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C-reactive protein is involved in natural killer cell-mediated lysis but does not mediate effector–target cell recognition

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SUMMARY

Anti-CRP and complement treatment of human peripheral blood lymphocytes significantly reduces natural killer (NK) cell-mediated cytotoxicity to K562 target cells as well as to MOLT-4 target cells. Although not all activity is eliminated by treatment of effector cells with antibody and complement, the reduction of NK function indicates that C-reactive protein (CRP) is present on a significant proportion of NK cells. Higher concentrations of anti-CRP or anti-CRP $F(ab')_2$ fragments also reduce NK function; this suggests that CRP is not only present on these effector cells but may also play a role in NK-mediated killing. We initially suspected that CRP–ligand interactions might be involved in effector-target cell recognition. Several lines of evidence suggest that this is not the case. While $F(ab')_2$ anti-CRP will block NK function, Fab anti-CRP will not, suggesting that the NK response is not impaired when surface CRP (S-CRP) is blocked but is only inhibited when the S-CRP is cross-linked and modulated. Neither CRP–C polysaccharide complexes (CRP–CPS) nor concentrations of CPS ranging from 0·1 μg/ml to 200 μg/ml have any effect on NK cell-mediated killing. Treatment of target cells with a ligand for CRP or CRP prior to co-culture with NK effectors does not augment NK function. Single cell assays clearly demonstrate that high concentrations of anti-CRP have no effect on the formation of effector–target cell conjugates. Although these concentrations of anti-CRP do not block effector–target cell conjugation in the single cell assay, they do block the killing of conjugated target cells. In total, this evidence strongly suggests that although CRP appears to be involved in NK-mediated killing, it is not involved in effector–target cell-mediated recognition.

INTRODUCTION

C-reactive protein (CRP) is an acute phase protein that is present in extremely low concentrations in normal human serum. In situations of acute inflammation, levels increase as much as 1000-fold resulting in serum concentrations as high as 200–400 μg/ml. The primary binding specificities of CRP are phosphocholine (PC) (Volanakis & Kaplan, 1971), polycations including protamine sulphate (Siegel, Rent & Gewurz, 1974; Siegel *et al.*, 1975; DiCamelli *et al.*, 1980), certain polysaccharides (Higginbotham, Heidelberger & Gotschlich, 1970; Uhlenbruch *et al.*, 1979) and chromatin (Robey *et al.*, 1984). CRP minimally enhances blastogenesis but significantly enhances a human cytotoxic T-cell (CTL) response (Vetter *et al.*, 1983; Vetter, Gewurz & Baum, 1985). CRP binds to a subpopulation of peripheral blood lymphocytes (PBL) (James, Hansen & Gewurz, 1981a, b), and recent studies have shown that lymphocytes can produce CRP and express it on their surface (Kuta & Baum, 1986). The similarities between NK effector cells and the cells that bind anti-CRP led us to investigate the role of CRP in the NK response. Although CRP, CRP–CPS complexes and heat-aggregated CRP did not alter the NK response, NK function was nearly eliminated in the presence of high concentrations of anti-CRP. Lower concentrations of anti-CRP and C also dramatically reduced NK-mediated killing of K562. These earlier investigations suggest that CRP is involved in the NK response to K562. We have extended these studies to show that anti-CRP also inhibits NK-mediated lysis of MOLT-4 target cells. The possible role of CRP-ligand interactions in target cell recognition by NK effectors was investigated.

Several mechanisms have been previously proposed to explain NK-target cell recognition at the effector level: these include, transferrin (Vodinelich *et al.*, 1983; Newman, Warner & Dennert, 1984), laminin (Hiserodt, Laybourn & Varani, 1985),

Abbreviations: BSS, balanced salt solution; BSS-gel, BSS containing 1% gelatin; CRP, C-reactive protein; CRP–CPS, CRP–C polysaccharide complexes; CTL, cytotoxic T lymphocyte; FBS, fetal bovine serum; NK, natural killer cell; PBL, peripheral blood lymphocytes; PC, phosphocholine; RPMI–FBS, RPMI containing 10% FBS; S–CRP, cell surface CRP; % SR, percentage specific ^{51}Cr release; VBS, veronal-buffered saline.

