

C-REACTIVE PROTEIN MEDIATES THE SOLUBILIZATION OF NUCLEAR DNA BY COMPLEMENT IN VITRO

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C-reactive protein (CRP)¹ is the prototypical acute phase protein; serum levels of CRP increase by as much as 2,000-fold during the first 24–48 h after the onset of certain types of inflammation (1, 2). Although CRP was discovered over fifty years ago (3), subsequent studies have yet to uncover a unique function for CRP. It is believed that CRP resembles immunoglobulins because, when bound to certain ligands, it can promote reactions of precipitation (3, 4), complement fixation (5), and phagocytosis (6). Therefore, it has been reasonable to hypothesize a unique function for CRP involving any or all of these phenomena.

Recently (4), chromatin was described as being a unique ligand for CRP; it was demonstrated that binding of CRP to the nucleosome core particles, the basic structure present in chromatin, occurred with an affinity of at least 2,400-times the affinity of CRP binding to any other previously described ligand. Chromatin is a term used to describe nuclear DNA complexed to its natural proteins, most of which are histones. Individually, DNA and histones are highly water-soluble substances because of the respective polyanionic and polycationic charges. However, chromatin itself is an insoluble, sticky material as a result of charge neutralization when the histones are electrostatically bound to DNA.

In view of the wide range of inflammatory conditions in which insoluble chromatin might be released from damaged cells and in which CRP levels in serum are elevated, we sought methods to verify in vitro our previous hypothesis (4) about the in vivo function of CRP, namely, that it mediates the removal of chromatin from the body after cell death. In the present study, we performed experiments using the hemolytic activity of serum against sensitized sheep erythrocytes and chromatin containing [³H]DNA as an indicator of very complex events in serum brought on by CRP-chromatin complexes. We found an association between the degree of chromatin solubilized by serum and the CRP concentration. Additionally, we found that some systemic lupus erythematosus (SLE) patients may have a depressed mechanism to solubilize chromatin, which

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¹ *Abbreviations used in this paper:* CRP, C-reactive protein; ELISA, enzyme-linked immunosorbent assay; PC, phosphorylcholine; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SLE, systemic lupus erythematosus; TLCK, *N*- α -*p*-tosyl-L-lysine chloromethyl ketone.

may contribute to the induction of either anti-DNA antibody formation or pathogenic immune complexes found in many of these patients.

Materials and Methods

Reagents. Fresh human serum was prepared from clotted whole blood obtained from healthy volunteers and stored at -20°C until use. Serum minus C3 was purchased commercially (Cappel Laboratories, Cochranville, PA). Serum genetically deficient in C2 was provided by Dr. Michael Frank, National Institute of Allergy and Infectious Diseases (NIAID), and purified C2 was purchased from Cordis Laboratories, Inc., Miami, FL. Serum that had been passed through a column of Bio-Rex-70 (Bio-Rad Laboratories, Richmond, CA) and was lacking C1q, and purified C1q prepared as described (26), were gifts from Dr. Andrea Tenner, NIAID. Rabbit antiserum to human histone H2B was provided by Dr. Michael Bustin, NCI, NIH. A WiDr cell line derived from human colon carcinoma specimens was provided by Mr. Robert Cunningham and Dr. Philip Noguchi, CDB/FDA. [^3H]thymidine (sp act, 60 Ci/mmol) was from New England Nuclear, Boston, MA. Brij 35 and *N*- α -*p*-tosyl-L-lysine chloromethyl ketone (TLCK) were from Sigma Chemical Co., St. Louis, MO.

Preparation of Human CRP from Rheumatoid Arthritis Plasma. Citrated plasma was obtained by plasmapheresis from a rheumatoid arthritis patient hospitalized at the Clinical Center, NIH, Bethesda, MD. Heparin (5,000 U) was added to 500 ml plasma, followed by 50 ml of a buffer containing 1.5 M NaCl, 0.5 M Tris, pH 7.4, and 0.1 M CaCl_2 . The heparinized plasma was then passed through a 1.5×12 cm column containing phosphor-ylcholine (PC)-Sephrose (7), and the PC-Sephrose was washed overnight with 900 ml of a buffer consisting of 0.15 M NaCl, 0.05 M Tris, pH 7.4, and 0.01 M CaCl_2 (Tris/saline/ Ca^{2+}), together with 1,000 U heparin. Additional washes were with 50 ml of the same Tris/saline/ Ca^{2+} buffer containing 0.3% Brij 35, and with 50 ml Tris/saline/ Ca^{2+} buffer containing NaCl to a final concentration of 2 M. Finally, the PC-Sephrose was washed with 50 ml of the Tris/saline/ Ca^{2+} buffer and the CRP was eluted with 0.1 M PC in the same buffer. Those fractions having an $A_{280} > 1.0$ were pooled and dialyzed exhaustively against 0.15 M NaCl, 0.05 M Tris, 0.01 M EDTA, pH 7.4, followed by dialysis against the Tris/saline/ Ca^{2+} buffer. After the readdition of Ca^{2+} to the eluted protein sample, a precipitate formed in the dialysis bag. The precipitated material was found to be 90% serum amyloid P component, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (8), amino acid analysis, and amino-terminal sequence analysis of the first 20 amino acids, all of whose results were in complete agreement with the properties previously described (9). CRP was found exclusively in the soluble fraction. The purity of the CRP was verified by SDS-PAGE (8).

