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The interaction of C-reactive protein and serum amyloid P component with nuclear antigens

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Abstract

The pentraxins are a family of proteins characterized by cyclic pentameric structure, calcium-dependent ligand binding and sequence homology. The two main representatives of this family are the serum proteins, C-reactive protein (CRP) and serum amyloid P component (SAP). In man CRP is an acute phase reactant which increases up to 1000 fold during the acute phase response whereas SAP is a constitutive protein expressed at about 30 μg/ml. These proteins activate complement through the classical pathway and participate in opsonization of particulate antigens and bacteria. In the past several years it has been determined that both of these pentraxins interact with nuclear antigens including chromatin and small nuclear ribonucleoproteins (snRNPs). Both CRP and SAP have nuclear transport signals which facilitate their entry into the nuclei of intact cells. Furthermore, these pentraxins have been shown to affect the clearance of nuclear antigens *in vivo*. It is now believed that one of the major functions of the pentraxins could be to interact with the nuclear antigens released from apoptotic or necrotic cells. This interaction could mitigate against deposition of these antigens in tissue and autoimmune reactivity.

Abbreviations: CRP – C-reactive protein; HSA – human serum albumin; PC – phosphocholine; SAP – serum amyloid P component; snRNP – small nuclear ribonucleoprotein; SLE – systemic lupus erythematosus.

Introduction

Chromatin and other nucleic acid-protein complexes are the primary targets of pathogenic autoantibodies in systemic autoimmune diseases such as systemic lupus erythematosus (SLE). There is increasing evidence that these autoantibody responses are driven by immunization with self antigen [1]. Studies of the clearance of autoantigens have primarily focused on the clearance of DNA or immune complexes containing DNA [2–7]. However, recent evidence indicates that the form of DNA which is present in the circulation and the autoantigen driving the immune response is not protein-free DNA but rather chromatin, the physiological form of DNA complexed with histones. DNA is found in the circulation with a size distribution consistent with the size of the repeating subunit of nuclear material in the nucleus, the nucleosome. This is also the form of DNA released from cells dying by apoptosis. Levels of anti-chromatin antibodies correlate better with disease activity than do levels of anti-dsDNA antibodies [8]. T cell clones capable of helping anti-DNA B cells recognize determinants on chromatin [9]. Despite the potential importance of exposure of the immune system to chromatin in the pathogenesis of SLE, very little is known about the normal pathways of clearance and processing of chromatin. Recent evidence suggests that the pentraxin family of proteins may be important in the recognition and clearance of chromatin. This article will review the interactions of these proteins with chromatin and other nuclear antigens (overview of pentraxin properties, Table 1).

Table 1. Comparison of CRP and SAP

CRP	SAP
Structural features	
cyclic pentamer	cyclic pentamer
non-glycosylated	glycosylated
Physiologic features	
normal serum levels <0.5 μg/ml	normal serum levels 30–40 μg/ml
increases up to 1000 fold during acute phase response	no increase during acute phase response
activates classical complement pathway	activates classical complement pathway
opsonin	
Binding properties	
phosphocholine	phosphoethanolamine
polysaccharides and proteins	polysaccharides and proteins (including amyloid deposits)
chromatin >> H1-stripped chromatin	chromatin = H1-stripped chromatin
DNA-free histone	DNA-free histones
not DNA	DNA
snRNP in nuclei	chromatin in nuclei and nucleoli
Other properties	
solubilizes chromatin in presence of complement	solubilizes chromatin by displacing H1
actively transported from cytoplasm to nucleus	actively transported from cytoplasm to nucleus

Characteristics of pentraxins

The pentraxins are a family of proteins which are found in the serum of virtually all vertebrates which have been examined and are present in species as primitive as the horseshoe crab. They are defined by their cyclic pentameric structure, by their sequence homology, and by their calcium-dependent substrate binding [10–12]. In man, two members of the pentraxin family are found in the serum, C-reactive protein (CRP) and serum amyloid P component (SAP). No deficiencies or polymorphisms are seen in either protein. The crystal structures of both human CRP and SAP have recently been determined [13, 14].