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lectin-like recogntion structures (Stutman *et al.*, 1980) and a 90,000 molecular weight (MW) surface molecule designated NKTa (Hercent *et al.*, 1983). Target cell structures that may contribute to recognition by NK cells include: transferrin and laminin receptors, high levels of asialo GM_2 (Durdik *et al.*, 1980; Young *et al.*, 1981), low levels of surface sialic acid (Yogeeswaran *et al.*, 1982), and a 140,000 MW target cell antigen (Moingeon *et al.*, 1985).

Studies presented here confirm the previous observation that surface CRP (S-CRP) is involved in the NK response; however, they clearly demonstrate that this involvement is not at the level of target cell recognition. We suggest, therefore, that NK cell S-CRP must be required at some later stage of the cytolytic event.

MATERIALS AND METHODS

Isolation of human PBL

Mononuclear cells were isolated according to the methods of Böyum (1968) with the following modifications. Blood from normal, healthy donors was drawn into preservative-free heparin, diluted 1/2 with Mishell–Dutton balanced salt solution (BSS), layered on Ficoll–Paque (Pharmacia Fine Chemicals, Piscataway, NJ) and centrifuged at 400 *g* for 25 min. The mononuclear cell layer was harvested, washed twice with BSS containing 0·1% gelatin (BSS-gel) and was finally resuspended in RPMI supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Calbiochem-Behring Corp., La Jolla, CA), 2 mM L-glutamine, 50 μg/ml gentamicin, 100 U/ml penicillin, 100 μg/ml streptomycin, and 25 mM HEPES (RPMI-FBS). BSS-gel was used for washes solely to conserve more costly supplies. If monocytes were to be removed, cells were resuspended in 50 ml RPMI containing 20% FBS and approximately 300 mg of carbonyl iron (CAF Corp., New York, NY). Cells were then rotated for 60 min at 37° and iron filings were removed using a magnet. Approximately 25% of the cells were removed by this treatment and cells thus treated were depleted of most monocytes as demonstrated by greatly reduced proliferative responses to mitogen and absence of the monocyte population by fluorescence-activated cell sorter profile. After monocyte removal, lymphocytes were resuspended in RPMI-FBS.

Target cells

The K562 myeloid cell line and the MOLT-4 T-cell leukaemia cell line were maintained in RPMI-FBS. They were passed three times a week by resuspending cells in fresh media at a concentration of 2×10^5 cells/ml. The HEp-2 cell line was passed twice weekly by removing monolayers using a trypsin–versine solution. Cells to be used as targets were labelled with ^{51}Cr (New England Nuclear, Boston, MA) by incubating cells in 1 ml of RPMI + 10% FBS that contained 200 μCi of ^{51}Cr at 37° for 1 hr. After labelling, cells were washed three to five times in BSS-gel and were resuspended in RPMI-FBS before use.

Protamine or protamine-CRP treatment of target cells

Single cell suspensions of HEp-2 and K562 were washed twice with veronal-buffered saline (VBS) or RPMI-FBS, respectively; 5×10^5 cells were then incubated with 25–100 μg of protamine sulphate. Protamine sulphate was homogeneous upon acid electrophoresis and had a molecular weight of 5000 (Sigma Chemical Co, St Louis, MO). HEp-2 and K562 were incubated with protamine sulphate in either VBS or RPMI-FBS for 30 min at 37° and were washed once with the appropriate buffer. For CRP-protamine treatment, 5×10^5 protamine sulphate treated cells were incubated for 30 min at 37° with 50 μg of CRP in buffer that contained 1 mM Ca^{2+}.

^{51}Cr-release assay

Concentrations ranging from 5×10^4 to 40×10^4 effector cells per well were incubated in V-bottomed microtitre trays at 37° in 5% humidified CO_2 with 1×10^4 ^{51}Cr-labelled target cells. After either 4 hr or 18 hr, 100 μl of the culture supernatant was harvested and the radioactivity of the supernatant was measured. Spontaneous release values were obtained by collecting supernatants from target cells incubated with media alone, and maximum release values were obtained by incubating target cells with 0·5% Triton × 100. The formula used for calculation of the percentage specific release (% SR) was:

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

Anti-CRP

Initial experiments were performed using the IgG fraction of goat anti-CRP antibodies prepared and characterized as previously described (James *et al.*, 1981a). Characterization of this antibody involved both Ouchterlony diffusion analysis and passive haemagglutination using antigens coupled to erythrocytes by chromic chloride. No antibody activity against IgG, C3, serum amyloid P component, CPS or other antigens contained in normal human serum was detectable. Similar results were obtained with several commercial antibodies and some experiments utilized an IgG cut of goat anti-human CRP prepared by American Biosystems, Marine of St Croi, MN. Antigen for preparation of this antiserum was supplied by Dr Karen James, DuPage County Hospital, DuPage, IL. Sheep anti-CRP (N. L. Cappel Laboratories, Cochranville, PA) was also used for some studies.