Assays for Serum Complement Consumption by CRP-Chromatin Complexes. Chromatin from rabbit liver was prepared by the method of Rizzo and Bustin (10). The microcomplement fixation technique as described by Champion et al. (11) was modified after optimizing conditions by incubating 246 μg chromatin with from 140 to 1 $\mu\text{g}/\text{ml}$ CRP and 50 μl human serum in a total volume of 1 ml. The buffer used was 0.15 M NaCl, 0.05 M Tris, pH 7.4, 0.1 mM CaCl_2 , and 0.12 mM MgCl_2 . After the above mixture was incubated for 1 h at 37°C , 0.5 ml sensitized sheep red blood cells (3.7×10^8 cells/ml) was added and the mixtures incubated at 37°C for 20–30 min. After the allotted time, the mixtures were centrifuged and the optical density of each supernatant was determined by spectrophotometry at 520 nm (sheep red cells [Cappel Laboratories] were sensitized by treating the cells in 2 ml buffer at 37°C for 30 min with 10 μl rabbit anti-sheep red blood cell serum [Cappel Laboratories]).

Chromatin Solubilization Assays. Chromatin containing [^3H]DNA was prepared as follows: Rabbit lung fibroblasts or human WiDr cells from colon carcinoma specimens were grown to 30–40% confluency in twelve 150-cm² tissue culture flasks (Corning Glass Works, Corning, NY) in basal Eagle's medium (for the rabbit lung fibroblasts) or Eagle's minimum essential medium (for the WiDr cells), each supplemented with 10% fetal calf serum and, initially, 1 $\mu\text{Ci}/\text{ml}$ of [^3H]thymidine. After 24 h, the medium was replaced

with medium containing 10% fetal calf serum but no [^3H]thymidine. When the cells were finally confluent, they were washed twice in medium and, in 15 ml of hypotonic buffer containing 15 mM NaCl, 5 mM Tris, pH 7.4, 1 mM TLCK, and 1 mM CaCl_2 , the cells were swollen by standing at 4°C for 30 min. Nuclei containing the [^3H]DNA were prepared by lysing the swollen cells with a type B Dounce homogenizer in buffer, and washing the resulting nuclei with buffer containing 1% Triton X-100. Nuclei were washed by centrifuging a suspension of the nuclei at 6,000 rpm for 2 min, withdrawing the supernatant from the nuclear pellet, and gently resuspending the pellet in fresh buffer containing no TLCK or Triton X-100. Chromatin was prepared from the nuclei by the method described by Rizzo and Bustin (10). An average specific activity of $\sim 1.5 \mu\text{Ci}/\text{mg}$ DNA was obtained after several preparations. The [^3H]nuclei and [^3H]chromatin were prepared on the same day that the experiments were conducted.

[^3H]Chromatin at concentrations of 2–5 mg per 10 ml in basal Eagle's medium was evenly suspended by gently homogenizing with a type B Dounce homogenizer. To several polypropylene tubes (12 × 75 mm) containing 100 μl CRP of varying concentrations was added 200 μl [^3H]chromatin suspension, 200 μl human serum, and 500 μl basal Eagle's medium. The tubes were then collectively placed into a Dubnoff metabolic shaking incubator at 37°C for allotted times; the reaction was stopped by removing the contents of each tube and centrifuging for 5 min at 15,000 rpm in an Eppendorf microfuge at 25°C. The supernatant was then filtered on a 0.22- μm -pore membrane (Millipore/Continental Water Systems, Bedford, MA). The quantity of solubilized [^3H]DNA was determined by placing 100 μl of the filtrate into 10 ml Aquasol (New England Nuclear) and determining the radioactivity using an LS 7800 scintillation counter (Beckman Instruments, Inc., Fullerton, CA).

Studies on CRP in SLE Plasma. Patients with SLE were seen in the outpatient or inpatient services of the Arthritis and Rheumatism Branch, NIADDK at the Clinical Center of NIH. All patients satisfied preliminary ARA criteria for SLE. Plasma from these patients was citrated and stored at -70°C until use.

The quantity of CRP in the plasma of SLE patients was determined by an enzyme-linked immunosorbent assay (ELISA) developed using purified CRP and affinity-purified goat anti-CRP, following the method of Voller et al. (12). The sensitivity of the assay was 0.2 ng/ml.