CRP is normally present in blood at less than 0.5 μg/ml but levels increase up to 1000 fold in response to infection, inflammation or trauma. Therefore, it has been widely used as an indicator of the presence and degree of acute inflammation. CRP was named for its calcium-dependent interaction with the C polysaccharide of the pneumococcus [15]. However, binding to fibronectin and to other protein substrates has been reported [16] as well as binding to nuclear components which will be reviewed here. CRP binding to ligands occurs in two ways. First there is a calcium-dependent binding which is strongly inhibited by phosphocholine (PC), a molecule containing a strongly positively charged choline moiety and a negatively charged phosphate moiety. The second type of binding is through interactions with polycations. This binding is not inhibited by PC and is inhibited by calcium [17].

The other member of the pentraxin family which is present in the serum is not an acute phase reactant in man. SAP is present at about 30 μg/ml in human serum [18]. SAP is present as the P component of amyloid deposits and has recently been used to image amyloid deposits in patients suspected of having amyloidosis [19]. Like all members of the pentraxin family SAP undergoes a calcium-dependent binding interaction with various ligands. For SAP these ligands include phosphoethanolamine and polysaccharides including agarose and zymosan in addition to nuclear components.

Pentraxin binding to chromatin. The first indication that CRP could interact with nuclear antigens came from the work of Gitlin et al. [20] in 1977. They examined the synovial tissue of patients with rheumatoid arthritis and detected CRP localized to cell nuclei. The pattern of staining was not clearly defined and it was uncertain whether or not this reaction occurred *in vivo*. However, it was clear from this early study that CRP interacted very strongly with the nuclei of certain cells. Neither the identity of the cell type nor the type of perturbation of the cell required for binding was determined.

The first direct evidence that the pentraxins could interact with nuclear antigens came from the work of Robey et al. [21]. They demonstrated that rabbit CRP bound in a spotty pattern to the nuclei of cells treated with bradykinin, phospholipase A_2 or lysolecithin to permeabilize them. Binding was inhibited by excess CRP or by PC. They attributed this binding to chromatin and directly demonstrated CRP binding to chromatin. The interaction was of very high avidity as measured by a solid phase assay. No binding to histones or to DNA was observed and the binding was thought to be directed to a neoantigen produced by histone-DNA interaction. They proposed for the first time that CRP was involved in the clearance of chromatin from damaged cells.

There has been some controversy over the ability of CRP to bind to chromatin. Pepys's group had reported that CRP binds weakly to chromatin at physiological ionic strength [22]. Much of this controversy has arisen because of the nature of the chromatin preparation used for binding studies. We reanalyzed the binding of CRP to chromatin and determined that the presence of histone H1 strongly affects the ability of CRP to bind to chromatin [23]. When chromatin was depleted of H1, a marked decrease in CRP binding was seen which was restored by reconstitution with H1. Studies by others which failed to detect binding to chromatin used H1-stripped chromatin [22]. The other major difference between these studies is the source of chromatin used. The Pepys group has used chromatin from birds which contains histone H5 predominantly in place of H1. The amino acid sequence of histone H5 is markedly different from that of H1. Since CRP binding to chromatin is strongly dependent on H1 it is unlikely to bind avidly to chicken chromatin which is relatively deficient in H1. Mammalian chromatin contains only H1.

In 1987, Pepys and Butler examined the ability of SAP to interact with DNA and chromatin [22]. They concluded that SAP was the major calcium-dependent DNA-binding protein in serum. (There are other DNA-binding proteins in serum which do not require calcium for binding.) However, since naked DNA is not normally present under physiological conditions, the relevance of this finding is uncertain. They further reported that SAP bound more avidly than CRP to chicken chromatin and to core particles prepared from it.

