POSSIBLE ROLE FOR C-REACTIVE PROTEIN IN THE HUMAN NATURAL KILLER CELL RESPONSE*

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C-reactive protein (CRP)[1] is an acute-phase protein in man that is of considerable interest, in part because serum concentrations increase from 20- to several hundred-fold during inflammation or acute tissue injury (1, 2). The association of elevated levels of CRP with activation of certain immune responses led to the postulation that CRP is involved in initiation or regulation of lymphocyte function (3). Recent evidence of similarities between CRP-binding cells and the effector cell responsible for natural killer (NK) cell-mediated lysis prompted our investigation of the role of CRP in the NK response. NK and CRP-binding cells are large granular lymphocytes (LGL), bear IgG Fc receptors, represent only a small percentage of the total lymphocyte population, and rosette similarly with sheep erythrocytes (4–8).

These experiments were initiated to determine whether NK cells bear CRP, whether cell-bound CRP is involved in the effector phase of the NK response, and whether CRP or CRP-complex binding would alter NK function (9–12), that is, they were designed to examine whether CRP or CRP complexes are involved in NK cell-mediated killing. The results demonstrate that CRP is present on at least certain NK effectors, that CRP or a molecule that co-caps with CRP is required for optimal NK function, and that cell-surface CRP and not fluid-phase CRP is involved in the mediation of NK activity.

Materials and Methods

Purification and Characterization of CRP and CPS. CRP was prepared from pooled human serous fluids by phosphocholine-affinity, DE-52, and Sephacryl S-200 (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, NJ) column chromatography by methods previously described (13). CRP preparations were concentrated by ultrafiltration, sterile filtered, and stored at 4°C. When examined by reduced sodium dodecyl sulfate-polyacrylamide gel electrophoresis (14), purified CRP produced a single band corresponding to CRP subunits with a molecular weight of 21,500 ± 500. A single peak of ^{125}I was obtained after sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of labeled CRP (15). Purified CRP was concentrated to 10 mg/ml by ultrafiltration and no IgG, IgA, IgM, C3, or serum amyloid P component were detected using radial immunodiffusion plates with limits of sensitivity of 5 μg/ml. Ouchterlony analysis of the concentrated material revealed a single precipitation line with

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[1] *Abbreviations used in this paper*: C, complement, CPS, pneumococcal C-polysaccharide; CRP, C-reactive protein; H-CRP, heat-modified CRP; LGL, large granular lymphocytes; NK, natural killer; PBL, peripheral blood lymphocytes; S-CRP, surface CRP; % SR, percent specific release.

anti-CRP and no reactivity with anti-whole human serum. Heat-modified CRP (H-CRP) was prepared by heating purified CRP for 2 min at 63°C. Pneumococcal C-polysaccharide (CPS) was obtained by the methods of Liu and Gotschlich (16) as previously modified (13).

Anti-CRP. Initial experiments were performed with the IgG fraction of goat anti-CRP prepared and characterized as previously described (13). Characterization of this antibody involved both Ouchterlony diffusion analysis and passive hemagglutination using antigens coupled to erythrocytes by chromium chloride. No antibody activity against IgG, C3, serum amyloid P component, CPS, or antigens contained in normal human serum was detectable. In other experiments we used sheep anti-CRP (N. L. Cappel Laboratories, Cochranville, PA) or goat anti-CRP that had been affinity purified by passage over CRP-Sepharose (Pharmacia Fine Chemicals) (12). Before dilution, the concentration of the affinity-purified antibody was 1 mg/ml. Similar results were obtained in all cases. All antisera were extensively dialyzed into media immediately before use.

Lymphocyte Preparation. Lymphocytes were obtained from heparinized peripheral blood of normal individuals and were separated on lymphocyte-separation medium (17). Cells were collected in sodium heparin (20 U/ml) or EDTA (1 mg/ml). Peripheral blood lymphocytes (PBL) were then washed and resuspended in RPMI 1640 medium containing 0.05% gelatin. In some experiments, a subpopulation of cells was removed by adherence to tissue culture dishes. Normal donors were tested for NK levels; optimal responder cell numbers ranged from 5×10^4 to 2×10^5 per 0.2 ml culture.

