted in a similar amount of phagocytosis when compared to the same intermediates prepared from E. CPS-CRP. Therefore, the ingestion of E. CPS mediated by CRP appears to be dependent on the generation of opsonic fragments of C.

Since the C-dependent hemolysis of E. CPS was previously shown to be a function of the amount of CRP supplied for sensitization (9), we varied this parameter to determine its effect on phagocytosis of C-treated E. CPS-CRP. Sensitization of E. CPS with increasing concentrations of CRP followed by treatment with 1 CH_{50} unit of C resulted in an increased fraction of monocytes ingesting these cells (Fig. 1). By contrast E. CPS sensitized with increasing doses of CRP, but not subsequently incubated with C, were not ingested. Incubating E. CPS-CRP with monocytes for longer periods (up to 3 hr) resulted in a slight increase of the fraction of monocytes ingesting these cells, but this was always less than 10%.

TABLE III

Phagocytosis of E. CPS treated with CRP or antibody and complement by human monocyte monolayers[a]

Red Cell Intermediate	% of Monocytes with Ingested Red Cells[b]
E. CPS	2 (1-5)
E. CPS-A(IgG)	72 (66-86)
E. CPS-A(IgG)-C	79 (74-90)
E. CPS-CRP	5 (2-12)
E. CPS-CRP-C	71 (54-88)
E. CPS-CRP-C14	16 (3-21)
E. CPS-CRP-C1-3	39 (31-56)
EA(IgM)	3 (1-6)
EA(IgM)C	5 (3-6)

[a] Monolayers composed of 98% or more morphologically identifiable monocytes.

[b] Percentage (range) of monocytes clearly ingesting one or more of the red cell intermediates after lysis of attached red cells with NH_4Cl (0.83%) in TRIS buffer.

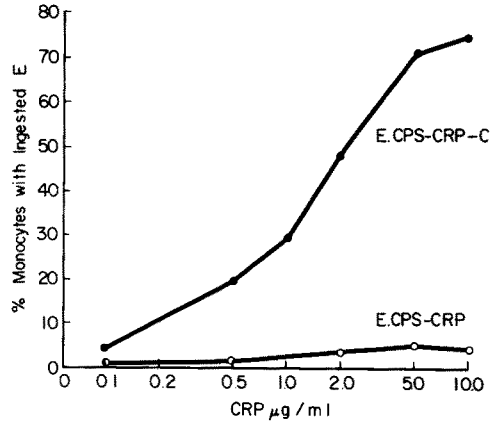

Figure 1. The effect of increasing the dose of CRP used for the sensitization of E. CPS on the phagocytosis of E. CPS-CRP-C by monocytes. The indicator cells were reacted with a constant amount of appropriately absorbed human serum C (1 CH_{50} unit) after sensitization with CRP.

TABLE IV

Requirement for the presence of CRP for the phagocytosis of E. CPS cells sensitized with CRP and reacted with complement

Red Cell Indicator	Treatment of Indicator Red Cells[a]	Anti-CRP HA Titer (log_2)[b]	% of Monocytes with Ingested E	CPM-Ingested Cr^{51}-Red Cells[c]
E. CPS-CRP-C		11	82	1907
	0.01 M EDTA	2	13	259
	0.01 M EDTA + CRP[d]	12	76	1880
E. CPS-A(IgG)-C			86	2257
	0.01 M EDTA		84	3248
E. CPS			3	282
E. CPS-CRP			5	515

[a] Indicator red cells incubated with EDTA (0.01 M) in GVB without divalent cations for 30 min at 37°C to remove bound CRP.

[b] Hemagglutination performed on microtiter plates with goat anti-CRP.

[c] Ingestion is the CPM on the coverslips after lysis of external or attached red cells with NH_4Cl-TRIS buffer. Mean CPM for three determinations each run in triplicate.

[d] Indicator cells resensitized with CRP (2 μg/ml) in GVB containing divalent cations.