CRP was removed from SLE plasma using either the PC-Sepharose method outlined above for the removal of CRP from rheumatoid arthritis plasma or by antibody affinity chromatography. For the antibody affinity chromatography method, affinity-purified sheep anti-CRP Ig was immobilized to Sepharose using activated Sepharose CH-4B (Pharmacia Fine Chemicals, Piscataway, NJ). Before adding the anti-CRP Sepharose to the SLE plasma, the resin was washed in two column volumes of 4 M MgCl_2 . After reequilibration of the resin in 0.15 M NaCl, 0.05 M Tris, pH 7.4, we added 2 ml resin to 50 ml heparinized SLE plasma and rotated the mixture overnight at 4°C. The resin was washed first with Tris/saline (100 ml), then with Tris/saline/0.3% Brij 35 (50 ml), then with Tris/saline containing 2 M NaCl (50 ml), and, finally, with 4 M MgCl_2 . Those fractions having an $A_{280} > 0.1$ (full scale) were pooled, dialyzed against water, and dried for evaluation by SDS-PAGE.

Results

Complement Consumption by CRP-Chromatin Complexes. Complement was pre-treated with chromatin and increasing concentrations of CRP at 37°C. It was then assayed in a hemolytic assay. With increasing amounts of CRP and, therefore, increasing chromatin-CRP complexes, there was increasing depletion of complement (Fig. 1). Fig. 1 further illustrates that chromatin alone does not deplete the hemolytic complement of serum within the incubation time used (1 h); nor does CRP alone, i.e., without chromatin (data not shown). Preincubation of the serum for 30 min at 56°C abolished the hemolytic activity. Results parallel

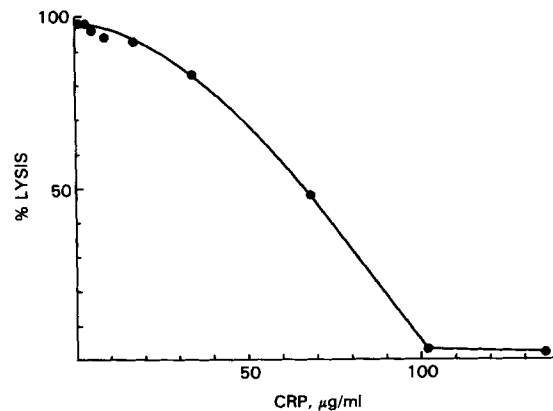


FIGURE 1. Ability of CRP-chromatin complexes to deplete complement. Complement was pretreated with chromatin and varying concentrations of CRP, and then used in a hemolytic assay. Curve shows the lysis of sensitized sheep red cells by pretreated complement. Plot represents the percent lysis as a function of CRP concentration used in the pretreatment.

to those in Fig. 1 were obtained with rabbit CRP and rabbit serum except, instead of 50 μ l human serum, 200 μ l of rabbit serum (Cappel Laboratories) and a 2 h preincubation period were required. It should be noted that the highest concentrations used in all of our experiments closely correspond to the concentration of CRP found in serum during the acute phase of inflammation (2).

Solubilization of Chromatin by Complement Is Mediated by CRP. Recently (13), Volanakis demonstrated that insoluble complexes of pneumococcal C-polysaccharide could be solubilized by complement (13). In an effort to see if CRP-chromatin complexes could be solubilized by complement, we prepared [3 H]chromatin by growing cells in medium supplemented with [3 H]thymidine to specifically radiolabel the DNA. We were thus able to easily measure the solubility of DNA in a complex solution containing serum, media, and varying quantities of CRP by quantifying the amount of radioactivity in supernatants after high speed centrifugation.

The amount of [3 H]DNA solubilized by complement was directly proportional to the concentration of CRP present (Figs. 2 and 3). Fig. 2 represents the complement-induced solubilization of isolated chromatin, whereas Fig. 3 shows the solubilization by complement of DNA from chromatin still in isolated nuclei. In Fig. 2, the chromatin was first mechanically homogenized for even suspension; this may have denatured the chromatin to some extent, resulting in more soluble DNA at low CRP concentrations compared with that in the isolated nuclei experiment (Fig. 3).

Nevertheless, these experiments clearly indicate that solubilization of chromatin by serum is directly dependent on the amount of CRP present. It is strongly suggested that complement is the solubilizing mechanism, because serum minus C3 did not solubilize chromatin even at high concentrations of CRP (Fig. 3). Additionally, complement is further implicated because heat-inactivated serum (56°C, 30 min) did not solubilize the chromatin at high levels of CRP (Fig. 2).