Enrichment of LGL. Human PBL were enriched for NK activity by Percoll-gradient separation by the modified methods of Timonen et al. (5, 6, 18). Before density-gradient separation, phagocytic cells were removed: after incubation in RPMI medium containing 25% fetal calf serum and 10 mg/ml of carbonyl iron and rotation at 37°C for 30 min, they were removed with a pencil magnet. The remaining lymphocytes were gently layered on a discontinuous gradient composed of five 2.5-ml layers of Percoll (Pharmacia Fine Chemicals, Div. of Pharmacia Inc.) in RPMI ranging from 34 to 50% Percoll. No more than 5×10^7 cells were layered onto a single gradient. Cells were centrifuged at 550 *g* for 30 min at room temperature. Each layer of lymphocytes was removed from the gradient, resuspended in RPMI containing 10% fetal calf serum, washed, and counted. The exact percentage of lymphocytes obtained from each layer varied with the donor. Depending upon the percentage of lymphocytes recovered in the top two layers of the gradient, cells from these fractions were either pooled or examined separately. By this technique the LGL content of the phagocyte-depleted mononuclear cell preparation was enriched 5- to 10-fold. These enriched cells were incubated for 1 h at 37°C in a humidified 5% CO_2 environment with a 1:100 dilution of OKT3 antibody (Ortho Pharmaceutical Corp., Raritan, NJ), washed, and resuspended in a 1:10 dilution of baby rabbit complement (C) for 45 min. The remaining lymphocytes will be referred to as phagocyte- and T lymphocyte-depleted LGL.

Anti-CRP and C Pretreatments. PBL, phagocyte- and T lymphocyte-depleted LGL, or target cells were brought to a concentration no greater than 1×10^7 cells/ml in RPMI containing 10% fetal calf serum. Cells were incubated for 30 min at 37°C in the presence or absence of a 1:8 dilution of anti-CRP, washed, and resuspended in a 1:10 dilution of C for 30–45 min at 37°C. After incubation with antibody and C, cells again were washed and resuspended to appropriate cell concentrations in the presence of ^{51}Cr-labeled target cells.

K562 Target Cells. The K562 myeloid cell line was maintained in RPMI medium supplemented with 10% fetal calf serum, 2 mM glutamine, and 1% penicillin-streptomycin. They were passed twice a week by resuspension to a concentration of 2.5×10^5 cells/ml in fresh medium. K562 cells to be used as target cells were labeled with ^{51}Cr by incubating 5×10^6 cells/ml in RPMI containing 100 μCi of ^{51}Cr at 37°C for 1 h. After labeling, the cells were washed three to five times in RPMI containing 0.05% gelatin and resuspended in this medium before use.

^{51}Cr-Release Assay. A 4-h ^{51}Cr-release assay using 5×10^5 effector cells and 5×10^3 or 1×10^4 ^{51}Cr-labeled K562 was performed. For this assay, cells were incubated in RPMI containing 0.05% gelatin in 0.2-ml microtiter wells at 37°C in a humidified 5% CO_2 atmosphere. Half (100 μl) of the culture supernatant fluid was then harvested with a micropipet (Eppendorf; Brinkman Instruments Inc., Westbury, NY), and ^{51}Cr release was measured. Spontaneous and detergent

controls were considered acceptable only if spontaneous release was <15% of the detergent release value. The formula used for calculation of the percent specific release (% SR) was

$$\% \text{ SR} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{detergent release} - \text{spontaneous release}} \times 100.$$

S-CRP and Analysis of Capping. Cells from normal donors were incubated with biotinylated (19) affinity-purified goat anti-human CRP (12) for 30 min at 4°C, washed, and incubated with fluorescent avidin (E-Y Laboratories, San Mateo, CA) for 30 min at 4°C, and resuspended to 10^7 cells/ml. Aliquots of cells (200 μl) were then incubated at 37°C for various times before fixation with an equal volume of 2% paraformaldehyde, washed twice, and analyzed for capping according to criteria established by Schreiner and Unanue (20).