Preparation of Fab and $F(ab')_2$ fragments

The IgG cut of goat anti-human CRP and $F(ab')_2$ fragments (both at 10·5 mg/ml) were purchased from Kalestaad Laboratories (Chaska, MN). Fab anti-CRP was prepared by incubating 1 ml of whole IgG anti-CRP with 0·01 M cystein, 0·002 M EDTA, and 21·0 ng papain at 37° for 46 hr and then separating on Sephadex G-75. Fractions were collected, protein peaks were pooled and concentrated to original concentrations, and were then dialysed overnight against RPMI. Fab was sterile-filtered and aliquots were frozen.

Anti-CRP and C pretreatments

Lymphocytes were brought to a concentration no greater than 1×10^7 cells/ml in RPMI-FBS. Cells were incubated for 30 min at 37° in the presence or absence of anti-CRP. They were then washed and resuspended in a 1:10 dilution of baby rabbit complement (Pel Freez, Brown Deer, WI) and were incubated for 1 hr at 37°. After incubation with antibody and complement, cells were washed and resuspended to appropriate cell concentrations in the presence of ^{51}Cr-labelled target cells.

Purification of CRP

CRP was isolated from human pleural and peritoneal fluids by previously described methods (James *et al.*, 1981a). CRP-rich

fluids were passed through a three-column purification sequence that involved binding to phosphocholine (PC)–Sepharose with elution by citrate chelation, ion exchange chromatography using DE-52, and molecular sieving through Sephacryl S-200. CRP gave a single band on SDS–PAGE. When concentrated to 10 mg/ml, no IgG, IgA, IgM, C3, or serum amyloid P component could be detected using radial immunodiffusion plates with limits of sensitivity of < 5 μg/ml.

CPS preparation

CPS was isolated by the method of Liu & Gotschlich (1963) from the R36A strain of *Streptococcus pneumoniae*. The extracted CPS was lyophilized and reconstituted to appropriate concentrations prior to use.

Single cell cytotoxicity assay

The single cell cytotoxicity assay was performed according to the methods of Grimm & Bonavida (1979) with modifications. Briefly, 2×10^6 K562 and 1×10^6 macrophage-depleted lymphocytes were incubated for 5 min at 30°. Cells were then centrifuged for 5 min at 250 ***g*** and all but 0·5 ml of the supernatant was removed. Cells were gently resuspended and the percentage of conjugates was determined. The remaining cells were mixed with RPMI-FBS that contained agarose at a final concentration 0·5%, and 1 ml of cells was distributed evenly over a 35-mm tissue culture dish that had been precoated with 1 ml of 1% agarose in RPMI-FBS. Solidified agarose was covered with 2 ml of RPMI-FBS and conjugates were incubated for 6 hr at 37° in a humidified 5% CO_2 incubator. After incubation the medium was removed and the plates flooded with 3 ml of 0·2% trypan blue solution for 5 min. The trypan blue was removed and plates were washed with cold PBS and covered with a small volume of RPMI-FBS to prevent dehydration. The viability of conjugated target cells was then evaluated microscopically.

RESULTS

Anti-CRP and complement inhibits NK-mediated killing of both K562 and MOLT-4 target cells

The experiments previously reported have utilized K562 target cells exclusively. Experiments were now performed to determine whether or not anti-CRP inhibited NK-mediated killing of another standard NK target. Effector cells were incubated in the presence or absence of a 1/4 dilution of anti-CRP, were washed, and were then incubated for 1 hr with complement. After antibody and complement treatment, cells were resuspended to the original cell volumes. Cell concentrations were not re-adjusted to account for cells lost due to antibody or complement treatment. Each group of cells was then assayed separately on ^{51}Cr-labelled K562 and MOLT-4 target cells. The results are presented in Fig. 1. Although the degree of inhibition varied from experiment to experiment and was not always as great as observed in Fig. 1, killing of K562 and MOLT-4 targets was always inhibited equally. No inhibition was seen in controls using equivalent concentrations of normal goat immunoglobulin. As an additional control, cells were incubated in the presence of media, Leu 11b, or anti-CRP. Leu 11b is a monoclonal antibody that binds to the Fc receptor on NK cells. NK activity of $43 \pm 2\%$ with media or Leu 11b was reduced to background levels in the presence of anti-CRP and to $20 \pm 1\%$ in the presence of Leu 11b and complement.