Serum genetically deficient in C2 and containing no hemolytic activity toward

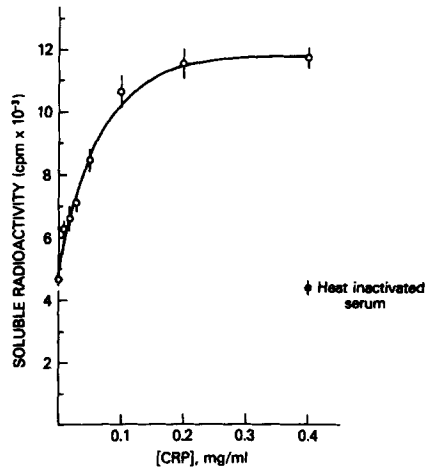


FIGURE 2. Complement-induced solubilization of chromatin as a function of CRP concentration. With increasing CRP there was increasing amounts of ^3H -labeled DNA released from chromatin. Heat inactivation to prevent complement activity prevented [^3H]DNA release (●).

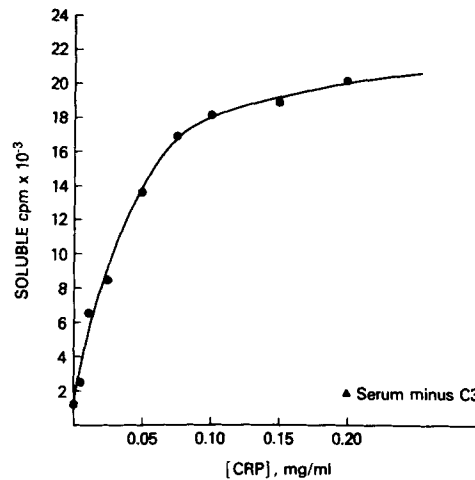


FIGURE 3. Complement-induced solubilization of chromatin in isolated nuclei as a function of CRP concentration. With increasing CRP, increasing amounts of [^3H]DNA were released. (▲) Amount of DNA released by C3-deficient serum.

sensitized sheep red blood cells caused the solubilization of chromatin at high CRP concentrations (100–200 $\mu\text{g}/\text{ml}$). However, the amount of chromatin solubilized in 1 h at 37°C was only 20% of that obtained using normal serum or C2-deficient serum to which purified C2 had been added. These results indicate that the classical complement pathway is the primary pathway involved in solubilizing chromatin, although the alternative pathway is also involved.

There was no solubilization of chromatin via CRP using serum minus C1q (prepared by Bio-Rex-70 chromatography [26]) even after purified C1q was added back. However, after purified C1q was added back, the serum's hemolytic activity against sensitized sheep red blood cells was restored to ~60–70% of that

obtained using whole serum (data not shown). These results suggest that passing the serum through Bio Rex-70 removed some factor(s), in addition to C1q, which may be required for CRP to activate complement.

When 1 mM TLCK, a protease inhibitor, was included in the incubation medium, the amount of solubilized DNA at high concentrations of CRP was ~20% of that in the absence of inhibitor. This suggests that proteolysis is a major process in the solubilization of chromatin.

A time course study (Fig. 4) indicated that most of the solubilization process is complete after 1 h and suggests that the solubilization mechanism is very complex and not a simple first-order reaction. However, serum constituted only 20% of the volume of the reaction mixture for these experiments. If we double or triple the percent of serum, we double and triple the rates of solubilization and the percent solubilized (data not shown). For example, using 100 $\mu\text{g}/\text{ml}$ chromatin, 100 $\mu\text{g}/\text{ml}$ CRP, and 60% serum, there was 100% solubilization of the [^3H]DNA after 1 h at 37°C. Under the same conditions, but with no added CRP, there was only 17% solubilization of the [^3H]DNA after 1 h.

Virtually all of the DNA solubilized was insoluble in 5% trichloroacetic acid. However, after treatment of the solubilized DNA with 1% DNase I for 1 h at 37°C, virtually all of the DNA was soluble in 5% trichloroacetic acid. This indicates that the [^3H]thymidine radiolabel was in the DNA instead of some other component.

We were unable to determine the sizes of the solubilized DNA because of the slow degradation in serum. However, after 1 h incubation at 37°C, all of the solubilized DNA eluted in the void volume of a TSK-300 column (molecular weight cutoff, ~300,000) in high performance liquid chromatography. After 18 h at 37°C, only 22% of the total radioactivity eluted (data not shown). This

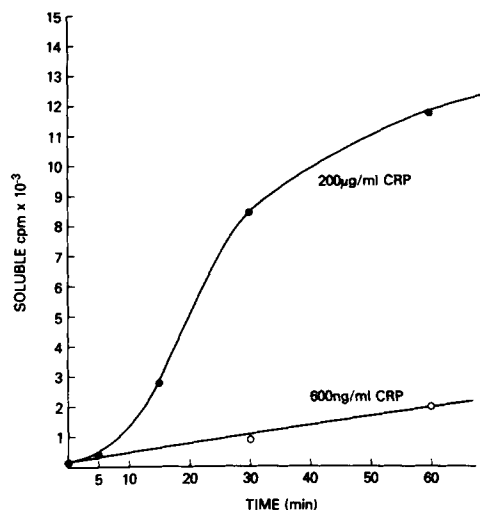


FIGURE 4. Complement-induced solubilization of chromatin as a function of time. The concentration of chromatin used for each experiment was ~200 $\mu\text{g}/\text{ml}$. High (200 $\mu\text{g}/\text{ml}$) and low (600 ng/ml) concentrations of CRP were studied. Incubations were at 37°C for the indicated times followed by evaluation of the cpm in the soluble fractions.

indicates that there are slow-acting nucleases in serum that are hydrolyzing the DNA and which complicate the characterization of the initial solubilized product.