Results

Treatment of PBL with Anti-CRP and C Results in a Loss of Functional NK Activity. When human lymphocytes were sequentially treated with anti-CRP and C and assayed for NK activity, their ability to kill K562 target cells was substantially depleted. Three representative experiments are presented in Table I. In these experiments functional activity was totally eliminated at a concentration of 1×10^5 cells per culture and substantially depleted at higher cell concentrations. Although NK function could be depleted, anti-CRP and C did not remove enough cells for loss of viability to be detectable by trypan blue exclusion. Comparable concentrations of $F(ab')_2$ anti-CRP (21) and anti-IgG (22) were used as control antibodies. Treatment with either of these antibodies and C, or with C alone, did not alter the NK response (Table I). Similar results were obtained when anti-CRP that had been eluted from a CRP affinity column was used. When PBL were depleted of adherent cells before treatment with antibody and C, the pattern of results was not altered, as seen in Table I, experiment 3. In most experiments, the concentration of anti-CRP used for C-mediated depletion of effector cells alone (1:8 dilution) had no effect on NK activity. These experiments indicate that CRP or an antigenically similar molecule is found on at least some of the PBL that mediate the natural killer cell response.

TABLE I

Treatment of Effectors with Anti-CRP and Complement Removes NK Activity

Antibody‡	Complement§	Percent specific ^{51}Cr release					
		Experiment 1		Experiment 2		Experiment 3*	
		Effector cell number					
		2×10^5	1×10^5	2×10^5	1×10^5	4×10^5	1×10^5
Whole αCRP	+	9 ± 1‖	0 ± 0	11 ± 11	0 ± 1	9 ± 3	0 ± 1
F(ab')2 αCRP	+	—	—	37 ± 9	11 ± 4	32 ± 3	4 ± 1
Whole αIgG	+	28 ± 1	19 ± 1	—	—	—	—
None	+	26 ± 3	16 ± 1	39 ± 10	16 ± 10	34 ± 1	7 ± 1
None	−	31 ± 2	21 ± 2	45 ± 7	15 ± 3	31 ± 6	5 ± 1

* Adherent cells were removed by adherence to tissue culture dishes in certain experiments, e.g., experiment 3.

‡ PBL were separated on LSM and incubated with a 1:8 dilution of anti-CRP for 30 min at 37°C.

§ Cells were washed and incubated with a 1:16 dilution of rabbit complement for 45 min at 37°C, then washed again before a 4-h incubation with 5×10^3 ^{51}Cr-labeled K562 targets. Cells not treated with antibody and/or complement were comparably incubated and washed.

‖ Mean ± SD of six replicate cultures.

Anti-CRP and C Treatment of Phagocyte- and T Lymphocyte-depleted LGL Lowers NK Response. Phagocytic cells were removed from the PBL with a magnet after carbonyl iron ingestion. The remaining cells were enriched for LGL by discontinuous Percoll-gradient separation and were treated with OKT3 monoclonal antibody and C to remove T lymphocytes. These phagocyte- and T lymphocyte-depleted LGL were incubated in the presence or absence of anti-CRP before C was added. The groups of cells incubated in the presence of anti-CRP demonstrated a substantially lower NK response than those incubated in the absence of anti-CRP, which indicates that the effector cell mediating an NK response has CRP antigenicity present on its surface (Fig. 1).

Pretreatment of PBL with High Concentrations of Anti-CRP Inhibits NK Response in the Absence of C. Incubation of PBL in the presence of a 1:8 dilution of anti-CRP and no C occasionally resulted in loss of functional activity. This prompted us to look at the effects of various dilutions of antibody alone on the NK response. The results (Fig. 2) indicate that preincubation of effectors with a twofold higher (1:4 or greater)