Requirement for CRP for ingestion of E. CPS-CRP-C by monocytes. It became of interest to determine whether CRP was required for the uptake of C-treated E. CPS-CRP, since it is known that the opsonic cleaved C fragments in the absence of IgG mediate ingestion only with activated macrophages (18, 19). The monocytes on coverslips in our experiments were not activated as indicated by their inability to ingest EA(IgM)C (Table III). Since CRP binding to CPS is known to be Ca^{++} dependent (2), the CRP could be removed from E. CPS-CRP-C by incubation with 0.01 M EDTA. This procedure was effective as seen by the drop in the hemagglutination titer of these red cells with anti-CRP (Table IV). The percentage of monocytes ingesting these CRP-depleted E. CPS-C intermediates was greatly reduced although binding to C receptors was not altered (Fig. 2*B*, Table IV). Phagocytosis was restored by sensitizing the cells again with the same amount of CRP (2 μg/ml) originally used for sensitization. In analogous experiments with antibody-coated E. CPS treated with C, incubation with 0.01 M EDTA had no detectable effect on the uptake of

these cells. Agglutination titers with anti-C3 were the same for all of the cells reacted with C before and after treatment with EDTA (data not shown); this was confirmed by the morphologic observation of attachment of these cells to the monocytes (Fig. 2*B*). The microscopic observation of the inhibition of ingestion after removal of CRP was supported by additional studies with ^{51}Cr-labeled indicator red cells. The CPM remaining on the coverslips after the lysis of attached red cells served as a measure of ingestion, and removal of CRP with EDTA greatly reduced ingestion of ^{51}Cr-labeled E. CPS-CRP-C (Table IV). The attachment of indicator cells as estimated by radioactivity on coverslips before the lysis of the external red cells indicated that subjecting the C-treated indicator cells to EDTA had no effect on this parameter.

Possible involvement of F_c receptors in CRP-dependent phagocytosis. Since CRP was necessary for ingestion, experiments were designed to determine the involvement of F_c receptors on monocyte membranes in the ingestion of E. CPS-CRP-C. One efficient method of blocking the IgG F_c receptors on monocytes is by the addition of aggregated IgG (37). Therefore F_c receptors were blocked by adding heat-aggregated HGG, that was free of CRP by radial immunodiffusion, to the monocyte monolayers before the addition of the red cells. This resulted in inhibition of the uptake of both E. CPS-CRP-C and its antibody analogue, E. CPS-A(IgG)C Table V). The aggregated HGG more effectively inhibited the IgG-coated cells than the CRP-coated cells. In addition, the attachment of the C treated red cells through receptors for opsonic C fragments was not compromised by the presence of HGG attached to the monocytes (Fig. 2*C*). This treatment did not alter the phagocytic ability of the monocytes *per se* as seen by the lack of interference with the uptake of opsonized yeast particles or latex beads after addition of the same inhibitory amounts of HGG (Fig. 2*D*, Table V). These results suggest that unblocked F_c receptors are necessary for optimal CRP-dependent phagocytosis and at least imply an interaction of CRP with monocyte F_c receptors.

DISCUSSION

These studies clearly establish that CRP bound to a cell surface can initiate the deposition of the C cleavage products

TABLE V

Inhibition of phagocytosis by human monocytes of E. CPS-CRP-C and E. CPS-A(IgG)C by aggregated HGG

Aggregated HGG[a]	% of Monocytes Ingesting the Following Particles[b]			
	E. CPS-CRP-C	E. CPS-A(IgG)C	Yeast + C	Latex beads
mg/ml				
	74	76	79	97
0.05	55	36		
0.10	42	15	74	90
0.50	24	10	72	98
1.00	19	14	69	85
2.00	16	7	63	91

[a] Concentration of aggregated HGG incubated with monocytes attached to coverslips 15 min before the addition of the particles.

[b] Mean percentage of monocytes ingesting one or more of the indicated particles.