All of the studies described above using chromatin and nuclei from rabbit lung fibroblasts were repeated using preparations from a WiDr cell line obtained from a patient with carcinoma of the colon. Rabbit antiserum to human H2B precipitated 43% of the total radioactivity in the solution containing solubilized DNA (data not shown), whereas normal rabbit serum precipitated no detectable radioactivity. This indicates that H2B, a core histone, is still bound to much of the DNA after solubilization.

The sensitivity of these assays depended on several factors, such as the specific activity and yield of the chromatin preparation. For unknown reasons, some chromatin would decompose, as determined by the amount of radioactivity in supernatants during the preparation process, but proper treatment of the nuclei with TLCK during preparation controlled the degradation. However, it is critical that residual TLCK be removed from the washed nuclei; otherwise, as mentioned above, the sensitivity of the assay is not as great. Provided there was no detectable decomposition of the chromatin during the purification process, the sensitivity of these preparations was within about $\pm 30\%$ of those in Figs. 2 and 3.

Evaluation of CRP in SLE plasma. It is well-known that patients with SLE have anti-DNA antibodies in their blood. We studied CRP levels in SLE plasma to see if a defect in the normal CRP-mediated solubilization of chromatin might be detectable in SLE plasma and thus present a possible reason for these patients having anti-DNA antibodies. Table I gives the values of CRP found in plasma obtained from eight SLE patients. We also evaluated seven normal samples and found the lowest amount of CRP in our small group of normals to be 190 ng/ml. Patients 7 and 8 had extremely low levels of CRP in their plasma. It is highly likely that these amounts are overestimated, representing the background in our assay. We do not have a human sample known to have no CRP, and thus cannot definitively establish background data.

For many of the SLE samples, PC-Sepharose did not completely remove the

TABLE I
Concentrations of CRP Levels in Plasma from SLE Patients as
Determined by ELISA

Patient	CRP ng/ml
1	14,900
2	2,300
3	2,002
4	661
5	270
6	116
7	36
8	21

Lowest value previously reported in the literature was 68 ng/ml (2). Values from seven normal blood samples were determined for these experiments: $\bar{x} = 639 \pm 477$ ng/ml; max, 1,380 ng/ml; min, 190 ng/ml; median, 562 ng/ml.

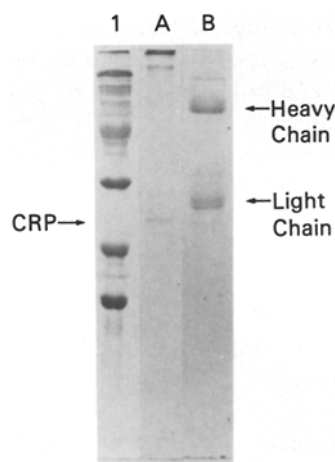


FIGURE 5. SDS-PAGE (8) of material from SLE plasma eluted from anti-CRP Sepharose. (1) Molecular weight standards; (A) nonreduced sample eluted from anti-CRP Sepharose; (B) reduced (with dithiothreitol) sample eluted from anti-CRP Sepharose. 50 ml heparinized plasma from SLE patient No. 3 (Table I) was passed first through PC-Sepharose, then three times through a column of sheep anti-mouse albumin Sepharose to remove crossreacting immunoglobulin. The plasma was then treated with sheep anti-CRP Sepharose as described in Materials and Methods. The sample was eluted with 4 M $MgCl_2$ before desalting and gel electrophoresis. The stacking gel was 7.5% bis-acrylamide; the separating gel was 13% bis-acrylamide.

CRP shown by ELISA to be present in the plasma. This is in contrast to our findings with rheumatoid arthritis plasma, where PC-Sepharose did remove most of the CRP (unpublished data). Therefore, we constructed an anti-CRP Sepharose resin to remove CRP from one sample (No. 3 in Table I) and evaluated the CRP on polyacrylamide gels.

Anti-CRP Sepharose was able to extract CRP from SLE plasma that had been previously passed through the PC-Sepharose (Fig. 5A). Also, when the sample used in lane A was chemically reduced with dithiothreitol, additional bands appeared, corresponding to heavy and light chains, respectively, of immunoglobulin. This result indicates that the one SLE sample tested contained antibodies against the CRP. It is possible that these antibodies blocked the binding of CRP to PC. In conclusion, two SLE patients had unusually low levels of CRP and a third patient had detectable antibodies reactive with her CRP.