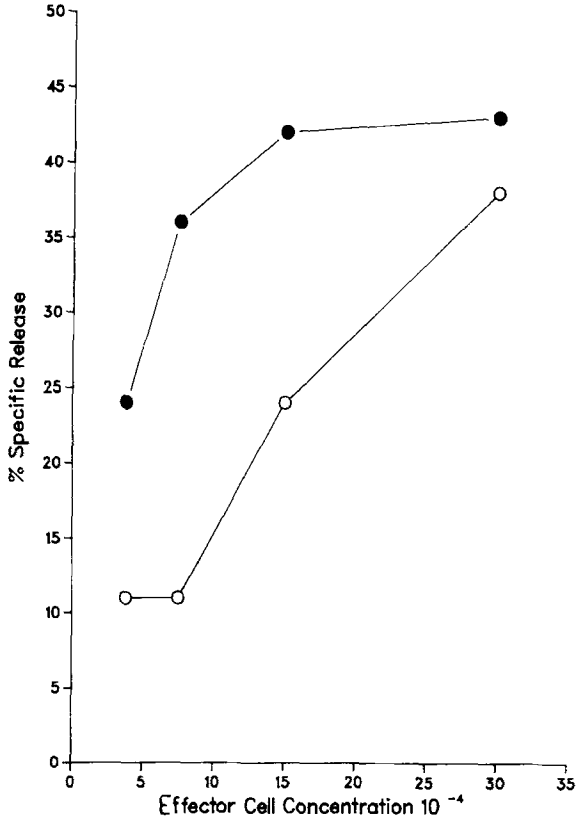

FIG. 1. Anti-CRP and C treatment is effective in removal of NK activity from phagocyte- and T cell-depleted LGL. Mononuclear cells were separated from peripheral blood, phagocytic cells were removed by carbonyl iron ingestion, LGL were purified on a Percoll gradient, and OKT3$^+$ lymphocytes were removed by treatment with OKT3 antibody and C. These enriched NK cells were then treated with anti-CRP and C (○) or with C alone (●). Each point represents the mean of six replicate cultures.

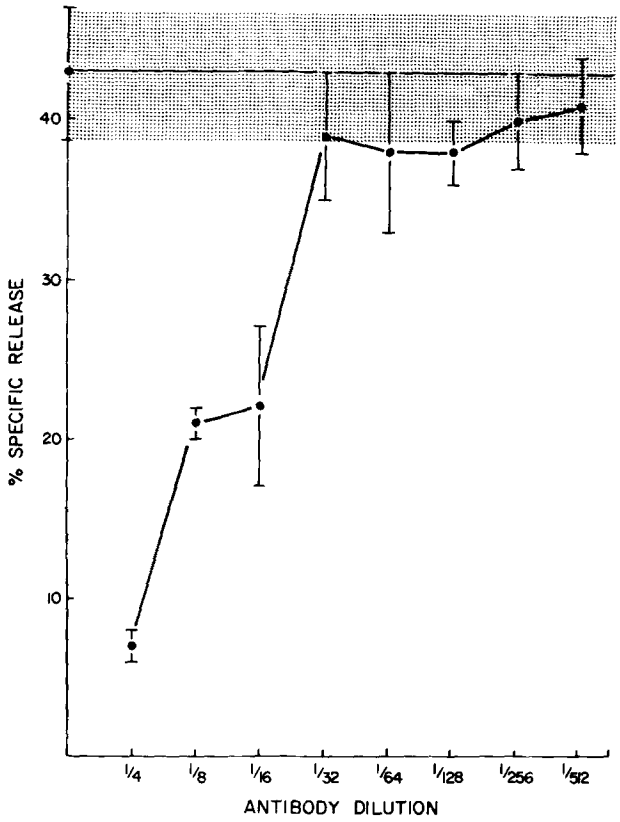

FIG. 2. Pretreatment with high concentrations of anti-CRP can inhibit an NK response in the absence of complement. Mononuclear cells were separated from peripheral blood and incubated with various dilutions of anti-CRP. Labeled K562 target cells then were added, and ^{51}Cr released was measured. The shaded area of the figure represents the mean and 1 SD of the NK response in the absence of anti-CRP. Each point is the mean ± SD of values obtained from four to six replicate cultures.

concentration of anti-CRP alone greatly reduces functional NK activity. When cells were incubated with Fab fragments of anti-CRP, there was no loss of NK activity, and inclusion of anti-CRP with no preincubation period did not inhibit the NK response (unpublished observations). When the effects of similar concentrations of anti-IgG antiserum were examined, no inhibition of the NK response was observed (Table II).

Binding Studies Reveal the Presence of Surface CRP (S-CRP) and Capping of S-CRP on PBL. Studies that demonstrated the presence of a small percentage of cells that bind complexed CRP initially failed to reveal a population of lymphocytes with detectable CRP on their surface (13, 15). The ability of anti-CRP and C to deplete NK activity prompted us to look for lymphocyte S-CRP by biotin-avidin amplification. Using this sensitive detection system, we enumerated cells bearing S-CRP in peripheral blood and found them in percentages that were similar to those of cells binding complexed CRP (Table III). We examined the cells that interact with biotinylated anti-CRP and fluorescent avidin to determine whether this interaction initiated capping. S-CRP redistributed to a polar cap in all positive lymphocytes by 8 min at

