Degradation of rat C-reactive protein by macrophages

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Rat C-reactive protein (CRP) is a serum glycoprotein belonging to the 'pentraxin' family of proteins. In this study we have shown the specific binding of ^{125}I -CRP to rat peritoneal macrophages at 4 °C. This binding was dependent upon incubation time, CRP and cell concentrations, and was not inhibited by either phosphorylcholine or human IgG. At 37 °C, the surface-bound ^{125}I -CRP was internalized and degraded. The degradation of ^{125}I -CRP was measured by the formation of ^{125}I -labelled trichloroacetic-acid-soluble CRP peptides by either precipitation assays or by h.p.l.c. of the incubation medium using a gel-filtration column. Since chloroquine and leupeptin inhibited

CRP degradation, it was concluded that degradation of CRP occurred in the lysosomal compartment of the macrophage. There was an absolute requirement for the presence of bivalent cations (Ca^{2+} and Mg^{2+}) in the incubation medium for the binding and degradation of CRP, which could be inhibited by EDTA but not by phosphorylcholine or human IgG. H.p.l.c. analysis of the medium obtained from incubation of macrophages with ^{125}I -CRP revealed the presence of ^{125}I -labelled low- M_r peptides, the formation of which was dependent upon incubation time.

INTRODUCTION

The interactions of human C-reactive protein (CRP) with cells of the immune system have been extensively studied (Gewurz, 1982; Koib-Bachofen, 1991; Kilpatrick and Volanakis, 1991). Most of these interactions involve the binding of CRP to the phosphorylcholine ligand, which is essential for CRP to function as an opsonin (Kilpatrick and Volanakis, 1991; Kushner, 1991). Human CRP has been shown to affect monocyte and macrophage function by modulating the generation of superoxide anion and tumouricidal activity (Hokama et al., 1986; Zahedi and Mortensen, 1986; Barna et al., 1987, 1988; Buchta et al., 1987; Gautam et al., 1987; Miyagawa et al., 1988a; Nunomura et al., 1990; Dobrinich and Spagnuolo, 1991). There are reports suggesting that human CRP peptides generated from the action of neutrophil and/or monocyte proteases on human CRP modulate the phagocytic function of the immune cells (Buchta et al., 1986; Robey et al., 1987; Shephard et al., 1989).

CRP is present in certain invertebrates and most mammalian species (Gewurz, 1982; Kolb-Bachofen, 1991; Kilpatrick and Volanakis, 1991; Kushner, 1991). Although there are significant variations in the CRP structure in these species, a common property that has been conserved throughout evolution is that of CRP binding to the phosphorylcholine ligand (Kolb-Bachofen, 1991).

The primary structure of rat CRP (M_r 125000), previously described as rat phosphorylcholine-binding protein (PCBP), has recently been derived (Rassouli et al., 1992). The unique features present in rat CRP sequence, that are not found in human CRP, are (i) two potential sites for glycosylation (Asn-128 and Asn-147) of which only one (Asn-128) is glycosylated; (ii) the presence of an extra C-terminal heptapeptide (Leu-205–Ser-211); and (iii) of the five apparently identical monomers of rat CRP, only two form a dimer linked by interchain disulphide bonds between Cys-208 and Cys-209. Besides these features, the isoelectric point and the concentrations of rat and human CRP in the serum are also very different. Rat CRP has a pI around 3.4 while the pI of human CRP is 7.9 (Baltz et al., 1982), and the normal physio-

logical concentration of rat CRP and human CRP is 600 and 0.1 $\mu\text{g}/\text{ml}$ of serum respectively (Gewurz, 1982; Saxena et al., 1985).