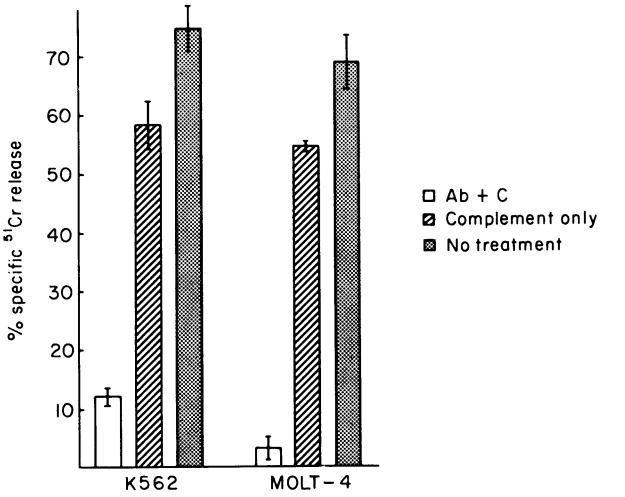

Figure 1. Anti-CRP and complement-mediated inhibition of NK activity against K562 and MOLT-4 target cells. 2×10^5 effector cells were incubated with 1×10^4 targets; E:T, 40:1. Similar inhibition was seen at all E:T ratios tested. Each point is the mean ± standard deviation of six replicate cultures.

F(ab′)$_2$ anti-CRP inhibits NK function in the absence of complement

We previously reported that anti-CRP and complement inhibited NK function while F(ab′)$_2$ anti-CRP and complement did not. The concentration of anti-CRP in these experiments was not sufficiently high to block NK function in the absence of complement. Increased concentrations of anti-CRP alone will block NK function. In these experiments we wanted to determine whether similar high concentrations of F(ab′)$_2$ anti-CRP were capable of blocking NK-mediated killing. Identical concentrations of native anti-CRP and F(ab′)$_2$ were examined for their ability to inhibit the NK response to K562. The results shown in Fig. 2 indicate that F(ab′)$_2$ anti-CRP molecules clearly inhibit as well as whole anti-CRP molecules. The percentage specific ^{51}Cr release in the absence of antibody was 52 ± 4, which is not significantly different from the values observed with a 1/128 dilution of either F(ab′)$_2$ or whole anti-CRP. The inhibition observed with either anti-CRP or F(ab′)$_2$ anti-CRP is highly dependent upon antibody concentration.

Fab anti-CRP does not inhibit NK activity

If anti-CRP inhibited NK-mediated killing by blocking accessibility of S-CRP on NK effector cells, we would expect Fab anti-CRP fragments to block cytotoxicity. If, on the other hand, capping events block function, Fab anti-CRP would not be expected to alter NK function. Similar concentrations of Fab anti-CRP and intact anti-CRP antibodies were examined for their ability to inhibit the NK response to K562 target cells. Although the results presented in Fig. 3 are only for the data obtained at a 20:1 effector to target cell ratio (E:T), three other E:T ratios were also examined yielding similar results. Although intact molecues inhibited as much as 60% of the NK

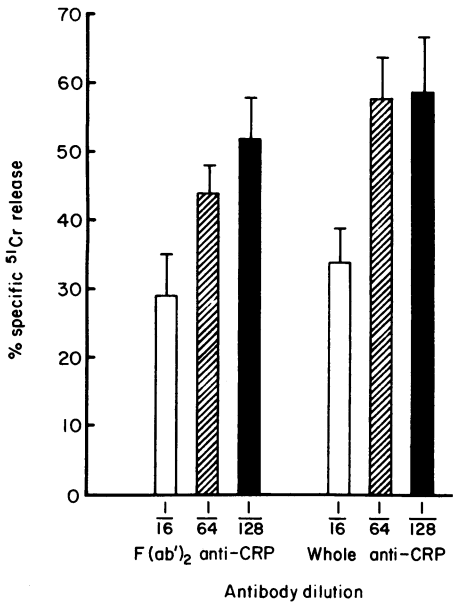

Figure 2. Effects of $F(ab')_2$ anti-CRP on the NK response. 2×10^5 human peripheral blood lymphocytes were incubated for 4 hr with 1×10^4 ^{51}Cr-labelled K562 target cells (E:T ratio, 20:1) in the presence of various dilutions of $F(ab')_2$ or intact monospecific goat anti-human CRP. Percentage specific ^{51}Cr release in the absence of antibody was $52 \pm 4\%$. This is a representative experiment and each point is the mean + standard deviation of six replicate cultures.