TABLE II

Preincubation with Anti-CRP Inhibits an NK Response

Preincubation*	Lytic units‡	Correlation coefficient§	% SR at 20:1 (E/T)‖
RPMI	13.6	0.996	22
Anti-CRP	3.1	0.938	5
Anti-IgG	13.9	0.996	22

* Cells were incubated with RPMI, a 1:4 dilution of anti-CRP, or Anti-IgG for 30 min at 37°C, washed, and resuspended in RPMI containing 1% gelatin for 30 min before ^{51}Cr-labeled K562 target cells were added.
‡ Expressed as mean lytic units at a 33% level/10^6 cells.
§ Measure of linearity of four experimental points.
‖ Effector/target.

TABLE III

S-CRP is Detectable on PBL

CRP-CPS*	FITC anti-CRP	B anti-CRP Fl avidin	Percent positive cells	Range	Percent of positive cells that cap‡
−	+	−	0.4 ± 0.6§	0.0–1.4	—
+	+	−	3.5 ± 0.6	0.3–8.7	100
−	−	+	3.9 ± 0.8	0.5–12.0	100

* Cells were incubated in the presence of CRP-CPS complexes before assay.
‡ Percent of positive cells that cap at 15 min at 37°C.
§ Mean ± SE of values obtained from 16 individuals.

TABLE IV

Effects of CRP or CRP-CPS Complexes on NK Response

Culture additions*	Percent specific ^{51}Cr release				
	Effector cell number				
	Experiment 1		Experiment 2‡	Experiment 3	
	3×10^5	1×10^5	3×10^5	2×10^5	1×10^5
RPMI	52 ± 4§	34 ± 3	23 ± 3	23 ± 5	9 ± 3
CRP (50 μg/ml)	47 ± 9	27 ± 3			
(100 μg/ml)	44 ± 13	27 ± 9	20 ± 2	19 ± 4	7 ± 2
(200 μg/ml)	42 ± 10	27 ± 1			
CPS (5 μg/ml)			21 ± 1	17 ± 0	4 ± 3
CRP-CPS (20:1)‖			20 ± 3	20 ± 3	10 ± 1
(50:1)			23 ± 2		
(100:1)	47 ± 5	23 ± 6	23 ± 1	16 ± 4	8 ± 3

* PBL effector cells were incubated with 5×10^3 ^{51}Cr-labeled K562 target cells in RPMI and <10 μl of CRP, CPS, or CRP-CPS.
‡ Macrophages were removed by adherence to plastic in experiments 2 and 3 but not in 1.
§ Mean ± SD.
‖ Appropriate ratios of CRP-CPS were obtained by using 100 μg/ml CRP and 5, 0.2, or 1 μg/ml of CPS, respectively.

TABLE V

Heat-modified CRP Does Not Alter NK Response to K562 Targets

Culture additions*	Percent specific ^{51}Cr release		
	Effector cell number		
	Experiment 1		Experiment 2
	2×10^5	1×10^5	3×10^5
Buffer only	29 ± 3‡	15 ± 1	9 ± 1
50 μg/ml CRP	31 ± 4	12 ± 4	9 ± 0
50 μg/ml H-CRP	30 ± 2	15 ± 3	8 ± 0
Buffer only	30 ± 1	15 ± 1	8 ± 1
100 μg/ml CRP	29 ± 1	13 ± 2	7 ± 1
100 μg/ml H-CRP	26 ± 3	14 ± 0	7 ± 0
Buffer only	25 ± 2	8 ± 1	7 ± 5
200 μg/ml CRP	26 ± 6	10 ± 2	3 ± 0
200 μg/ml H-CRP	21 ± 1	8 ± 1	3 ± 1

* PBL were incubated with K562 target cells and various concentrations of buffer, CRP, or H-CRP.

‡ Mean ± SD.

TABLE VI

K562 Target Cells Are Not Killed by Treatment with Anti-CRP and Complement

Preincubation*	Antibody‡	Complement§	Percent of cells recovered after treatment	
			Experiment 1	Experiment 2
None	−	−	100	72
	−	+	80	66
	+	+	100	66
CRP	−	−	90	68
	+	+	100	84
CRP-CPS	−	−	86	ND‖
	+	+	100	ND

* 5×10^6 K562 target cells were preincubated with buffer, CRP, or CRP-CPS complexes for 30 min at 37°C.

