Immunology 1992 **76** 324–330

Reactivity of anti-human C-reactive protein (CRP) and serum amyloid P component (SAP) monoclonal antibodies with limulin and pentraxins of other species

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Accepted for publication 6 February 1992

SUMMARY

Limulus polyphemus C-reactive protein (CRP) (limulin) has approximately 30% amino acid sequence homology and shares at least one idiotypic determinant associated with ligand-binding activity with human CRP (hCRP); limulin also shares amino acid sequence homology and lectin activity with human serum amyloid P component (hSAP). In the present study panels of 14 anti-hCRP monoclonal antibodies (mAb) directed to distinct hCRP epitopes and 11 anti-hSAP mAb directed to distinct epitopes of hSAP were tested for reactivity with limulin and pentraxins of other species including rabbit CRP (raCRP), rat CRP and hamster female protein (FP) by ELISA and Western blot analyses. None of the anti-human pentraxin mAb showed strong cross-reactivity with limulin; only five mAb reacted with limulin at all, and cross-reactivities of these mAb with the other pentraxins, when present, also were weak. Cross-reactivity of limulin with hCRP and hSAP was similar, and in light of comparable amino acid sequence homology, suggests this molecule can be considered the limulus SAP as well as the limulus CRP. Several anti-hCRP mAb cross-reacted strongly with rabbit CRP and rat CRP; a few anti-hSAP cross-reacted strongly with FP; and weak cross-reactions were observed between hCRP and hSAP, but cross-reactivities between the pentraxins generally were limited and weak. A rabbit polyclonal antibody raised to highly conserved limulin peptide 141-156 and strongly reactive with limulin reacted weakly with hCRP and raCRP but failed to react with rat CRP, hSAP or FP. These studies emphasize a limited but distinct antigenic similarity between limulin, hCRP and other pentraxins, and identify mAb reactive with potential regions of shared structure and/or function between pentraxins of different species.

INTRODUCTION

C-reactive protein (CRP) is a pentameric protein comprised of five identical subunits arranged in radial symmetry.[1] CRP is the prototypic acute phase protein in man, increasing in concentration up to 1000-fold in response to inflammation and tissue necrosis.[1-3] CRP is structurally distinct from all other human proteins except the serum amyloid P component (SAP), with which it shares amino acid and ultrastructural homology.[1-3] SAP also is a pentameric protein, but consists of 10 identical subunits, comprised of two face-to-face pentameric disks.[1-3] SAP is a minor (up to 15%) component of all forms of amyloid, and has been implicated in amyloidogenesis.[4] CRP and SAP are present in sera of a wide range of vertebrate species, including marine teleost fish and birds as well as many mammals.[5-8] Since vertebrate CRP and SAP have similar pentameric structure and amino acid sequence homology they have been grouped into a family of proteins termed 'pentraxins'.[9] The CRP pentraxins characteristically have the capacity for calcium-modulated binding to phosphorylcholine (PC) and phosphate esters, polycations and galactans, while SAP pentraxins characteristically show calcium-dependent binding to polysaccharides such as agarose via pyruvate acetal and zymosan via mannose groups.[1-3,10,11]

Invertebrate CRP has been well characterized in the horseshoe crab, where the lectin first described as 'limulin'[12,13] was found by immunologic cross-reactivity, ligand-binding activity and amino acid sequence homology to also represent the C-reactive protein of this species.[14-16] Limulin has a MW of

Abbreviations: CRP, C-reactive protein; FP, (hamster) female protein; hCRP, human CRP; hSAP, human SAP; KLH, keyhole limpet haemocyanin; limCRP, limulin or limulus CRP; PC, phosphorylcholine; raCRP, rabbit CRP; SAP, serum amyloid P component; TBS, Tris-buffered saline; VBS, veronal-buffered saline; ZX, soluble zymosan extract (to which hSAP binds).

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approximately 500,000;[15,16] is comprised of non-identical subunits of approximately 24,000 MW, encoded by three defined genes, which combine non-covalently to form a doubly stacked hexagonal structure;[15,16] and has phosphorylcholine- as well as lectin-binding activity.[12-14] Immunochemical cross-reactivity has been shown between the CRP of species as phylogenetically distant as human and the invertebrate horseshoe crab using polyclonal antibodies.[6,8,14,17-19] However, to our knowledge no cross-reactivity has been reported between CRP and SAP of man.