We have previously shown that rat CRP is synthesized by the hepatocytes (Collins et al., 1984), but is not internalized unless the terminal sialic acids from rat CRP are removed (Yang et al., 1992). We have also shown the inhibitory effect of rat CRP on platelet aggregation (Nagpurkar et al., 1988), and have described interactions of rat CRP with lipoproteins (Nagpurkar and Mookerjee, 1981; Nagpurkar et al., 1983; Saxena et al., 1985, 1986, 1987, 1989). The present study shows that after binding to rat peritoneal macrophages rat CRP is internalized, degraded and released into the incubation medium as low- M_r peptides. This process is not mediated through the phosphorylcholine-binding site of CRP. The internalization of rat CRP by macrophages may support a mechanism in which CRP may have a role in the clearance of phosphorylcholine-containing foreign pathogens by the macrophage.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (body wt. 250–300 g) were purchased from Charles River Canada, La Prairie, Quebec, Canada. Rats were fed Purina Chow.

Reagents and chemicals

Dulbecco's modified Eagle's medium (DMEM), Dulbecco's phosphate-buffered saline (DPBS), fetal-calf serum (FCS) and Penicillin–streptomycin solution were purchased from Gibco Laboratories, Burlington, Ontario, Canada. Carrier-free [^{125}I]Nal was from Dupont (Canada). Pronase was from Calbiochem, San Diego, CA, U.S.A. EDTA and all other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Preparation of rat CRP

Rat CRP was isolated from normal rat serum using a

Abbreviations used: CRP, C-reactive protein; DMEM, Dulbecco's modified Eagle's medium; DPBS, Dulbecco's phosphate-buffered saline; FCS, fetal-calf serum; TCA, trichloroacetic acid; PTA, phosphotungstic acid.

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Sephacrose-phenylphosphorylcholine affinity column as described (Nagpurkar and Mookerjee, 1981). Rat CRP was iodinated using [125 I]NaI and Iodo-gen iodination reagent as previously described (Yang et al., 1992). The specific radioactivity of 125 I-CRP was in the range of 180–350 d.p.m./ng. The purity of 125 I-CRP was checked by co-eluting the 125 I-CRP and non-labelled CRP from an h.p.l.c. gel-filtration column, or by PAGE of a mixture of 125 I-CRP and non-labelled CRP using 8–25 % gradient gels. In each case, > 96 % of the radioactivity corresponded with the unlabelled protein and > 98 % of the radioactivity was precipitable by 5 % (w/v) trichloroacetic acid (TCA)–1 % (w/v) phosphotungstic acid (PTA).

Cell culture

Non-stimulated macrophages were harvested from male Sprague–Dawley rats by peritoneal lavage with DPBS lacking Ca^{2+} and Mg^{2+} (Edelson and Cohen, 1976). In brief, cells were collected by centrifugation, washed ($\times 1$) with the DPBS and suspended in DMEM containing 20 % (v/v) FCS and 1 % (v/v) penicillin–streptomycin solution (medium A). An aliquot (1 ml; $3\text{--}5 \times 10^6$ cells) was used to inoculate plastic six-well tissue-culture plates which were then incubated for 2 h in a humidified incubator saturated with 5 % CO_2 and 95 % O_2 at 37 °C. After 2 h, each dish was washed ($\times 3$) with DMEM to remove non-adherent cells, and then maintained in medium A overnight for experiments on the following day. Each dish of adherent macrophages contained 30–40 μg of cell protein. Before the start of the experiment, cells were equilibrated for 30 min with either medium B or medium C. (Medium B: DPBS with Ca^{2+} and Mg^{2+} and supplemented with BSA-containing penicillin–streptomycin solution, and medium C: DMEM supplemented with BSA-containing penicillin–streptomycin solution).

