Macrophage Activation and Generation of Tumoricidal Activity by Liposome-associated Human C-Reactive Protein

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ABSTRACT

The effect of human C-reactive protein incorporated into multilamellar vesicles (CRP-MLV) was studied in assays of macrophage function. Peritoneal exudate macrophages from C57BL/6 mice phagocytosed CRP-MLV in vitro more rapidly than multilamellar vesicles bearing comparable amounts of immunoglobulin G. Exposure of peritoneal exudate macrophages in vitro to CRP-MLV resulted in development of tumoricidal activity against syngeneic T241 fibrosarcoma and B-16 melanoma cells and against allogeneic Sarcoma 1 cells. Peritoneal exudate macrophages obtained from mice given CRP-MLV i.p. demonstrated antitumor activity against the syngeneic T241 fibrosarcoma in a Winn-type assay, and when challenged in vitro with phorbol myristate acetate, they showed elevated superoxide anion production. Administration of CRP-MLV i.p. did not enhance natural killer activity of spleen cells, however. In superoxide anion assays, CRP-MLV were approximately 10 to 100 times more effective than free C-reactive protein. Results indicate that C-reactive protein is capable of activating macrophages, thus supporting the concept of C-reactive protein as an immunomodulator.

INTRODUCTION

During periods of acute inflammation, the concentration of CRP, an M. 105,000 protein normally found in trace amounts in serum, becomes sharply elevated to levels of 100-fold or more (12, 35). While CRP has been found in a wide range of animal species, including invertebrates (29), the biological significance of the molecule has not been fully established. Certain activities of CRP are analogous to immunological functions mediated by IgG, for example, the activation of C1q (6) and the ability to precipitate pneumococcal CPS (1). There is also evidence that CRP interacts with immunocompetent monocytes and lymphocytes, especially those bearing Fc receptors, and that such interaction results in modulation of immune responses (15, 23, 25). In a previous study, we found that treatment with liposome-associated human CRP inhibits lung metastasis and prolonged survival in C57BL/6 mice bearing the syngeneic T241 fibrosarcoma (8). While the mechanism of this tumor-inhibitory effect was not established, data presented here suggest that macrophages activated by liposome-associated CRP may be involved. Results herein demonstrate that liposome-associated CRP is readily endocytosed by macrophages and enhances both superoxide anion production and tumoricidal activity.

MATERIALS AND METHODS

Mice. Six- to 8-week-old male C57BL/6 mice were obtained from The Jackson Laboratory, Bar Harbor, Maine.

Tumors. The T241 fibrosarcoma, syngeneic in C57BL/6 mice, was maintained by serial passages in vivo (7). The tumor was free of murine leukemia virus as well as pneumonia virus of mice, reovirus type 3, K virus, encephalomyelitis virus, mouse hepatitis virus, lymphocytic choriomeningitis virus, minute virus of mice, mouse adenovirus, Sendai virus, and polyoma virus (assayed by MA Bioproducts, Walkersville, Md.). The BL-6 subline of B-16 melanoma (donated by Dr. I. J. Fidler, Frederick Cancer Research Facility, Frederick, Md.), a spontaneous tumor from C57BL/6 mice, and Sarcoma 1, a chemically induced fibrosarcoma of A/J mice, were maintained in vitro in Hanks' minimal essential medium supplemented with 10% fetal bovine serum, l-glutamine (292 µg/ml), penicillin (100 units/ml), streptomycin (100 µg/ml), and 1% of each of the following 100X solutions: nonessential amino acids; vitamins; and sodium pyruvate (Grand Island Biological Co., Grand Island, N. Y.).

CRP. CRP was purified to homogeneity from serum fluids, and the purity of the final preparation was verified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, as described previously (8, 36). The endotoxin level, as assayed by Limulus amebocyte lysate assay (Associates of Cape Cod, Inc., Woods Hole, Mass.), was less than 0.01 ng/µg CRP.