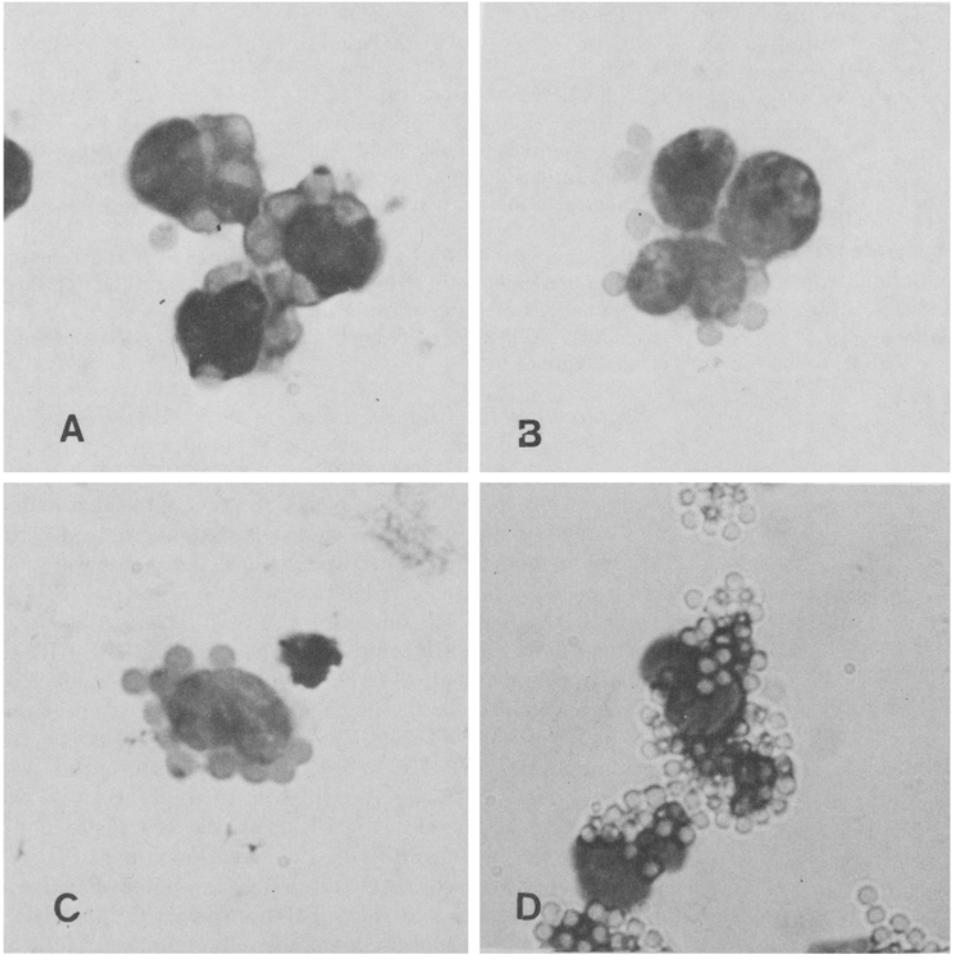

Figure 2. Photomicrographs of monocyte monolayers incubated with the following: *A*, E. CPS-CRP-C followed by lysis of attached red cells with NH_4Cl in TRIS buffer; *B*, E. CPS-CRP-C incubated in 0.01 M EDTA to remove CRP; *C*, heat-aggregated human γ-globulin (1.0 mg/ml) 15 min before the addition of E. CPS-CRP-C; *D*, heat-aggregated HGG as in C before the addition of latex beads. The stained monocytes were photographed under brightfield illumination (× 625).

C4b and C3b, which in turn can mediate the adherence of such cells to both B cells and monocytes expressing receptors for these C components. To demonstrate these activities, advantage was taken of an indicator cell previously utilized in this laboratory for detecting CRP-initiated C-dependent passive lysis, a CPS-coated E sensitized with CRP (9). CRP sensitization of E. CPS in the absence of exposure to C resulted in only minimal adherence to monocytes, and no binding at all to B cells; however, in the presence of serum C or purified C components, CRP could initiate C4b and C3b deposition and subsequent attachment to monocytes and B cells. CRP was also found to mediate effectively the ingestion of E. CPS by human monocytes after sufficient deposition of the cleaved C components C4b and C3b to permit attachment to monocytes; in the absence of C, CRP promoted only minimal phagocytosis. These observations point to several analogous functional activities between CRP and antibody; in the latter case such activities have usually been dependent on the F_c region of immunoglobulin.

It should be emphasized that the components of the assay systems employed were free of antibody by several criteria. Antibodies would not have a calcium-dependent binding to the CPS on the affinity column employed in the preparation of CRP and thus specific Ig would have been retained on the column; CRP prepared in this manner has previously been documented to be free of other serum constituents (9). Both the C source and FCS were extensively absorbed with sheep E followed by pneumococcal CPS on an affinity column; the removal of antibody specific for CPS or E in these reagents was indicated by the absence of both agglutination and phagocytosis of E. CPS when exposed to each of the reagents alone. IgM specific for E or CPS was eliminated both by the absorptions and by exclusion by its greater size in the final chromatography step in the preparation of CRP (9). These precautions strengthen our conclusion that the activities attributed to CRP in this report are independent of antibody.