Binding assays

Binding assays were performed in duplicate at 4 °C in tissue-culture dishes containing 30–40 μg of cell protein in medium B. Unless otherwise stated, binding assays were carried out using 16 μg of 125 I-CRP ($3\text{--}4 \times 10^6$ d.p.m.), and incubated for up to 6 h. At the end of the incubation period the supernatant was carefully decanted and the cells washed ($\times 3$) with 50 mM Tris/HCl (pH 7.4) containing 0.15 M NaCl and BSA (2 mg/ml) and finally washed ($\times 3$) with 50 mM Tris/HCl (pH 7.4) containing 0.15 M NaCl. The cell-surface-associated radioactivity (total binding) was determined by dissolving the cells in 0.2 M NaOH and counting an aliquot for radioactivity in a Beckman 5500 γ -counter. Another aliquot was used to measure the protein content (Lowry et al., 1951). Non-specific binding was determined by carrying out the binding assays in the presence of a 100-fold excess of non-labelled CRP, which was subtracted from the total binding to give specific binding. Experiments to study the effect of Ca^{2+} and Mg^{2+} on the binding of 125 I-CRP to macrophages were carried out using a fixed amount of 125 I-CRP in Ca^{2+} and Mg^{2+} -free medium, to which increasing concentrations of Ca^{2+} and Mg^{2+} were added. The effect of human IgG and phosphorylcholine on the binding of 125 I-CRP to macrophages was studied by including a fixed amount of 125 I-CRP in the presence of the indicated amounts of either IgG or phosphorylcholine in the incubations, and the binding was measured as described earlier.

Degradation assays

Degradation assays were performed in duplicate by addition of either fixed (16 μg), or increasing, amounts of 125 I-CRP to the

culture plates containing $3\text{--}5 \times 10^6$ cells in medium C (unless otherwise specified), and incubating at 37 °C for different time intervals. At the end of the incubation, a 200 μl aliquot of the medium was assayed in duplicate for degradation of CRP. Control incubations were carried out in the same medium in the presence of 125 I-CRP, but in the absence of cells. The radioactivity (expressed as ng of CRP) obtained from these incubations was subtracted from the experimental values and the degradation expressed as ng of CRP degraded/ml of incubation medium.

The degradation of 125 I-CRP was measured by two methods. Method 1: By the TCA–PTA precipitation method for the estimation of the amount of 125 I-labelled TCA-soluble material formed in the incubation medium as a result of degradation of CRP by macrophages. To the incubation medium (200 μl) was added 1 ml of ice-cold 5 % TCA–1 % PTA, followed by centrifugation at 12800 *g* for 5 min. The supernatant was carefully removed and an aliquot (600 μl) was counted for radioactivity. Method 2: this was carried out by analysing the incubation medium for the formation of 125 I-CRP-labelled degradation products by h.p.l.c. (Perkin Elmer series 4) using a gel-filtration column (TSK Spherogel 2000SW). An aliquot of the incubation medium was injected directly on to the column maintained in 50 mM sodium sulphate and 20 mM sodium dihydrogen phosphate (pH 6.8), and eluted at a flow rate of 1 ml/min. The absorbance of the eluent was continuously monitored at 280 nm with a variable-wavelength detector and aliquots were counted for radioactivity at various time intervals over the entire elution period. The column was previously calibrated using a mixture of known M_r standards.

Effect of chloroquine, leupeptin, EDTA, bivalent cations, IgG and phosphorylcholine on the degradation of CRP

Unless otherwise specified, these assays were carried out using macrophages which were incubated with 125 I-CRP (16 μg) in medium C at 37 °C for 24 h. The effect of chloroquine on the degradation of CRP was studied as a function of time by including 50 or 100 μM chloroquine in the incubation mixture. The effect of increasing concentrations of leupeptin on the degradation of CRP was studied by incubating macrophages with 125 I-CRP in the presence of leupeptin (0–300 μM). The effect of IgG and phosphorylcholine on CRP degradation was studied by incubating the macrophages with 125 I-CRP in the presence of the indicated amounts of either human IgG or phosphorylcholine. The effect of bivalent cations (Ca^{2+} and Mg^{2+}) on CRP degradation was studied by incubating macrophages and 125 I-CRP in DPBS without Ca^{2+} and Mg^{2+} but containing BSA and penicillin–streptomycin solution, to which increasing amounts of bivalent cations (Ca^{2+} and Mg^{2+}) were added. In another experiment macrophages were incubated in medium B to which increasing amounts of EDTA was added. In all of these experiments aliquots of the incubation medium at the indicated times were assayed for degradation of CRP.