Preparation of Liposomes. Multilamellar liposomes were prepared from PC and PS (Avanti Biochemicals, Inc., Birmingham, Ala.) at a 1:1 molar ratio as described previously (7, 8). Solutions of reagents to be encapsulated were made in PBS at a concentration of 27 µg protein per ml. In addition to CRP, liposomes were also prepared with human IgG and HSA (Calbiochem-Behring, Inc., La Jolla, Calif.) or with buffer alone. Liposomes incorporating MTP-PE (CGP19835; Ciba-Geigy, Ltd., Basel Switzerland) were prepared by evaporating 5 µg MTP-PE per µmol phospholipid and dispersing the dry film in PBS (11, 33). Liposomes containing DNP-PE (Avanti) were prepared similarly at a DNP-PE:PC:PS molar ratio of 1:4:3. The efficiency of protein encapsulation was determined by encapsulating 125I-labeled reagents prepared by the method of Bolton and Hunter (3). For CRP, encapsulation efficiency was 292 ± 3.5 (S.D.) ng/µmol phospholipid (2 determinations), and for human IgG and HSA, 220 and 90 ng were encapsulated, respectively, per µmol phospholipid. In some experiments, buffer-containing, formed PS:PC liposomes were incubated with various concentrations of 125I-labeled CRP in PBS at room temperature for 60 min, and after washing and centrifugation (10,000 x g for 15 min), the amount of surface-bound radiolabeled protein was determined. Buffer-containing DNP-PE:PS:PC MLV were coated similarly with 125I-labeled γ-globulin fraction of rabbit anti-DNP antibody (Miles-Yeda, Elkhart, Ind.).

Preparation of PEC. PEC were collected in phenol red and calcium- and magnesium-free HBSS from C57BL/6 mice that had been given i.p. 1.0 ml thioglycolate broth 6 days earlier and 0.5 ml PBS containing 2.5...
μmol MLV 24 hr earlier. The mean endotoxin content of 5 lots of thiglycollate was 3.1 ng/ml, while PBS contained less than 0.01 ng per ml. Differential cell counts were made on Wright-Giemsa-stained cytospin preparations, and nonspecific esterase staining was also used to determine macrophage concentration. Typically, PEM exposed in vivo to liposomal reagents consisted of 75% macrophages, 13% granulocytes, and 12% lymphocytes. Monolayers of adherent PEM were prepared by incubating PEM for 60 min at 37°C in DMEM (Grand Island Biological Co.) supplemented with 5% heat-inactivated fetal bovine serum (Sterile Systems, Logan, Utah). Adherent cells were characterized as over 90% macrophages by differential and cytochemical analysis described above. For observation of PEM by transmission electron microscopy, coverslips of adherent PEM which had been incubated for 3 hr with 2.5 μmol liposome-associated CRP per ml DMEM were washed in PBS and fixed in 3.75% glutaraldehyde in 0.1 M sodium cacodylate buffer containing 6% sucrose. PEM were postfixed in 1% osmium tetroxide and then stained with uranyl and lead acetate.

Assay for Macrophage Tumoroidal Activity. Monolayers of adherent PEM at 10⁵ cells per well of Microtest III plates (B-D Labware, Oxnard, Calif.) were incubated in DMEM for 24 hr with 100 nmol liposomes or culture medium alone, then washed, and exposed to 5000 [¹¹³I]labeled target cells. Methods for performing this assay have been described in detail (34). After 72 hr, cultures were washed, and adherent cells were lysed with 0.1 n NaOH for determination of radioactive content. The percentage of cytotoxicity was calculated from the following formula based on mean cpm in target exposed to control MLV or test MLV (CRP- or MTP-MLV).

% of cytotoxicity =  

\[ \frac{\text{Control PEM} - \text{test PEM}}{\text{Control PEM}} \times 100 \]

Phagocytosis and Binding of Liposomes. PEM, plated at 2 x 10⁶ cells/well of Microtest III plates (B-D Labware), were incubated in DMEM with 100 to 400 nmol liposomes containing 125I-labeled reagents. To inhibit uptake and examine only binding, some wells contained 5 HIM buffer or CRP-MLV, and DNP-PE:PS:PC MLV coated with a comparable amount of IgG. Binding of PEM to HSA-associated MLV (1.2 nmol/10⁶ PEM) was significantly (p < 0.001) less than binding to either PEM. Binding of PEM to HSA-associated MLV (1.2 nmol/10⁶ PEM) was significantly (p < 0.001) more than binding to human or rabbit IgG-coated MLV, however, as determined by including iodoacetate in the assays, receptor involvement in PEM phagocytosis of CRP-MLV was considered as base line and was subtracted from values for the percentage of lysis of other groups to yield net percentage of lysis.