‡ Target cells were treated with anti-CRP at 37°C for 30 min.

§ Cells were washed, treated with complement for 45 min, washed again, and counted for viability in the presence of trypan blue. Viabilities were always within 1% in any experiment. More than 200 cells were counted in every case.

‖ Not done.

37°C and capped off within 30 min (Table III). Although we were unable to detect S-CRP using fluorescein isothiocyanate anti-CRP alone, the sensitivity of the biotin-avidin system permitted visualization of S-CRP and observation of capping.

Exogenous CRP Does Not Alter NK Response. The addition of CRP to effector-target cell mixtures did not enhance or inhibit effector function, regardless of whether CRP was added alone or as CRP-CPS complexes (Table IV). This was examined with a variety of effector cell concentrations and with both high and low NK responders. Even minimal enhancement or inhibition would have been observed under the conditions tested. Because many mononuclear phagocytic cells bind CRP, experiments were performed in which adherent cells or carbonyl iron-ingesting phagocytes were

removed, thereby ensuring that optimal concentrations of CRP or CRP-CPS complexes would be available for binding to lymphocytes. Neither CRP nor complexed CRP affected ^{51}Cr release. Similar results were obtained when the effects of H-CRP were examined (Table V).

K562 Target Cells are not Killed by Treatment with Anti-CRP and C. Because S-CRP is present on NK effector cells, we wondered whether CRP or its ligand might also be present on target cells, providing a mechanism for effector-target interactions. To test this, K562 target cells were preincubated alone or in the presence of CRP or CRP-CPS complexes, washed, incubated with anti-CRP and C, and assessed for loss of viability by trypan blue exclusion. No loss of K562 cell viability was detected after incubation with anti-CRP and C, regardless of preincubation with CRP or CRP-CPS complexes (Table VI). In every case, the viability of groups treated with antibody alone was the same as that of groups treated with both antibody and complement.

Discussion

Treatment of human PBL with anti-CRP and C removes functional NK activity to K562 target cells. Similar treatment with anti-IgG or $F(ab')_2$ anti-CRP antibodies and C does not inactivate the population of cells responsible for this killing. Removal of phagocytic cells and $OKT3^+$ T cells from an LGL population does not alter the removal of NK activity by antibody and C, suggesting that CRP or an antigenically related molecule is present on the NK effector cell and that treatment with whole antibody and C removes this activity by C-mediated lysis of the effector cell population. Although treatment of NK effectors with anti-CRP and C removes nearly all functional NK activity, it does not detectably decrease the number of cells present, perhaps reflecting that NK effectors represent <5% of the PBL (5). These results suggest that CRP may be a selective marker for some human NK effector cells.

Although it has been reported (4) that CRP was detected on the surface of ~3% of PBL only after a preincubation with exogenous CRP in the presence of CPS, experiments with anti-CRP and C in which functional NK activity was depleted prompted us to search further for S-CRP. When sensitive biotin-avidin or double-antibody techniques were used for the detection of S-CRP, a similar percentage of cells that bind S-CRP was detected. It is possible that complexed CRP binds to S-CRP and amplifies the amount of fluorescein isothiocyanate-conjugated anti-CRP. Alternatively, CRP-CPS complexes might bind to a different cell surface molecule. Additional studies of S-CRP are described elsewhere.[2]

Anti-CRP depleted NK effector function in the presence of C; however, the role of S-CRP in the NK response is not clear. Early experiments in which an intermediate (1:8) dilution of anti-CRP in the absence of C was used resulted in variable loss of NK function, but this loss was consistently seen when larger amounts of anti-CRP were used. This suggested that either anti-CRP blocked a molecule required for an effector-target cell interaction or anti-CRP facilitated capping of a molecule required for an effective NK interaction. Capping studies indicate that removal of CRP could be responsible for loss of functional NK activity in the presence of high concentrations of anti-CRP alone. This supports the concept that removal of CRP or a molecule that co-caps directly with S-CRP affects the NK response.

[2] James, K., L. Baum, C. Adamowski, and H. Gewurz. C-reactive protein antigenicity on the surface of human lymphocytes. Manuscript submitted for publication.