Surface binding/internalization and degradation of rat CRP

Preliminary experiments, in which 125 I-CRP (16 μg) was bound to the macrophages at 4 °C for 4 h, showed that > 90 % of the surface-bound 125 I-CRP was released into the incubation medium when macrophages were incubated for 20 min at 4 °C in medium containing Pronase (1 mg/ml). Based on this information, 125 I-CRP (16 μg) was incubated with the macrophages at 4 °C for 4 h. The macrophages were then washed thoroughly after which the medium was replaced with medium C and the temperature raised to 37 °C and incubated for up to 4 h. After the specified times,

the medium was decanted and an aliquot was counted for TCA-soluble (degraded ^{125}I -CRP) and TCA-insoluble radioactivity. The medium was replaced with medium containing Pronase and the macrophages kept at 4°C for 20 min to release the surface-bound ^{125}I -CRP. An aliquot of this medium was counted for radioactivity (surface-bound ^{125}I -CRP). Macrophages were then digested with 0.2 M NaOH (600 μl) and the radioactivity of an aliquot counted (internalized ^{125}I -CRP).

RESULTS

Binding of rat CRP to macrophages

Figure 1(a and b) shows non-saturable specific binding of rat CRP to macrophages at 4°C as a function of time (Figure 1a), and CRP concentration (Figure 1b). The binding of CRP was also dependent on cell concentration, and maximum binding occurred when a cell concentration of 4×10^6 cells/ml was used (result not shown). The effect of human IgG and phosphoryl-

Figure 1 Binding of rat CRP to macrophages as a function of time and CRP concentration

(a) The binding of rat CRP to macrophages as a function of incubation time (○, total binding; ●, non-specific binding; △, specific binding). (b) The total binding of rat CRP to the macrophages after 4 h incubation in the presence of increasing CRP concentrations. Macrophages ($3-5 \times 10^6$) were incubated in 1 ml of medium B in six-well plastic tissue-culture plates with either ^{125}I -CRP (2 μg) (a) or increasing amounts of ^{125}I -CRP (b) at 4°C for up to 6 h. At the indicated times the amount of rat CRP bound to the macrophages was measured as described in the Materials and methods section. Non-specific binding was determined in the presence of a 100-fold excess of unlabelled CRP. Specific binding was obtained by subtracting non-specific binding from total binding. Results represent the average of at least two experiments which were very reproducible.

Figure 2 Effect of Ca^{2+} and Mg^{2+} and EDTA on the binding and degradation of rat CRP

(a) The effect of increasing concentrations of Ca^{2+} and Mg^{2+} on the binding of ^{125}I -CRP to the macrophages. Macrophages ($3-5 \times 10^6$) were incubated with ^{125}I -CRP (16 μg) for 4 h at 4°C in 1 ml of DPBS (without Ca^{2+} and Mg^{2+}) to which increasing concentrations of Ca^{2+} and Mg^{2+} were added. At the indicated time the amount of ^{125}I -CRP bound to the macrophages was determined as described in the Materials and methods section. (b) The effect of increasing concentrations of Ca^{2+} and Mg^{2+} and EDTA on the degradation of rat CRP by macrophages. Macrophages ($3-5 \times 10^6$) were incubated with ^{125}I -CRP for 12 h at 37°C in 1 ml of DPBS (without Ca^{2+} and Mg^{2+}) to which increasing concentrations of Ca^{2+} and Mg^{2+} were added (○). Experiments with EDTA were carried out in medium B to which increasing concentrations of EDTA were added (●). An aliquot (200 μl) of the incubation medium was assayed for the formation of TCA-soluble degraded ^{125}I -CRP as described in the Materials and methods section.