Chemiluminescence Assay. PEM (5 x 10⁵) in 4 ml phenol red-free HBSS containing 1% gelatin and 10⁻⁴ M luminal (5-amino-2,3-dihydro-1,4-pthalazinedione; Sigma) were placed in glass scintillation vials (Wheaton Scientific, Millville, N. J.) in semidarkness. Vial contents were then mixed rapidly with 10 nmol PMA or 2 mg zymosan (Sigma), opsonized with fresh human serum, and monitored immediately for production of chemiluminescence in a scintillation counter (Tracor, Elk Grove Village, Ill.) at room temperature. Vials were counted every 17 min for a total of 4 to 6 hr. Data were expressed as peak cpm (28).

Procedure for Winn Neutralization Assay. PEM from C57BL/6 mice that had been given i.p. injections of liposomal reagents 24 hr earlier were mixed in HBSS with syngenecic T241 tumor cells at a 50:1 ratio. Cell mixtures were incubated at 37°C for 60 min, and then 0.05 ml containing 10⁵ T241 cells was injected s.c. into the dorsal of each hind foot. Controls received tumor cells alone. After 50 days, mice were killed, feet bearing primary tumors were amputated and weighed, and the degree of lung metastasis was determined by a metastatic index as described previously (7, 8). Net tumor weights were calculated by subtracting the weight of non-tumor-bearing feet (150 mg).

Statistical Analysis. Statistical significance of data was determined by comparing results of test and control groups in the various assays by the 2-tailed Student t test.

RESULTS

Binding and Uptake of Liposomes. Initial experiments had indicated that PEM phagocytosed MLV containing 280 ng CRP per μmol phospholipid at a significantly greater rate (p < 0.005 by 2 hr) than MLV containing human IgG (220 ng/μmol) (Chart 1). Uptake of MLV containing IgG was also found to be significantly (p < 0.001) higher than was uptake of MLV containing HSA at 2 hr. The amount of protein bound externally to the liposome surface was not characterized in these early experiments, however. Subsequent studies were carried out with buffer-filled PS:PC MLV coated with 450 ng [¹²⁵I]-CRP per μmol and DNP-PE:PS:PC MLV coated with a comparable amount of rabbit IgG antibody to DNP. PEM took up significantly (p < 0.001) greater quantities of CRP-coated MLV than antibody-coated MLV by 1 hr (Chart 1), confirming previous observations with conventionally prepared MLV. Binding of PEM to CRP-MLV, however, as determined by including iodoacetate in the assays, did not differ significantly from binding to human or rabbit IgG-associated MLV in any experiment and typically was in the range of 25 to 45 nmol CRP-MLV or IgG-associated MLV per 10⁶ PEM. Binding of PEM to HSA-associated MLV (1.2 nmol/10⁶ PEM) was significantly (p < 0.01) less than binding to either CRP-MLV or IgG-associated MLV. Uptake of CRP-MLV by PEM was visually confirmed by transmission electron microscopy (Fig. 1).

Receptor involvement in PEM phagocytosis of CRP-MLV was examined by allowing PEM first to phagocytose uncoated MLV, or MLV coated with nonradiolabeled CRP or IgG antibody, and...
Macrophage Activation by Liposomal CRP

Chart 1. Phagocytosis of 125I-protein-labeled MLV by murine PEM. PEM were incubated with 250 nmol MLV for the times shown, then washed, and lysed, and the residual radioactivity was determined. O—O, CRP-MLV; •—•, human IgG-associated MLV; △, HSA-associated MLV; O—O, buffer:PS:PC MLV coated with 450 ng CRP per nmol lipid; •—•, buffer:DNP-PE:PS:PC MLV coated with 450 ng anti-DNP antibody per nmol lipid. Representative data from 2 experiments are shown. Bars, S.E.

Chart 2. Effects of nonradiolabeled CRP or IgG-coated MLV (450 ng protein per nmol lipid) on phagocytosis of 125I-CRP-coated MLV. Murine PEM were incubated for 90 min with various doses of nonradiolabeled MLV, then washed, and incubated an additional 90 min with 100 nmol 125I-CRP-coated MLV (450 ng/nmol lipid). PEM were washed again and lysed, and residual radioactivity was determined. Results are expressed as percentage of control, where control PEM received only 125I-CRP-coated MLV. △, uncoated buffer:PS:PC MLV; O, buffer:PS:PC MLV coated with CRP; •, buffer:DNP-PE:PS:PC MLV coated with anti-DNP antibody. Data from 3 experiments are summarized. Bars, S.E.

then MLV coated with 125I-CRP. When compared with uncoated MLV, all doses of unlabeled CRP-MLV significantly (p < 0.05) reduced subsequent uptake of 125I-CRP-MLV (Chart 2). Preincubation with antibody-coated MLV did not significantly diminish uptake of CRP-MLV except at the highest dose used (p < 0.05).