The ability of CRP and SAP to bind to mammalian chromatin in an ELISA format has been directly compared [24]. These studies showed that CRP and SAP bound to chromatin but that SAP binding did not require H1 to be present. Cross inhibition studies revealed that SAP inhibited CRP binding up to 75% but that CRP could only inhibit SAP binding by a maximum of 25%. This is most likely due to the strong interaction of SAP with DNA, to which CRP does not bind. Both proteins bound to histones H1, H2A and H2B.

The physiological relevance of chromatin binding by these two pentraxins cannot be fully examined in *in vitro* experiments. Under non-acute phase conditions SAP undoubtedly plays the most important role in any interaction of pentraxins with chromatin. However, during the acute phase CRP may be present at concentrations 10 times or more those of SAP. Under these conditions the role of CRP may be more important, especially if SAP is limiting. This is the time, during significant tissue damage due to trauma, burns or intense inflammation, when chromatin is likely to be released from apoptotic or necrotic cells.

SAP has also been shown to interact with the nuclei of cells *in vitro* following permeabilization or microinjection [25]. Breathnach et al. also presented evidence that SAP was present in the skin lesions of two patients with SLE [26]. However, the nature of the lesions or the generality of these findings could not be assessed.

CRP binding to chromatin subunits. Interaction of CRP with chromatin subunits was also examined [27]. Chromatin is organized into repeating nucleosome subunits. Nucleosomes consist of 200 BP of DNA wrapped around a core octamer of histones $[H2A\text{-}H2B\text{-}H3\text{-}H4]_2$ and one molecule of H1 on the exterior of the structure. The octamer can be broken down to a dimer of H2A and H2B and a tetramer of H3 and H4. It was shown that CRP would bind to the H2A-H2B dimer and the $[H3\text{-}H4]_2$ tetramer but that binding was inhibited in the presence of DNA. Similarly the interaction of CRP with H1 was eliminated when H1 was bound to DNA.

CRP binding to histones. Studies of CRP binding to chromatin revealed that the interaction was mediated by the histone moiety [23]. The interaction of CRP with histones was examined in detail. CRP reacted most strongly with histones H1 and H2A with much less binding to H2B and the arginine-rich histones, H3 and H4. This binding was completely inhibited by EDTA and by PC, suggesting interaction through the PC-binding site of CRP. In contrast, in studies using isolated cell nuclei, CRP bound primarily to H4 as determined by crosslinker studies [28]. Additional studies of CRP binding to histones have examined the determinants which were recognized by CRP [29]. Using digestion products of histones and synthetic peptides a short (8 amino acid), defined sequence from histone H2A was identified which bound to CRP in a calcium-dependent manner. Furthermore this peptide was capable of inhibiting the interaction of CRP with both PC-BSA and histone H2A. This was the first demonstration of a short well-defined protein sequence to which CRP could bind. Interaction of CRP with the C-terminal region of H1 was also seen. The interaction of CRP with H1 was confirmed by the work of Minota et al. [30].

Finally, it appears that interaction of pentraxins with nuclear antigens may be a conserved property. Saunero-Nava et al. examined the interaction of hamster female protein, the major hamster pentraxin, with chromatin and histones. Like human CRP and SAP [24], female protein bound to H1 and H2A as well as to chromatin. However, unlike human CRP and similar to human SAP, female protein bound to DNA [31]. All these interactions were inhibited by free PC suggesting an interaction through the PC-binding site.

Solubilization of chromatin by pentraxins. Robey et al. also described the solubilization of chromatin by CRP in the presence of serum [32]. CRP-chromatin complexes were shown to consume complement at physiological concentrations of CRP. Further, CRP was shown to induce the release of soluble DNA fragments in labeled nuclei. This reaction was strictly complement-dependent through the classical complement pathway. Although the mechanism was not clearly defined, the process was related to proteolysis by an unknown protease and to a serum nuclease. The physiological relevance of this process was not shown.