choline on the binding of CRP to macrophages was examined, and it was found that the CRP binding could not be inhibited by up to a 5-fold molar excess of either human IgG or a 50-fold molar excess of phosphorylcholine (over CRP) (results not shown). The binding of rat CRP to the macrophages was dependent on the presence of Ca^{2+} and Mg^{2+} and optimal binding occurred at approx. 1.5–3.0 mM Ca^{2+} and Mg^{2+} (Figure 2a). The role of Ca^{2+} and Mg^{2+} in the degradation of CRP is illustrated in Figure 2(b).

Degradation of rat CRP by macrophages

Figure 3(a and b) shows the degradation of ^{125}I -labelled rat CRP at 37°C by macrophages, as a function of incubation time (Figure 3a) and CRP concentration (Figure 3b). In both cases the degradation was linear; however, when the degradation was studied as a function of CRP concentration, a concentration

Figure 3 Degradation of rat CRP by macrophages as a function of time and CRP concentration

Macrophages ($3-5 \times 10^6$) were incubated at 37 °C in 1 ml of medium C either with a fixed amount of ¹²⁵I-CRP (16 μg) for different time intervals (a), or for 24 h in the presence of increasing concentrations of ¹²⁵I-CRP (b). At the indicated times, a 200 μl aliquot of the incubation medium was assayed for TCA-soluble degraded ¹²⁵I-CRP. The degradation is expressed as ng of CRP degraded/ml of incubation medium as described in the Materials and methods section. Results represent the average of at least two experiments which were very reproducible.

greater than 10 μg/ml was required to observe the degradation of CRP. The significance of this observation at the moment is not clear. It may be suggested that the degradation process may be dependent upon a certain critical CRP concentration, leading to an aggregated form which may be a pre-requisite for the process.

Surface binding, internalization and degradation of rat CRP as a function of incubation time

Figure 4(a) shows the gradual decrease of the ¹²⁵I-labelled rat CRP bound to the surface of Pronase-treated macrophages. The optimum amount of Pronase and the incubation time were determined from a separate experiment. The internalization and degradation of the surface-bound ¹²⁵I-labelled rat CRP by macrophages is shown in Figures 4(b) and 4(c).

Incubation of rat CRP in macrophage-conditioned medium

Macrophage-conditioned medium was used to determine whether any extracellular degradation of CRP occurred during incubation. Macrophage-conditioned medium was prepared by incubating macrophages (4×10^6) in medium C for 24 h, after which

Figure 4 Surface binding, internalization and degradation of rat CRP

Macrophages ($3-5 \times 10^6$) were incubated with ¹²⁵I-rat CRP (16 μg) at 4 °C for 4 h. Macrophages were then washed, fresh medium C replaced and cells incubated at 37 °C for 0, 30, 60, 90 and 240 min. Internalized and surface-bound counts were determined at the indicated times by incubating the macrophages with 1 mg/ml Pronase. Counts released into the medium represent surface-bound CRP (a), while those associated with the pellet represent internalized CRP (b). Degraded CRP (c) was estimated by assaying an aliquot of the medium for the formation of TCA-soluble degraded ¹²⁵I-CRP before Pronase treatment. In each case results are expressed as ng of CRP degraded/ml of incubation medium.

time the medium was carefully decanted and used in the following experiment. ¹²⁵I-CRP (16 μg) was incubated in the macrophage-conditioned medium for up to 24 h, and the medium assayed at different time intervals for degradation of CRP. There was virtually no measurable degradation of CRP by the macrophage-conditioned medium for up to 24 h. It was therefore concluded that the degradation of CRP could be solely attributed as being due to the macrophages.

Effect of IgG, phosphorylcholine, bivalent cations, chloroquine and leupeptin on the degradation of CRP

Addition of up to a 5-fold molar excess of human IgG (over CRP) did not inhibit the degradation of CRP (result not shown).