Discussion

In previous work (4), it was shown that CRP bound strongly and specifically to chromatin and chromatin fragments and it was suggested that an important function of CRP might be to mediate the removal of chromatin from damaged tissue. In the present paper, we support this hypothesis with data clearly showing that the solubilization of chromatin by complement is mediated by CRP.

The significance of this is twofold: First, it indicates that the body has an efficient mechanism with which to deal with nuclear components released from damaged tissue. Because chromatin is the densest, most insoluble component of a cell, it is reasonable to conclude that a special mechanism is required for the

clearance of this material from sites of cell death. It is important to note that, with low concentrations of CRP, nucleases in plasma did not appreciably solubilize the chromatin. Second, the present results suggest that chromatin which is not rapidly cleared (due to a defect or depression in the CRP-complement mechanism) may act in some individuals as an immunogen, possibly resulting in the production of antichromatin antibodies, as seen in SLE plasma. It should be noted that many SLE patients have very depressed levels of complement, especially when their disease is active (14); therefore, even if the CRP levels in an SLE patient were normal, the depressed complement levels could account for impaired chromatin solubilization. Even if chromatin were not critical as an immunogen, an impaired CRP-complement solubilization mechanism might provide antigen for either antigen-antibody complexes or the binding of antigen to basement membranes.

Our present work closely relates to work from other laboratories. In 1977, Gitlin et al. (16) showed that CRP localized to the nuclei of cells in synovial biopsy samples from rheumatoid arthritis patients. Later (4), it was shown that chromatin bound CRP very strongly, implying that the unknown ligand for CRP, previously thought to be in nuclei of synovial tissue, was chromatin. In 1981, Volanakis (13) demonstrated that pneumococcal C-polysaccharide-CRP complexes were solubilized by complement. The present paper links these previous results by demonstrating that complement solubilizes CRP-chromatin complexes.

The mechanism by which CRP mediates the solubilization of chromatin by complement is probably quite complex, as suggested by the time course study presented in Fig. 4. It appears that, first, CRP binds to the chromatin (4). Next, CRP either directly turns on the complement pathways or undergoes a conformation change in molecular structure due to binding to chromatin; this is then followed by complement activation.

The experiments in which C1q-deficient serum did not support chromatin solubilization via CRP, even after C1q was added back (see Results), suggest that there are some additional factor(s) in serum which can bind to CRP immobilized to chromatin and that the resulting complex may activate complement. The same results were reported by Volanakis (13) for pneumococcal C-polysaccharide-CRP complexes. He found that serum which had been passed through Bio-Rex-70 (hence, C1q-deficient) did not support solubilization when C1q was added back (13).

Similarly, de Beer et al. (27) reported that aggregated CRP bound, strongly and specifically, low density lipoprotein and very low density lipoprotein. Therefore, whether complement activation by CRP-chromatin complexes requires low density lipoproteins should be investigated. We are currently investigating the possibility that low density lipoproteins were removed from serum by Bio-Rex-70.

As far as the nature of the complement is concerned, the experiments with genetically C₂-deficient serum showed that the alternative complement pathway plays a minor role in the chromatin solubilization process but that the classical pathway seems to be the major route. Again, this parallels the results reported by Volanakis on the complement requirements for the solubilization of pneumococcal-C-polysaccharide-CRP complexes (13).

Treatment with proteinase K solubilizes chromatin by digesting the histones bound to the DNA, thereby removing them, leaving the DNA highly charged and, thus, soluble. A similar mechanism may be initiated by CRP-chromatin complexes in which the complement enzymes digest histones or strip certain histones from the DNA.

As noted above, rabbit anti-human H2B precipitated 43% of the solubilized product, indicating that some of the core histones are probably still bound to the solubilized DNA. Further characterization of the solubilized product was complicated by the slow degradation of the solubilized DNA in serum, and possibly by the solubilized product binding C1q (28) or other serum factors to activate complement (29). However, in the absence of CRP (Fig. 1), insoluble chromatin did not appreciably consume the hemolytic activity of serum in the duration of our experiments.

We have shown that chromatin-bound rabbit CRP diminishes the hemolytic activity of rabbit serum. This is the first time that CRP from a nonhuman species has been shown to interact with the complement system from the same species. Because it has been believed that bound CRP did not deplete complement in other species, the precise involvement of CRP with complement has been unclear. Further experiments using chromatin and CRP from other species may illuminate the interaction of CRP with complement throughout all of nature.