Shephard's group confirmed the solubilization of chromatin by CRP in the presence of complement and showed this material to be of nucleosome-sized fragments [33]. Furthermore they showed that the presence of CRP enhanced the digestion of chromatin by micrococcal nuclease suggesting that the linker DNA became more susceptible to nuclease attack. They suggested that CRP interaction with chromatin could induce an alteration of chromatin structure. Finally, they noted a decrease in the rate of *in vitro* transcription by nuclei which had been treated with CRP. The mechanism for such an effect is uncertain.

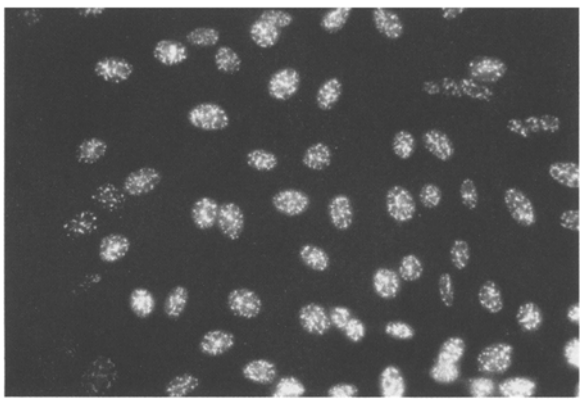

Figure 1. Reactivity of CRP with HEP-2 cells by immunofluorescence. Slides were stained with CRP, anti-CRP mAb 2C10 and FITC anti-mouse IgG in the presence of calcium. Adapted from reference 35.

In 1990 Butler et al. [34] reported that SAP was capable of solubilizing chromatin. Unlike earlier reports by Robey et al. for CRP, this process was not dependent on the presence of serum complement and did not result in chromatin cleavage. Butler et al. proposed that the strong interaction of SAP with chromatin displaced histone H1. Since H1 neutralizes the charge of the linker DNA, chromatin containing H1 is insoluble at physiological ionic strength. They specifically found that the interaction of SAP with native chromatin resulted in the induction of solubility of both the chromatin and the SAP, both of which normally precipitate in the presence of calcium and physiological ionic strength. In these studies CRP did not solubilize chromatin but precipitated with chromatin on an equimolar basis with H1.

CRP binding to extractable nuclear antigens. Although CRP interacts with chromatin in physiological buffers the spotty fluorescence reported by Robey is not likely due to chromatin binding. We observed that this pattern is reminiscent of the binding of autoantibodies to extractable nuclear antigens. We decided to reexamine the binding of CRP to nuclear

antigens directly in a manner used for examining the antinuclear antibodies. Tissue culture cells that were fixed and permeabilized were incubated with CRP and binding detected by a monoclonal antibody to CRP (Figure 1). Using two-colored fluorescence with antibodies to the RNP particle from a patient with mixed connective tissue disease and CRP, the pattern of fluorescence was identical for CRP binding and anti-RNP binding indicating that the two reactants recognized the same nuclear particle(s) [35]. Using immunoprecipitation CRP was shown to precipitate the U1 RNA species and the U4-U6 species characteristic of autoantibodies to the small nuclear ribonuclear protein complex Sm/RNP. By a blotting analysis analogous to Western blotting CRP reacted with the D protein of Sm and the 70 kDa protein of RNP.

Jewell et al. examined the interaction of CRP with purified snRNP particles using fusion proteins prepared from the D protein [36]. The binding determinants were localized to the C-terminal region of the D protein. A comparison of this sequence with previous determinants recognized by CRP on H2A and H1 was used to construct a consensus binding determinant for CRP binding (Figure 2).