Earlier investigations by others had indicated that CRP promoted phagocytosis under certain experimental conditions. Hokama *et al.* (20) were the first to note an increased rate of uptake of carbonyl iron particles and several bacterial species by human leukocytes in the presence of CRP. Williams and Quie (38) did not observe increased phagocytosis of bacteria as measured by the bactericidal activity of leukocytes, but Ganrot and Kindmark (22, 23) later showed that CRP enhanced the extent and rate of phagocytosis of a wide variety of pathogenic bacteria by human leukocytes in serum-free systems. None of these studies addressed the question of whether CRP exerted its effect on the particle to be ingested or on the phagocytic cell itself; certainly, the possibility that CRP acts on leukocytes must be considered in view of the enhanced leukocyte (neutrophil) motility induced by CRP as noted by H. F. Wood (39). The studies reported here have focused on the opsonic effect of CRP on one of its substrates rather than its effect on the phagocytic cell itself, since the CRP was bound to a red cell surface and not available for binding to the monocytes directly. However, we have recently obtained evidence showing that CRP enhances hexose monophosphate shunt activity in human leukocytes (unpublished results). Therefore, CRP may augment phagocytosis by two distinct routes: serving as an opsonin as described here and possibly increasing the activities of phagocytic cells.

The exact mode of interaction of cell-bound CRP with the monocytes used in the phagocytic system here is not fully understood, but several interesting characteristics of the opsonic properties of CRP were shown. Sensitization of E. CPS with CRP alone resulted in only minimal adherence and ingestion by monocytes; yet CRP was required for ingestion of E. CPS-CRP-C once the initial attachment of the indicator cells occurred via C receptors. This requirement was shown by the decreased uptake of E. CPS with cleaved C components on its surface after the removal of CRP from E. CPS-CRP-C. It is unlikely that the CRP-dependent phagocytosis described here occurred only through the C receptors since this has been shown to be a property of activated macrophages (18, 19). Evidence in support of this is that the monocytes in these studies were unable to ingest EA(IgM)C. Analogous to opsonic IgG antibody (35, 36), there was a dependence on the number of CRP molecules per red cell for uptake of C-coated E. CPS by monocytes. The inhibition of the ingestion but not the attachment of E. CPS-CRP-C by aggregated HGG suggests an involvement of monocyte F_c receptors in the uptake of these cells. The amounts of aggregated HGG employed did not exert a generalized inhibitory effect on phagocytosis since the uptake of both opsonized yeast and latex beads were not altered. It should be noted that the inhibition by the aggregated HGG was greater for the IgG-than the CRP-coated E. CPS, suggesting that the receptors involved may not be identical. This agrees with our earlier findings which did not reveal any evidence for binding of CRP or CRP-CPS complexes to the F_c-receptors on B lymphocytes (40), and with the absence of binding of E. CPS-CRP to B cells in the studies reported here. Furthermore, CRP aggregated by bisdiazobenzidine (BDB) failed to inhibit phagocytosis of IgG-coated E under conditions where aggregated HGG was clearly inhibitory (unpublished results). In agreement with the apparent lack of an interaction between CRP and the F_c receptor, amino acid sequence studies of approximately 50% of the CRP molecule have not detected homology with the CH3 domain of IgG (41), a region which is known to bind to the F_c receptor (42, 43). Sequence homologies with other domains of IgG remain to be established. Because of these discrepant findings we are unable to conclude that CRP mediates phagocytosis by monocytes through their F_c receptors.

The biologic activities of CRP and their relationship to host defense may depend on both the cell types with which CRP interacts and on its ability to activate the C cascade upon interaction with its substrates *in vivo*. The CRP-initiated C-dependent opsonic adherence and phagocytosis described here lead us to propose that similar activities occur *in vivo*, especially since CRP has been demonstrated to be deposited on damaged tissues at sites of inflammation (44, 45). Such CRP-initiated C activation not only could serve to generate the phlogistic C cleavage products at inflammatory sites, but also mediate the subsequent clearance of damaged cells or cellular constituents from these sites. The potentiation of the activation of the C system and mediation of phagocytic functions by CRP stand in marked contrast with its ability to inhibit platelet aggregation (46) and certain T cell responses (47). This enhancement of some activities coupled with the suppression of others, along with its rapid appearance after an inflammatory stimulus points to a regulatory role for CRP in various immunopathologic mechanisms and underscores its biologic significance.

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