Generation of Tumoricidal Activity. PEM incubated in vitro with CRP-MLV or MTP-MLV developed cytotoxic activity against syngeneic T241 sarcoma and B-16 melanoma target cells, as well as against allogeneic Sarcoma 1 cells. Syngeneic normal skin fibroblasts were not affected by liposome-exposed PEM (Table 1). Administration of liposomal reagents in vivo also induced tumoricidal PEM in 2 experiments using B-16 melanoma target cells. PEM exposed to CRP-MLV and MTP-MLV demonstrated 32% (p < 0.001) and 35% (p < 0.001) mean cytotoxicity, respectively, compared to activity of PEM from control -MLV-treated mice.

Superoxide Anion Production. Superoxide anion release was studied in PEM taken from animals that had been given thioglycolate i.p. followed by liposomal reagents or free CRP. When challenged with 100 nM PMA, PEM from animals receiving either CRP-MLV or MTP-MLV produced significantly higher levels of superoxide dismutase-inhibitable O2− than did PEM from mice receiving thioglycolate alone (Table 2). Activity of PEM from control (buffer) MLV-treated animals was not significantly different from that of thioglycolate-induced PEM. PEM from mice given free CRP equal to that encapsulated in MLV (0.75 μg) did not demonstrate elevated O2− release, although responses of PMA-stimulated PEM exposed to 5 or 10 μg of free CRP were significantly higher than those of thioglycolate controls (Table 3). However, only when a dose of 100 μg free CRP was given was the magnitude of the O2− response equivalent to that seen with CRP-MLV.

NK Cell Activity. Spleen cells from mice receiving CRP-MLV or an equivalent amount of free CRP i.p. did not demonstrate elevated NK cell activity against YAC-1 target cells (Chart 3). Elevated NK activity was seen only in animals given polyriboinosinic:polyribocytidylic acid.

Production of Chemiluminescence. PEC from mice given liposomal reagents i.p. were tested for production of chemiluminescence. In MTP-MLV-treated groups, PEC produced consistently higher responses than did cells from CRP-MLV- or buffer:MLV-treated animals. Chemiluminescence of PEC from CRP-MLV-treated mice was, however, significantly (p < 0.01) higher than that displayed by PEC from animals given buffer:MLV (Table 4).

PEC Activity in a Winn Assay. The ability of PEC from liposome-treated animals to inhibit growth of syngeneic T241 sarcoma was examined in a Winn-type assay. Primary tumor weights and extent of lung metastasis were significantly (p < 0.05) reduced in animals given tumor cells mixed with PEC from CRP-MLV- or MTP-MLV-treated donors, when compared to values for animals given tumor cells alone (Table 5). In animals
Human CRP is recognized as having opsonizing activity. In studies of both human and murine phagocytic cells, CRP has provided by results of the chemiluminescence and Winn assays, there is considerable evidence for species cross-reactivity in the cellular interactions of CRP. Murine macrophages avidly phagocytosed CRP-MLV, and after exposure to CRP-MLV there is evidence for CRP enhancement of macrophage activity, although the mechanism by which this effect is produced remains to be defined. Although human CRP was used in these studies with mouse macrophages, there is considerable evidence for species cross-reactivity in the cellular interactions of CRP. Murine macrophages avidly phagocytosed CRP-MLV, and after exposure to CRP-MLV in vitro or in vivo, they displayed enhanced production of superoxide anion and increased tumoricidal capacity. Further evidence for CRP enhancement of macrophage activity was provided by results of the chemiluminescence and Winn assays, in which the peritoneal cells tested contained predominantly macrophages.