The interaction of the pentraxins with nuclear antigens was confirmed by Pepys's lab [37]. Using confocal microscopy and monoclonal antibodies it was again definitively shown that CRP interacted with the identical particles as the anti-snRNP antibodies. They further showed that SAP bound to chromatin in these cells. The binding of SAP to nucleoli was also observed. That this interaction with nucleoli is physiological and possibly due to a nucleolar localization sequence is supported by nuclear transport studies [25]. Microinjection of SAP into Vero cells results in diffuse nuclear staining with prominent nucleolar staining as well (personal observation). It is also of interest that the CRP peptides when coupled to human serum albumin (HSA) induce very strong nucleolar localization of the carrier [25] suggesting that the nucleolar localization signal is present in both pentraxins.

Nuclear transport of the pentraxins

One of the questions which was raised by the early findings of Gitlin et al. [20] was the mechanism by which CRP deposited in the nuclei of cells in vivo. Proteins which may enter a cell with a damaged cell membrane are not expected to enter the nucleus. Proteins of greater than 40 kD are excluded by the nuclear pore. Nuclear proteins, however contain a nuclear transport signal which allows them to be transported rapidly and efficiently into the nucleus. Our studies showed that CRP and SAP are both specifically transported into the nucleus when microinjected into tissue culture cells [25]. When CRP was microinjected, a speckled nuclear fluorescence pattern was generated (Figure 3). This process was energy-dependent, as is the case for all known nuclear transport proteins. Thus CRP appeared to bind to the nucleus of the living cell with same pattern observed in fixed tissue culture cells. SAP also was specifically transported to the nucleus. However, the pattern of nuclear fluorescence was homogeneous with strong staining of the nucleolus as well. This pattern was consistent with the known interaction of SAP with chromatin and showed that SAP was transported to the nucleolus. It is unknown whether a nucleolar transport signal is required for this reaction with the nucleolus or whether it is due to a binding activity of SAP for the nucleolus. The nuclear localization sequence in CRP and in SAP were also determined. Peptides corresponding to these sequences were constructed and coupled to HSA. These peptides induced nuclear transport of HSA, a characteristic of nuclear localization signals. The nuclear localization sequence in CRP has an amino-terminal proline followed by an octapeptide containing 5 basic amino acids, similar to the 'classical' nuclear localization sequence present in the SV-40 large T antigen.

Biological significance of pentraxin interaction with nuclear antigens

There are very few studies which address the biological significance of the pentraxin interaction with nuclear antigens. We have examined the effect of CRP on the course of autoimmune disease in a mouse model of SLE, the (NZB X NZW) F_1 female hybrid mouse. This mouse strain has disease characteristics which are similar to those of the human condition and has been widely used as an animal model of SLE [38]. Mice were treated with intraperitoneal injections of chromatin immobilized on a solid phase matrix in the presence or absence of CRP. Mice do not normally express significant amounts of CRP and the major acute phase reactant in the mouse is SAP. It was first demonstrated that chromatin injections accelerated the course of disease in these mice resulting in markedly decreased survival [39]. In these mice the acceleration of death which was induced by chromatin was prevented by simultaneous

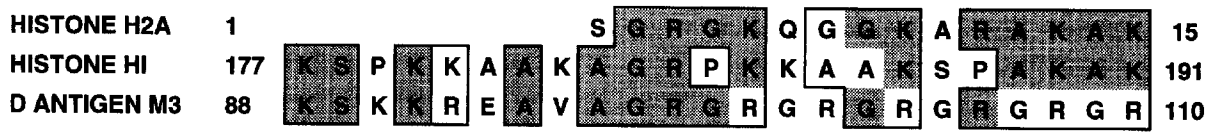

Figure 2. Sequence alignment of CRP binding determinants. The protein sequences were aligned using the default settings of the GAP program of the GCG Sequence Analysis Software Package. Identical amino acids are outlined and shaded. Conserved amino acid substitutions are outlined. Sequences were obtained from Swissprot accessed through the GCG package. Adapted from reference 36.