Figure 5 Effect of chloroquine and leupeptin on the degradation of rat CRP by macrophages

(a) The effect of chloroquine on CRP degradation was studied by incubating macrophages ($3-5 \times 10^6$) in 1 ml of medium C with ^{125}I -CRP (16 μg) for 24 h at 37 °C either in the absence (○) or presence of 50 μM (●) or 100 μM (△) chloroquine. At the indicated time an aliquot (200 μl) of the incubation medium was assayed for TCA-soluble degraded ^{125}I -CRP as described in the Materials and methods section. (b) The effect of leupeptin on CRP degradation was studied by incubating macrophages ($3-5 \times 10^6$) with ^{125}I -CRP (16 μg) for 24 h at 37 °C in the presence of increasing concentrations of leupeptin. The incubation medium (200 μl aliquot) was assayed for the formation of TCA-soluble ^{125}I -CRP as described in the Materials and methods section.

This result was consistent with the earlier result which showed that human IgG had no effect on the binding of CRP to macrophages. Phosphorylcholine (up to an 800-fold molar excess) also did not inhibit CRP degradation, suggesting that the degradation of rat CRP by macrophages is not mediated through the phosphorylcholine-binding site of rat CRP.

The degradation of ^{125}I -CRP by macrophages was dependent on the presence of Ca^{2+} and Mg^{2+} in the incubation medium (Figure 2b). The optimum Ca^{2+} and Mg^{2+} concentration required for maximum degradation was between 1.5 and 3.0 mM. Figure 2(b) shows the inhibitory effect of increasing amounts of EDTA on the CRP degradation. The degradation of rat CRP was also dependent on the incubation temperature, since at 4 °C the degradation of rat CRP was < 3 % of that at 37 °C (result not shown).

The effects of chloroquine and leupeptin are shown in Figures 5(a) and 5(b). The effect of chloroquine was studied at concentrations of 50 and 100 μM (Figure 5a), while the effect of leupeptin was studied in the presence of increasing concentrations

(0–300 μM) of leupeptin (Figure 5b). Both effectively inhibited the degradation of CRP in a dose-dependent manner, suggesting that CRP degradation occurred in the lysosomal compartment of the macrophages.

Analysis of CRP degradation by h.p.l.c.

Figure 6 shows the h.p.l.c. gel-filtration profile of the ^{125}I -labelled low- M_r peptides from the incubation medium, formed as a result of macrophage-mediated ^{125}I -CRP degradation. There was a marked increase in the formation of ^{125}I -labelled low- M_r peptides formed during 4, 8, 12 and 24 h incubations of rat CRP with macrophages. In contrast, in parallel incubations without macrophages (Figure 6, lower panel), there was no similar time-dependent formation of ^{125}I -labelled low- M_r peptides.

DISCUSSION

In this study, we have provided evidence for the macrophage-mediated binding and degradation of rat CRP. We have shown very clearly the internalization and degradation of rat CRP by macrophages. This process was contingent upon the presence of Ca^{2+} and Mg^{2+} in the incubation medium, and was dependent upon the duration of the incubation and the CRP concentration. Consistent with the binding of rat CRP to macrophages, the degradation of rat CRP was also linear with incubation time, which suggested that the degradation of rat CRP might be an unregulated process.

To investigate whether the degradation of CRP by macrophages was intracellular, the effect on the degradation of CRP by chloroquine and leupeptin, known inhibitors of lysosomal protein hydrolysis, was examined. Chloroquine, a weak base that raises the pH in the lysosomes (Mellman et al., 1986), and leupeptin, a lysosomal protease inhibitor (Seglen, 1983), both inhibited the CRP degradation, thus providing evidence that CRP is internalized and degraded in the lysosomes. The fact that 'macrophage-conditioned medium' did not degrade CRP (see the Materials and methods section) provided further proof that CRP degradation was intracellular, and not due to the action of macrophage proteases released into the incubation medium. To the best of our knowledge this is the first report showing the internalization and lysosomal degradation of rat CRP (Nagpurkar et al., 1991). More recently the receptor-mediated internalization and degradation of human CRP by the human monocytic cell line U-937 has been reported (Tebo and Mortensen, 1991).