Human CRP is recognized as having opsonizing activity. In studies of both human and murine phagocytic cells, CRP has

### Table 2

**Superoxide anion release by peritoneal macrophages from mice given i.p. injections of liposomes**

<table>
<thead>
<tr>
<th>Treatment of peritoneal macrophage donor</th>
<th>Superoxide anion (nmol/10⁶ PEM) at various times after challenge with PMA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>Thioglycollate alone</td>
<td>0.5 ± 0.27</td>
</tr>
<tr>
<td>Buffer:MLV</td>
<td>0.6 ± 0.61</td>
</tr>
<tr>
<td>CRP-MLV</td>
<td>3.1 ± 0.61</td>
</tr>
<tr>
<td>MTP-MLV</td>
<td>2.0 ± 0.58</td>
</tr>
</tbody>
</table>

* Animals received 1.0 ml thioglycollate i.p. and, 5 days later, 0.5 ml PBS containing 2.5 µmol MLV. PEM were obtained 20 hr later.

**Table 3**

**Superoxide anion release by peritoneal macrophages from mice given i.p. injections of free CRP**

<table>
<thead>
<tr>
<th>Treatment of peritoneal cell donor</th>
<th>Peak cpm after challenge with</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thioglycollate alone</td>
<td>19 ± 3</td>
</tr>
<tr>
<td>Buffer:MLV</td>
<td>87 ± 43</td>
</tr>
<tr>
<td>CRP-MLV</td>
<td>41 ± 12</td>
</tr>
<tr>
<td>MTP-MLV</td>
<td>250 ± 95</td>
</tr>
</tbody>
</table>

* Animals received 1.0 ml thioglycollate i.p. and, 5 days later, 0.5 ml PBS containing 2.5 µmol MLV. PEM were obtained 20 hr later.

**Table 4**

**Chemiluminescence of PEC from mice given i.p. injections of liposomes**

<table>
<thead>
<tr>
<th>Treatment of adherent PEM</th>
<th>Peak cpm from 5 replicates per variable. Five such experiments were done.</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>[250 ± 95 to 100,617 ± 7,474] (PMA)</td>
</tr>
<tr>
<td>Buffer:MLV</td>
<td>[502 ± 88 to 3,220 ± 883] (PMA)</td>
</tr>
<tr>
<td>CRP-MLV</td>
<td>[338 ± 149 to 19 ± 3] (PMA)</td>
</tr>
</tbody>
</table>

* Animals received 1.0 ml thioglycollate i.p. and, 5 days later, 0.5 ml PBS containing 2.5 µmol MLV. PEM were obtained 20 hr later.

### DISCUSSION

Our studies demonstrate that human CRP associated with liposomes enhances macrophage activity, although the mechanism by which this effect is produced remains to be defined. Although human CRP was used in these studies with mouse macrophages, there is considerable evidence for species cross-reactivity in the cellular interactions of CRP. Murine macrophages avidly phagocytosed CRP-MLV, and after exposure to CRP-MLV in vitro or in vivo, they displayed enhanced production of superoxide anion and increased tumoricidal capacity. Further evidence for CRP enhancement of macrophage activity was provided by results of the chemiluminescence and Winn assays, in which the peritoneal cells tested contained predominantly macrophages.

Human CRP is recognized as having opsonizing activity. In studies of both human and murine phagocytic cells, CRP has...
been found to promote phagocytosis of *Streptococcus pneumoniae* or erythrocytes coated with pneumococcal CPS (21, 26). Such CRP-enhanced phagocytosis has been shown to require the participation of complement. Complement was not added in our CRP-MLV uptake experiments, and addition of complement-intact fresh human serum to CRP-MLV did not increase the rate of phagocytosis (data not shown). Interactions of CRP-MLV with complement occurring in *in vivo*; however, cannot be ruled out, since multilamellar liposomes coated with CRP have been shown to activate the classical pathway of complement (32, 37).

Hsu and Juliano (13) found that coating liposomes with proteins capable of interacting with macrophage surface receptors enhanced phagocytosis markedly. Both IgG and fibronectin enhanced liposome uptake in this manner without the addition of complement. These same investigators also found that receptors for the Fc portion of IgG and for fibronectin were distinct, based on experiments showing independent uptake of IgG and fibronectin-coated liposomes. We have not demonstrated total independence in uptake of IgG and CRP-coated MLV. Phagocytosis of IgG-coated MLV inhibited to some extent subsequent phagocytosis of CRP-MLV, suggesting that the Fc receptor may be important in uptake of CRP-coated vesicles. Such data confirm earlier findings of Mortensen and Duszkiewicz (24), who observed that uptake of CRP-coated erythrocytes by murine macrophages was inhibited by both CRP and IgG. James et al. (14, 15) have pointed out that human leukocytes and other cells capable of binding to complexed CRP (CRP bound to a ligand such as CPS) also have Fc receptors. These intriguing reports suggest a relationship between CRP binding and Fc receptors, but the physicochemical nature of the membrane components involved remains to be determined.