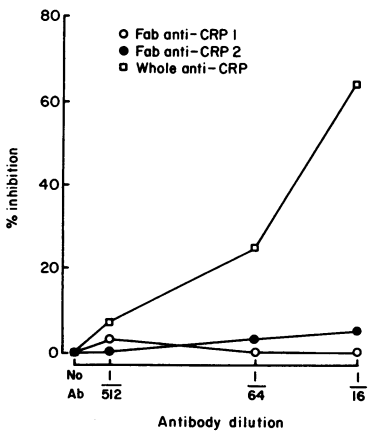

Figure 3. The effects of Fab anti-CRP antibodies on the NK response. 2×10^5 normal human peripheral blood lymphocytes and 1×10^4 ^{51}Cr-labelled K562 target cells (E:T, 20:1) were incubated for 4 hr in the presence of Fab or intact anti-CRP antibodies. This experiment is representative of three such experiments using two different Fab cuts of the same monospecific anti-CRP antibody.

activity at a 1/16 dilution, no concentration of either of two Fab anti-CRP preparations tested exhibited any inhibitory activity whatsoever.

High concentrations of CPS do not inhibit the NK response

If cell surface CRP (S-CRP) facilitates effector–target cell binding by recognizing a ligand on target cells, high concentrations of ligand should bind all of the CRP present on the surface of the NK effector cell and prevent target cell recognition and killing. The concentrations of ligand previously examined were optimal to facilitate formation of CRP–CPS complexes in serum and might not be expected to block S-CRP totally. We therefore examined the effects of 1–200 μg/ml of CPS on NK-mediated lysis of K562 target cells. Macrophage-depleted mononuclear cells ($2{\cdot}5 \times 10^4$–20×10^4) were incubated with 5×10^3 K562 target cells in a 4-hr ^{51}Cr-release assay. Enhancement (14 ± 1–23 ± 1) was seen with CPS concentrations of 100–200 μg/ml at an effector to target cell ratio of 5:1 in two out of four experiments. However, there was never any inhibition of NK activity regardless of the CPS concentration. In addition to this, preincubation of effectors with CPS did not alter our ability to inhibit NK function with anti-CRP.

Protamine or protamine-CRP treatment of target cells did not facilitate NK cytotoxicity

Although previous experiments indicated that CRP–ligand interactions were not required for effector–target cell recognition and therefore might not be the sole recognition mechanism, it was possible that these interactions might increase NK-mediated killing if ligand or CRP were present on the target cell. Experiments were therefore designed in which K562 or HEp-2 cells were coupled with ligand or ligand and CRP. These cells were then evaluated to determine whether or not NK-mediated killing was augmented. Initially, cells were incubated with various concentrations of protamine sulphate, followed by CRP and ^{125}I-labelled anti-CRP to determine the optimal protamine sulphate concentration for CRP binding. Twenty-five or 100 μg/ml of protamine sulphate sensitized 5×10^5 HEp-2 or K562 to bind 7 or 4 μg of CRP, respectively. These concentrations were chosen for further experiments. In Fig. 4a, K562 were treated with either 25 μg/ml protamine or 25 μg/ml protamine and 50 μg/ml CRP to determine whether or not the NK activity against K562 could be enhanced. The activity of NK effectors against modified K562 was not significantly different from the activity against unmodified K562. In Fig. 4b, HEp-2 were treated with either 100 μg/ml protamine alone or 100 μg/ml protamine sulphate plus 50 μg/ml CRP in order to determine whether or not a target cell that is normally resistant to NK-mediated lysis (Weston, Levy & Koren, 1980) could be altered by such treatment to create a susceptible target. No significant lysis of modified or unmodified HEp-2 cells was observed after incubation with NK effectors. When HEp-2 target cells were incubated with anti-HEp-2 and complement or with monocyte ADCC effectors, ^{51}Cr release was observed (not shown), indicative that these cells could serve as targets if recognized. Thus, the presence of protamine or protamine and CRP did not alter the killing activity generated against either NK-susceptible or NK-resistant target cells.