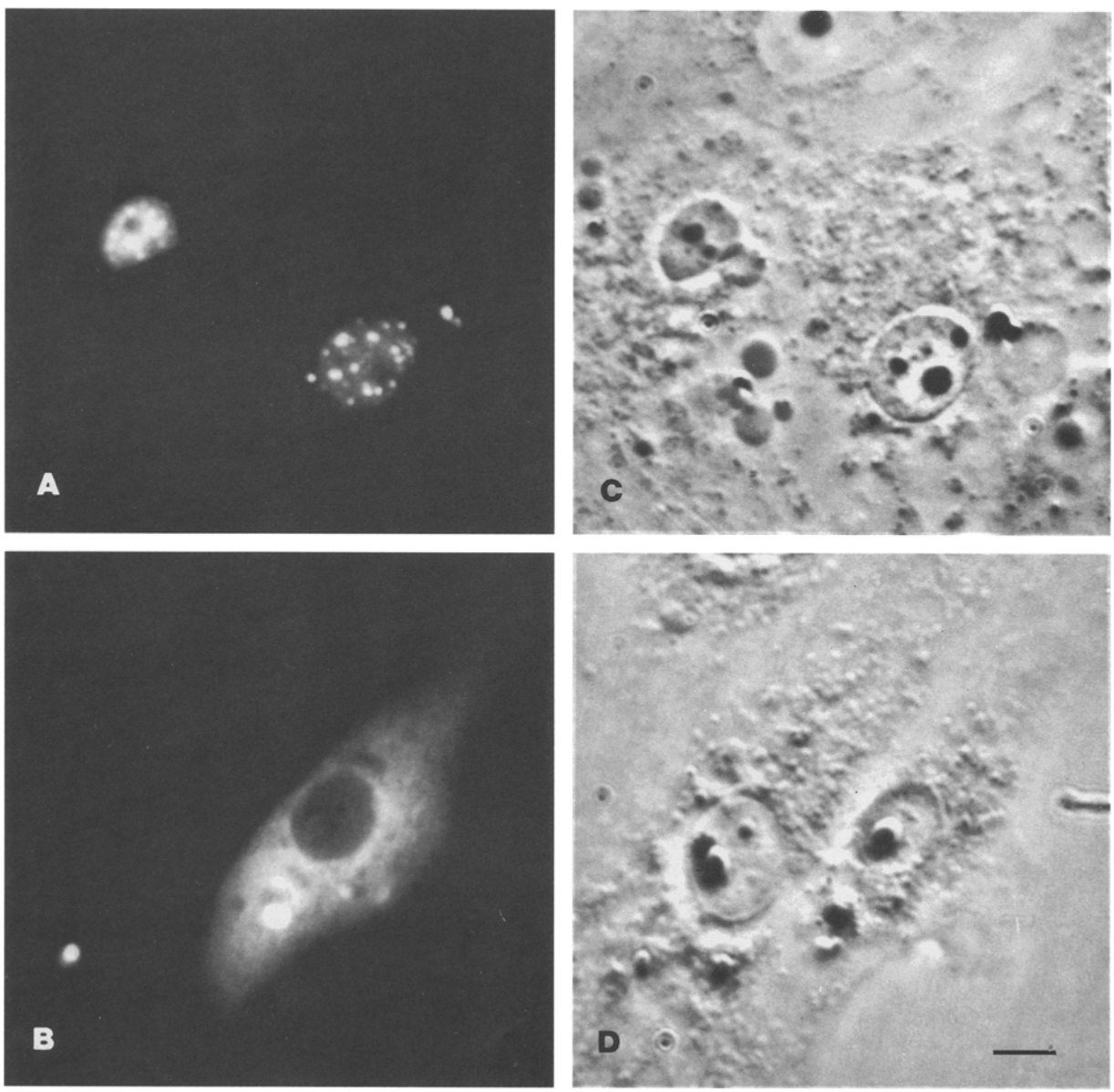

Figure 3. Nuclear localization of CRP after microinjection. FITC-labeled CRP (A and C) or FITC- labeled HSA (B and D) was microinjected into the cytoplasm of VERO cells. Images were obtained after 15 min incubation at 37 °C. C and D are phase microscopy images of the same fields presented in A and B, respectively. Bar = 5 μm. Adapted from reference 25.