Generation of murine macrophage tumoricidal activity by liposomes containing various biological response modifiers such as lipopolysaccharide or MTP has been studied extensively by Fidler and coworkers (10, 11, 31, 34). In a previous study (7), we also observed that liposomes encapsulating lipopolysaccharides induced macrophage tumoricidal responses *in vitro* and *in vivo*. Reduced lung metastasis in mice bearing the T241 fibrosarcoma. The lipopolysaccharide preparation used, however, was not purified but contained a crude supernatant from mitogen-stimulated lymphocytes. In a subsequent study (8), we showed that liposomes containing CRP purified to homogeneity also produced similar inhibition of lung metastasis. Other serum proteins such as IgG, albumin, transferrin, haptoglobin, and fibronectin with or without liposomes did not inhibit lung metastasis, and free CRP, in concentrations 70 times greater than that given with liposomes, was also ineffective. Free CRP in our *in vitro* studies enhanced macrophage superoxide anion activity but only in concentrations that were 10 to 100 times greater than the effective liposome-encapsulated dose. In other studies comparing efficacy of free and liposome-encapsulated MDP, a component of mycobacterial cell wall, approximately 4000 times more free MDP was required to produce the same results as those of the liposome-encapsulated reagent (34).

To further judge the efficacy of CRP-MLV activity in our experiments, MTP-coupled liposomes were used, and they represented a standard, well-characterized liposomal reagent known to activate macrophages (11, 33). Although a quantitative comparison of the effects of these agents was not carried out in these studies, under the same conditions in which MTP-MLV consistently enhanced macrophage functions, CRP-MLV produced similar results. The possibility that endotoxin may have enhanced effects of CRP-MLV or free CRP cannot be ruled out, since low levels were present in thioglycolate and other reagents.

Doses of endotoxin too low to be stimulatory in themselves have been found to be necessary for generating macrophage tumoricidal responses in the presence of pneumolysins (20, 27), but the relationship between endotoxin and CRP in terms of effects on macrophage function has not yet been studied.

Treatment of CRP preparations with Polymyxin B, a polycation which binds to endotoxin (22), was found to remove some (approximately 40%) but not all of the macrophage-activating effect of free CRP in a tumoricidal assay (data not shown). It is possible, however, that Polymyxin B might also bind to CRP in addition to endotoxin, since CRP has been shown to bind to polycations (9).

In studies of human monocytes exposed to liposomes containing MDP, development of tumoricidal activity was found to correlate closely with enhanced superoxide anion release (19). Elevated oxidative metabolism, as illustrated by enhanced superoxide anion release and production of chemiluminescence, has also been shown to occur in macrophages exposed to *Corynebacterium parvum* or mycobacterial products, reagents which induce tumoricidal activity (5, 17). The components of this augmented respiratory burst have been found to be essential to the enhanced microbicidal capacity of activated macrophages (16), while the precise relationship between products of oxidative metabolism and tumoricidal potential is not yet well understood (4). Nevertheless, development of tumoricidal capacity and elevated oxidative metabolism appear to be closely related occurrences.

Results of the Winn-type assay support findings from the previous study in which i.v. administration of CRP-MLV was found to inhibit lung metastasis in tumor-bearing animals (8). The mechanisms related to the antitumor effects of CRP-MLV appear to involve macrophages primarily, since to date we have found no evidence for activated NK cells in spleens of CRP-MLV-treated mice. On the other hand, administration of polystyrenosin:polynucleotidic acid clearly produced elevated NK cell activity in our experiments, under conditions in which CRP-MLV was also ineffective. Free CRP in our *in vitro* studies enhanced macrophage superoxide anion activity but only in concentrations...
had no such effect. A relationship between NK cell function and CRP has been suggested by findings that NK cells have CRP, or an antigenically similar molecule, as a surface component and that antisera to CRP inhibits NK activity (2). However, addition of either free CRP or CRP complexed to CPS was not found to alter NK cell responses (2). Thus, at this stage, the possible involvement of NK cells in explaining the biological effects of CRP remains an unresolved question.

The findings presented here indicate that CRP associated with liposomes can interact with macrophages and enhance their activity. These data also strongly suggest that one biological role for CRP may be as modulator of immune effector cell function.

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