Anti-CRP in a single cell cytotoxicity assay blocks killing but not effector to target cell recognition

Monocyte-depleted normal human lymphocytes were incubated for 1 hr with a 1:2 dilution of anti-CRP and were assayed in a single cell cytoxicity assay (Table 1). A 2:1 ratio of pretreated or untreated lymphocytes and targets was incubated at 30° for 5–10 min and the percentages of effector–target cell conjugates were measured. Expected numbers of conjugates were observed;

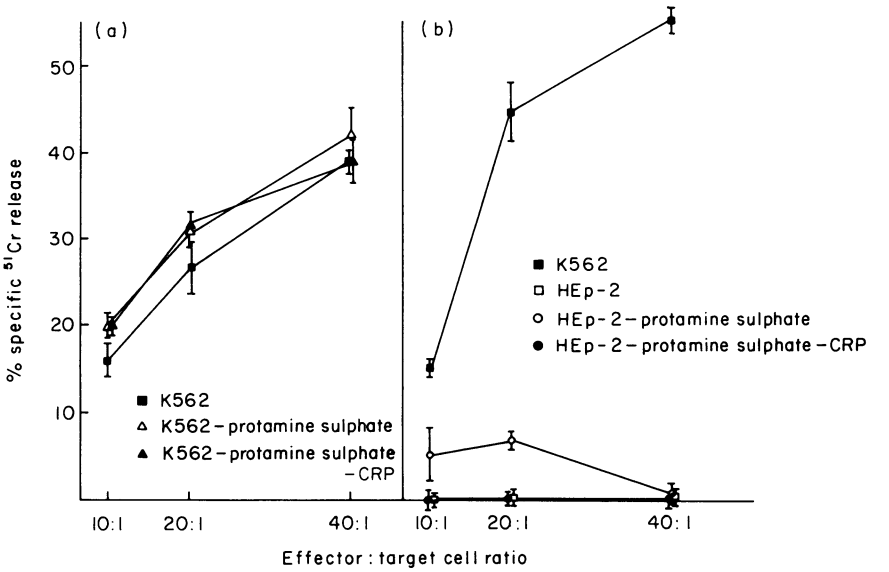

Figure 4. The effects of protamine or protamine and CRP treatment of K562 or HEp-2 target cells on NK-mediated lysis. ^{51}Cr-labelled K562 target cells (a) or HEp-2 target cells (b) were treated with 25 μg/ml of protamine sulphate or 100 μg/ml of protamine sulphate, respectively, followed by 50 μg/ml of CRP. These target cells were then incubated with various concentrations of normal human PBL and their ability to serve as target cells was compared to untreated K562 and/or HEp-2. These are representative experiments and each point is the mean ± standard deviation of six replicates.

Table 1. Anti-CRP blocks killing but not conjugation

Exp. no	Anti-CRP	% effectors conjugated	% conjugated targets killed
1	–	5	21
	+	6	3
2	–	7	22
	+	7	2
3	–	3	44
	+	4	23

NK effector cells and K562 target cells were incubated for 5 min at 30°, spun, and resuspended. The percentage of conjugates was visually determined. Cells were then distributed in agarose, incubated for 6 hr, and the viability of conjugated targets was examined using trypan blue exclusion.

there were no differences between the percentages of conjugates formed with pretreated and untreated effector cells in any of three similar experiments. After a 6-hr incubation at 37° in an agarose matrix, immobilized complexes were observed. Anti-CRP was not toxic to the pretreated lymphocytes; however, anti-CRP-treated lymphocytes killed far fewer bound target cells than untreated effector cells.

DISCUSSION

CRP is expressed as a membrane protein in a small but functionally significant subpopulation of lymphocytes (James *et al.*, 1982, 1983; Baum *et al.*, 1983). It is also produced by lymphocytes (Kuta & Baum, 1986). When surface-CRP is bound by immunoglobulin molecules, NK cells are inactivated. Since CRP appears to bind ligands present in some cell membranes and binds to CRP receptors (James *et al.*, 1982, 1983) under physiological conditions, we considered the possibility that CRP–ligand or CRP–receptor interactions might be responsible for the target cell recognition mediated by CRP-positive NK effector cells.