This suggests that CRP is involved in the NK response. Although we know that involvement is at the level of the effector cell, the mechanism of action of CRP in the NK response is not yet clear. We initially thought that CRP-CPS or CRP-ligand complexes could serve as a recognition mechanism by bridging effector and target cells in the NK response. However, inasmuch as anti-CRP plus C did not facilitate lysis of K562 and the inclusion of CRP or CRP-CPS complexes during the effector-target cell interaction did not abrogate or enhance the NK activity observed, it seems unlikely that CRP is involved in bridging between effectors and targets. Studies are in progress to determine whether modulation of CRP is involved in the delivery of an internal signal to the NK effector cell.

Although CRP or an antigenically similar molecule is present on a large percentage of the NK cells responsible for killing K562 target cells, when high numbers of effector cells were examined, some killing activity remained. This residual activity could represent (*a*) effector cells that do not express S-CRP; (*b*) effector cells that express such a low density of S-CRP that lysis with anti-CRP and C is not effective; or (*c*) limited quantities of the antibody specifically reactive with S-CRP. The heterogeneous nature of NK effector cells (23) make these possibilities likely. MOLT-4 cells and measles-infected HEp-2 are being used as target cells to determine whether the presence of S-CRP is limited to those NK effector cells that kill K562 targets.

NK cells have been characterized primarily by functional activity. Abo et al. (24, 25) and Lohmeyer et al. (26) have isolated monoclonal antibodies that detect human NK cells; the former group termed the antigen reactivity with their antibody "HNK-1." HNK-1 was detected on 15 ± 7% of PBL, and these were associated with both NK activity and antibody-dependent cell-mediated cytotoxicity (24). However, HNK-1 expression did not correlate with NK activity in cord blood of newborns or in the blood of elderly individuals (25). Additionally, the percent of HNK-1-positive cells was much larger than the 1–5% of mononuclear cells considered to be responsible for NK activity (27, 28). The two monoclonal antibodies of Lohmeyer et al. (26) recognized antigens present on suppressor and cytotoxic effector cells or those on monocytes, neutrophils, and null cells as well as NK cells. Thus, a single antibody that recognizes only NK cells has not yet been described. The monoclonal antibodies to human NK cells appear to detect a determinant different from S-CRP, because they react with more PBL than does anti-CRP and do not deplete NK function in the absence of C (24).

The IgG anti-CRP used in our studies was generated with highly purified CRP as antigen (13) and was derived from an antibody preparation that had been shown to be free of all known contaminants (4). Activity was retained upon further purification by CRP-affinity column chromatography, emphasizing that the reactivity observed was due to an interaction with an antigen or antigens present on purified CRP. To further establish that removal of functional NK activity was due to reactivity with CRP, we have prepared several monoclonal antibodies directed against CRP. Seven of nine of these monoclonals detected percentages of PBL displaying S-CRP similar to those detected with the antibody used in the experiments described herein.[2] Kearney et al. (29) and Kilpatrick et al. (30) have described an IgM monoclonal antibody to CRP that did not detect lymphocytes or neutrophils but did react with all peripheral blood monocytes. This monoclonal anti-CRP antibody did not bind to lymphocytes detected by HNK-1. Perhaps this monoclonal anti-HNK-1 antibody

recognizes a determinant of CRP that is not available for binding on NK cells or binds to a subpopulation of NK that does not bear CRP. Studies are currently in progress to determine whether HNK-1 is present on the cells that interact with our polyvalent anti-CRP and to determine the effects of our monoclonal anti-CRP antibodies on NK activity.

Summary

Functional NK activity can be removed from human PBL and from phagocyte- and T cell-depleted LGL preparations by treatment with antisera specific for C-reactive protein (CRP) in the presence of complement (C). Pretreatment of NK effector cells with high concentrations of anti-CRP in the absence of C also depletes functional activity. These results indicate that CRP or an antigenically similar molecule is present on a population of NK effector cells. Fluorescent antibody studies in which biotin-avidin amplification was used confirm the presence of surface CRP (S-CRP) on a small percentage of nonphagocytic peripheral blood mononuclear cells. S-CRP readily caps off, which suggests that removal by capping obviates killing by this cell population. This indicates that S-CRP or a molecule that co-caps with S-CRP may be required for successful effector-target cell interaction. The addition of exogenous CRP or CRP-CPS complexes, however, does not alter NK responses. A subpopulation of lymphoid cells responsible for functional NK activity therefore appears to bear surface CRP.

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