Our current hypothesis about the binding site for CRP on chromatin is that CRP binds to the nucleosome core where exposed amino groups on histones are adjacent to phosphate moieties of DNA. The stereochemical arrangements of these functionalities would mimic the phosphate-quaternary amine configuration seen in PC, the putative ligand for CRP. However, the histone-DNA binding region(s) appears to be favored over PC because the affinity of nucleosome core particles for the PC-binding site on CRP exceeds the affinity of PC for CRP by several orders of magnitude (4). We also believe that histones and DNA are required for CRP to bind to chromatin, because it was previously demonstrated (30) that CRP does not bind to histones or DNA alone in a PC-inhibitable, Ca^{2+} -dependent manner, as is the situation with CRP bound to chromatin (4).

It has been known for several years that phosphate with heparin, a polyanion, will solubilize chromatin by disrupting the nucleosome core structure (31). This supports our argument that disruption of the nucleosome by CRP and complement may be the mechanism by which chromatin is solubilized, although the mechanism of disruption may not be the same.

The finding of antibodies reactive with CRP in a sample of SLE plasma should not come as a complete surprise, as antibodies to several plasma components have been reported in SLE (15). There have been several unsuccessful attempts to link CRP to SLE (17–20). Nonsensitive detection methods were used that could detect only elevated levels of CRP. In each study, CRP was elevated in some but not all cases. The authors failed to address whether non-elevated CRP levels were “normal” or abnormally low. The lowest value ever reported (2) for CRP in normal individuals is 68 ng/ml, found in one of a group of 153 healthy blood donors whose range was 68–8,200 ng/ml and whose median and mean were 580 and 109 ng/ml, respectively. Two of the SLE patients in our group of

eight had 36 and 21 ng/ml CRP, respectively (Table I), both lower than the previously reported low for normals.

By activating complement at a site of exposed cellular chromatin, CRP could be an important inducer of inflammation since certain peptides from the complement system are potent chemoattractants and activators of phagocytic leukocytes (21). Thus, binding of CRP to chromatin at the site of inflammation may act as a major signal for the immune response to repair and protect tissue damage.

Although CRP was discovered over fifty years ago, and has been associated with the great majority of diseases in which there is active inflammation or tissue destruction (22–25), its function has not been proposed. On the basis of the data presented here and previously (4), we suggest that one function of CRP, common to inflammatory lesions, is mediation of the removal of chromatin and chromatin fragments from sites of inflammation or sites where cell death has occurred, so as to allow the initiation of tissue repair.

Summary

We have studied the interaction of C-reactive protein (CRP)-chromatin complexes with serum. The amount of chromatin solubilized by serum is directly proportional to the amount of CRP present. Serum minus C3 did not appreciably solubilize chromatin within the time allowed in these experiments regardless of the amount of CRP present. This indicates that, in addition to CRP, complement is critical to the solubilization process. Studies using genetically C2-deficient serum and purified C2 indicate that the classical complement pathway is primarily involved in the solubilization, however, there may be minor involvement by the alternative pathway. We used an enzyme-linked immunosorbent assay to determine the amounts of CRP in plasma from eight patients with systemic lupus erythematosus; two of the eight had levels of CRP far lower than previously reported for normal individuals, and an additional sample had antibodies reactive with CRP. Together, these results suggest that one of the functions of CRP is to mediate the removal of exposed nuclear DNA by complement-dependent solubilization of chromatin. A defect in this mechanism could (a) facilitate the production of antibodies against chromatin components exposed due to tissue damage or (b) contribute to immune complexes containing the chromatin components released from damaged tissue because they are not rapidly cleared.