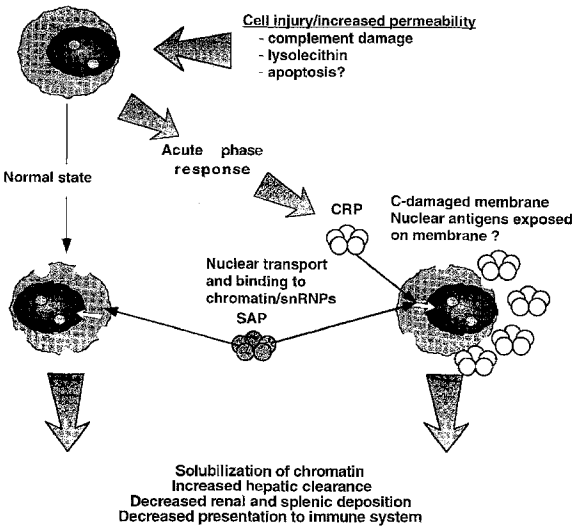

Figure 4. Possible roles of CRP and SAP in the clearance of cell debris/autoantigens.

introduction of CRP. A transient decrease in IgG antibodies to histones, DNA and dinitrophenol was also observed in mice treated with CRP.

As noted above, one physiological function postulated for CRP and also for SAP is the clearance of chromatin from damaged cells. The effect of the acute phase response and the individual pentraxins on chromatin clearance has recently been examined. Clearance of H1-stripped chromatin and nucleosome core particles was studied [40]. It was determined that chromatin and core particles are cleared by different pathways and at different rates. Core particle clearance was slow and could be inhibited by ssDNA whereas chromatin clearance was more rapid and varied in strains of mice with high and low baseline levels of SAP. The acute phase response decreased the clearance rate of chromatin. Both CRP and SAP, when injected with chromatin, reproduced this effect suggesting that these acute phase reactants could be the mediators of this altered clearance profile. In addition, acute phase mice showed a decrease in the percentage of chromatin deposited in the kidneys and a relative increase in the hepatic clearance of chromatin. Thus it is possible that these acute phase reactants could protect the animal from chromatin deposition in the kidney. Since chromatin is now felt to be one of the major autoantigens involved in SLE the acute phase reactants may play an important role in modulating SLE activity.

Summary

CRP and SAP are similar in many respects, and some of these features may be important in protection against development of autoimmunity. Both CRP and SAP are capable of binding nuclear proteins which can lead to complement activation and enhanced processing of this material by phagocytic cells. In addition they both have the capacity to be specifically transported to the nucleus of living cells where they bind to chromatin and snRNPs. These nucleic acid-protein complexes are some of the major autoantigens recognized by autoantibodies in SLE. Furthermore these pentraxins can cause a major change in the clearance of chromatin from circulation. Thus the interaction of the pentraxins with nuclear antigens may serve an important role in clearance and processing of nuclear antigens. These properties may be beneficial in preventing the deposition of nuclear antigens in target organs. An overall scheme of the possible interactions between the pentraxins and nuclear antigens and other cellular debris is presented in Figure 4. Normally as cells are damaged by a variety of insults the membrane allows access to SAP and/or CRP. The nuclear transport signal would promote entry to the nucleus and facilitate processing and clearance of the nuclear material. During the acute phase response when there is extensive cellular damage mediated by agents such as TNF-α and lysolecithin there is a massive increase in levels of CRP. The increased CRP would facilitate the removal of exposed cellular components. Finally in states such as SLE where this is an inadequate CRP response the result could be a decreased ability to clear these complexes and enhanced presentation of these antigens to the immune system.

Acknowledgements

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