Based on our data from Figure 4, after 4 h at 37 °C 21 % of the ^{125}I -CRP bound to the macrophages at 4 °C was surface bound, 24 % was internalized, while 24 % was degraded as TCA-soluble material in the incubation medium. The time-dependent formation of low- M_r ^{125}I -labelled CRP peptides in the incubation medium was observed only in the incubation medium containing the macrophages. There are reports demonstrating an immunomodulating role for peptides bearing a tuftsin-related sequence generated from the breakdown of human CRP (Buchta et al., 1986; Robey et al., 1987; Shephard et al., 1989). It remains, however, to be established whether the low- M_r peptides derived from rat CRP have any biological role, either in the macrophage-mediated degradation process or as modulators of phagocytic function.

There have been at least two reports (Tebo and Mortensen, 1990; Crowell et al., 1991) which have claimed that the binding of human CRP to the human monocytic cell line U-937 is mediated through the FcR receptors. However, our results showed that rat CRP binding to macrophages and degradation

Figure 6 Formation of ^{125}I -labelled TCA-soluble CRP peptides by macrophages

^{125}I -labelled rat CRP (16 μg) was incubated in 1 ml of medium C at 37 °C either in the presence or absence of macrophages for 4, 8, 12 and 24 h. An aliquot (50 μl) of the incubation medium was injected directly on a TSK Spherogel 2000 gel-filtration column maintained in 50 mM sodium sulphate and 20 mM sodium dihydrogen phosphate buffer (pH 6.8) at a flow rate of 1 ml/min. The column was previously calibrated using standards of known M_r . The eluent was continuously monitored for absorbance at 280 nm. An aliquot (50 μl) was counted for radioactivity over the entire elution period. The numbers (c.p.m.s) associated with the low- M_r peptides refer to the difference in radioactivity associated with the low- M_r peptides obtained from incubations with (upper panel) and without macrophages (lower panel).

was not inhibited by human IgG. This suggested that the IgG-specific receptors on macrophages (the FcR receptors) (Munn et al., 1991) are probably not involved in the binding and degradation of rat CRP. In support of our observation, there are reports that have clearly demonstrated the presence of receptors on phagocytic cells involved in the binding and degradation of CRP which are distinct from the FcR receptors (Ballou et al., 1989; Zeller et al., 1989; Ballou and Cleveland, 1991).

There have also been studies in which peritoneal macrophages were used to study interactions with either human CRP (Miyagawa et al., 1988b) or rat CRP (Nunomura et al., 1990), but these studies have primarily concentrated on the effect of CRP on macrophage migration and generation of superoxide ion.

The lack of inhibition by phosphorylcholine of the binding of CRP to macrophages and its subsequent degradation suggests that the phosphorylcholine-binding site of CRP is not utilized in the binding and degradation process. Our observations are consistent with previous reports which showed that phosphorylcholine did not inhibit the binding of human CRP to monocytes

(U-937) (Tebo and Mortensen, 1990; Crowell et al., 1991). Based on our results, we propose that there should be a site on rat CRP other than the phosphorylcholine-binding site through which this binding and degradation of CRP occurs. It should be pointed out that in support of a second binding site on CRP, there have been recent reports suggesting that a second binding site could be present on CRP the specificity of which is directed towards carbohydrate determinants on the cell surface (Kolb-Bachofen, 1991). The phosphorylcholine-binding site on CRP could then be used to bind phosphorylcholine-containing macromolecules and deliver them to the macrophages for their ultimate clearance. Further experiments are in progress to investigate this point.

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