If CRP were involved in effector–target cell recognition, then acute-phase levels of serum CRP might regulate the NK response *in vivo*. The physiological functions of the extremely high concentrations of CRP that are present during the acute phase have not yet been clearly defined; a role for serum CRP in NK cell regulation would, therefore, be of considerable interest. Previously reported data clearly showed that, in our hands, the exogenous addition of highly purified CRP does not significantly alter the human natural killer cell response (Baum *et al.*, 1983). Reports from other laboratories suggest that CRP does inhibit the NK response (Kimura *et al.*, 1982; Shibuya *et al.*, 1983; Saijo *et al.*, 1984). Additional studies have confirmed our original findings, making it unlikely that serum CRP blocks effector–target cell recognition during the acute-phase response. These studies did not, however, rule out involvement of the cell surface molecule in effector–target cell recognition. The studies contained in this publication were designed to determine whether or not CRP is involved in NK cell recognition.

Inhibition of NK activity is not restricted to the conventional K562 target cell; lysis of the NK-sensitive MOLT-4 cell line is also blocked by anti-CRP. This finding suggests that the inhibition of NK activity by anti-CRP is not target cell restricted.

Earlier studies have shown suppression of the NK response using relatively low concentrations of anti-CRP and C. Anti-

CRP alone at these low concentrations did not inhibit NK activity in the absence of C. In addition, $F(ab')_2$ fragments used at similar low concentrations had no effect on NK activity. When anti-CRP or anti-CRP $F(ab')_2$ fragments were used at higher concentrations, inhibition of the NK response was observed in the absence of C. Similar concentrations of Fab fragments of anti-CRP had no effect on the NK activity. These results suggest that cross-linking is involved in inhibition. This supports previous fluorescent data that correlate loss of function with capping events (Baum *et al.*, 1983).

Several independent observations indicate that the inhibition observed with anti-CRP is significant and not trivial. Although anti-CRP inhibits NK function, none of the following do: normal goat or sheep immunoglobulin, anti-immunoglobulin, Fab anti-CRP, or Leu 11b. Both Fab anti-CRP and Leu 11b bind NK cells but do not inhibit function. Since Leu 11b recognizes the Fc receptor on NK cells and will fix complement, it does inhibit NK activity in the presence of complement. Normal immunoglobulin and anti-immunoglobulin do not bind to NK cells but serve as controls for other effects that might be mediated by the antisera.

If S-CRP were involved in target cell recognition via S-CRP-ligand interactions, NK activity should be inhibited by the addition of CRP ligands such a C-polysaccharide (CPS). High concentrations of CPS did not inhibit NK activity. Although the addition of free ligand did not inhibit NK activity, if S-CRP was involved in target cell recognition, enhancement of effector–target cell ligand interactions might enhance NK cytotoxicity. The CRP ligand protamine was used to modify the cell surface of K562 target cells. This procedure did not increase killing above base-line. The killing of K562 may have already been optimal in this system, therefore the NK-resistant cell line HEp-2 was examined. Protamine modification of HEp-2 cells did not render them susceptible to NK-mediated lysis. These results led us to conclude that the addition of ligand to the assay system did not enhance target cell recognition by NK effectors. A similar percentage of large granular lymphocytes express receptors for CRP as well as S-CRP (James *et al.*, 1983). If CRP-receptors on NK cells were mediating binding to NK targets, then target cells with high concentrations of CRP on their surface would be more susceptible to NK recognition. When K562 or HEp-2 were treated with protamine and CRP, their cell surface CRP concentrations were considerably higher and yet neither functioned as a better target as a result of additional CRP.

Finally, the role of S-CRP was tested in the single-cell cytotoxicity assay. High concentrations of anti-CRP did not inhibit effector–target cell conjugation, neither did they kill NK effectors. Although effector–target cell conjugation was unaffected by anti-CRP treatment, the number of targets killed by anti-CRP-treated effectors was dramatically lower than the number killed by untreated effectors.

CRP is present on the NK effector cell; anti-CRP or $F(ab')_2$ anti-CRP block NK-mediated killing of K562 or MOLT-4 target cells. Neither a CRP ligand (CPS) nor Fab anti-CRP blocked NK-mediated killing, and killing could not be enhanced by attaching either ligand or CRP to target cells. Single cell assays clearly demonstrate that anti-CRP blocks the killing of target cells but does not block conjugate formation. The evidence presented here confirms the role of CRP in the NK response and clearly indicates that S-CRP is not involved in effector–target recognition events.

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