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References

1. Morley, J. J., and I. Kushner. 1982. Serum C-reactive protein levels in disease. *Ann. NY Acad. Sci.* 389:406.

2. Claus, D. R., A. P. Osmand, and H. Gewurz. 1976. Radioimmunoassay of human C-reactive protein and levels in normal sera. *J. Lab. Clin. Med.* 87:120.
3. Tillet, W. S., and T. Francis, Jr. 1930. Serological reactions in pneumonia with a non-protein fraction of pneumococcus. *J. Exp. Med.* 52:561.
4. Robey, F. A., K. D. Jones, T. Tanaka, and T.-Y. Liu. 1984. Binding of C-reactive protein to chromatin and nucleosome core particles. A possible physiological role of C-reactive protein. *J. Biol. Chem.* 259:7311.
5. Kaplan, M. H., and J. E. Volanakis. 1974. Interaction of C-reactive protein complexes with the complement system. I. Consumption of human complement associated with the reaction of C-reactive protein with pneumococcal C-polysaccharide and the choline phosphatides lecithin and sphingomyelin. *J. Immunol.* 112:2135.
6. Hakema, Y., M. K. Coleman, and R. F. Riley. 1962. In vitro effects of C-reactive protein on phagocytosis. *J. Bacteriol.* 83:1017.
7. Oliveira, E. B., E. C. Gotschlich, and T.-Y. Liu. 1980. Comparative studies on the binding properties of human and rabbit C-reactive proteins. *J. Immunol.* 124:1396.
8. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T₄. *Nature (Lond.)* 227:680.
9. Anderson, J. K., and J. E. Mole. 1982. Large scale isolation and partial primary structure of human plasma amyloid P-component. *Ann. NY Acad. Sci.* 389:216.
10. Rizzo, W. B., and M. Bustin. 1977. Lectins as probes of chromatin structure. Binding of concanavalin A to purified rat liver chromatin. *J. Biol. Chem.* 252:7062.
11. Champion, A. B., E. M. Prager, D. Wachter, and A. C. Wilson. 1974. Complement fixation. In *Biochemical and Immunological Taxonomy of Animals*. C. A. Wright, editor. Academic Press, Inc., (London) Ltd., London. 397-416.
12. Voller, A., D. E. Bidwell, and A. Bartlett. 1976. Enzyme immunoassays in diagnostic medicine. Theory and practice. *Bull. WHO.* 53:55.
13. Volanakis, J. E. 1982. Complement-induced solubilization of C-reactive protein-pneumococcal C-polysaccharide precipitates: evidence for covalent binding of complement proteins to C-reactive protein and to pneumococcal C-polysaccharide. *J. Immunol.* 128:2745.
14. Schur, P. H., and J. Sandson. 1968. Immunologic factors and clinical activity in systemic lupus erythematosus. *N. Engl. J. Med.* 278:533.
15. Shapiro, S. S., and P. Thiagarajan. 1982. Lupus anticoagulants. *Prog. Hemostasis. Thromb.* 6:263.
16. Gitlin, J. D., J. I. Gitlin, and D. Gitlin. 1977. Localization of C-reactive protein in synovium of patients with rheumatoid arthritis. *Arthritis Rheum.* 20:1491.
17. Pepys, M. B., and M. L. Baltz. 1983. Acute phase proteins with special reference to C-reactive protein and related proteins (pentaxins) and serum amyloid A protein. *Adv. Immunol.* 34:141.
18. Morrow, J. W., D. A. Isenberg, H. F. Parry, and M. L. Snaith. 1981. C-reactive protein in sera from patients with systemic lupus erythematosus. *J. Rheumatol.* 8:599.
19. Bertouch, J. V., P. J. Roberts-Thompson, P. H. Feng, and J. Bradley. 1983. C-reactive protein and serological indices of disease activity in systemic lupus erythematosus. *Ann. Rheum. Dis.* 42:655.
20. Zein, N., C. Ganuza, and I. Kushner. 1979. Significance of serum C-reactive protein elevation in patients with systemic lupus erythematosus. *Arthritis Rheum.* 22:7.
21. Ruddy, S. 1981. Plasma protein effectors of inflammation: complement. In *Textbook of Rheumatology*. W. Kelly, E. Harris, S. Ruddy, and C. Sledge, editors. W. B. Saunders Co., Philadelphia. 83-96.
22. Anderson, H. C., and M. McCarty. 1950. Determination of C-reactive protein in the

- blood as a measure of the activity of the disease process in acute rheumatic fever. *Am. J. Med.* 8:445.
23. Kroop, I. G., and N. H. Shackman. 1954. Level of C-reactive protein as a measure of acute myocardial infarction. *Proc. Soc. Exp. Biol. Med.* 86:95.
 24. Shetlar, M. R., J. A. Bullock, C. L. Shetlar, and R. W. Payne. 1955. Comparison of serum C-reactive protein, glycoprotein, and seromuroid in cancer, arthritis, tuberculosis, and pregnancy. *Proc. Soc. Exp. Biol. Med.* 88:107.
 25. Hedlund, P. 1961. Clinical and experimental studies on C-reactive protein (acute phase protein). *Acta Med. Scand. Suppl.* 361:1.
 26. Tenner, A. J., P. H. Lesaure, and N. R. Cooper. 1981. Purification and radiolabeling of human C1q. *J. Immunol.* 127:648.
 27. de Beer, F. C., A. K. Soutar, M. L. Baltz, I. M. Trayner, A. Feinstein, and M. B. Pepys. 1982. Low density lipoprotein and very low density lipoprotein are selectively bound by aggregated C-reactive protein. *J. Exp. Med.* 156:230.
 28. Agnello, V., R. I. Carr, D. Koffler, and H. G. Kunkel. 1969. Gel diffusion reactions of C1q with aggregated globulin, DNA and other anionic substances. *Fed. Proc.* 28:2447.
 29. Cooper, N. R. 1983. Activation and regulation of the first complement component. *Fed. Proc.* 42:134.
 30. Tanaka, T., and F. A. Robey. 1983. A new sensitive assay for the calcium-dependent binding of C-reactive protein to phosphorylcholine. *J. Immunol. Methods.* 65:333.
 31. Bornens, M. 1977. Solubilization of chromatin with heparin and the isolation of nuclear membranes. *In Methods in Cell Biology.* D. M. Prescott, editor. Academic Press, Inc., New York. 163–175.