We have investigated immunochemical cross-reactivity between pentraxins by using mAb raised against both human CRP (hCRP) and human SAP (hSAP) as well as a polyclonal antibody raised against highly conserved limulus CRP peptide 141-156 which is similar to hCRP peptide 135-150 and hSAP peptide 133-148.

MATERIALS AND METHODS

Purification of CRP

Limulus CRP (limulin; limCRP) was purified by PC-sepharose 4B affinity and gel filtration column chromatography. Horseshoe crab haemolymph (40 ml) was diluted with 120 ml of 75 mM Tris-buffered saline (TBS; pH 7·3) containing 2 mM calcium chloride and loaded on a 30-ml PC-sepharose 4B column. The column was washed with the same buffer until $OD_{280} = 0{\cdot}01$, and limCRP was eluted with TBS containing 7·5 mM citrate. The eluate was concentrated, dialysed against 10 mM TBS containing 2 mM EDTA, and chromatographed on a 1×30 cm superose 12 gel filtration fast protein liquid chromatography (FPLC) column at flow rate of 0·25 ml/min. The peak reactive with anti-limulin antibody was concentrated and dialysed against 10 mM TBS containing 2 mM calcium chloride. The protein concentration was determined by Pierce Coomassie protein assay (Pierce, Rockford, IL).

hCRP and rabbit CRP (raCRP) were purified as previously described.[20,21] Rat CRP was purified by a modification of the method used to purify raCRP[22] with the CRP-containing eluate from Sephacryl S-200 passaged through a second PC-sepharose 4B affinity column. The preparation was concentrated, dialysed against TBS, sterilized by filtration and kept at 4°.

Hamster female protein (FP)

FP was generously provided by Dr J. E. Coe.[23]

Purification of hSAP

hSAP was prepared from outdated plasma obtained from the American Red Cross as described by Potempa *et al.*[11] Briefly, hSAP was isolated by Bio-gel 0·5A affinity column chromatography using TBS containing 2 mM calcium chloride, eluted with TBS containing 10 mM EDTA. The eluate was dialysed extensively against TBS, precipitated with 10 mM calcium, spun for 30 min at 4000 ***g*** in a Beckman Centrifuge J6B and washed twice. The pellets were redissolved in TBS and maintained at 4°.

Preparation of mAb

The anti-hCRP mAb were described previously.[22,24] Anti-hSAP mAb were prepared using the same methodology and native hSAP as well as hSAP digested with alpha-chymotrypsin as immunogens.[25]

Preparation of limulin peptide 141-156 and anti-limulin peptide antibody

The synthetic 16-mer L-G-Q-D-Q-D-S-V-G-G-K-F-D-A-T-Q identical to limulin residues 141-156 was synthesized with an applied Biosystems 430A automatic peptide synthesizer. This sequence shows significant identity (63 and 69% respectively) to the corresponding sequences of human and rabbit CRP. The peptide was coupled to bovine serum albumin using glutaraldehyde and rabbits were immunized as previously described.[26]

Enzyme-linked immune solid phase assay (ELISA)

The ELISA binding assay was performed as described previously with minor modifications. In brief, mAb which reacted preferentially with hCRP or hSAP in the native conformation were quantified by capture assays in which 10 μg/ml keyhole limpet haemocyanin (KLH-PC) (for hCRP)[20] or solubilized zymosan extract (ZX) (for hSAP)[5] were immobilized on polystyrene plates with assays carried out in buffer containing 2 mM calcium chloride; for assay of mAb reactive with modified hCRP or hSAP, the pentraxins (5 μg/ml) were directly immobilized on polystyrene plates in 10 mM sodium bicarbonate buffer, pH 9·6. In both groups of assays the wells were washed with 50 mM veronal-buffered saline (VBS) containing 0·1% Tween 20 (v/v) (washing buffer) and blocked with 1% BSA in washing buffer (which also was used as diluent). The affinity-purified mAb at 20 μg/ml were diluted serially and incubated with the immobilized pentraxins for 30 min at 37°. After washing, peroxidase-conjugated goat anti-mouse IgG (H+L) (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) was added to the wells for 30 min at 37°. Substrate [2,2′-azinobis (3-ethylbenz-thiazoline-sulphonic acid)]; (Sigma Chemical Company, St Louis, MO) was added and the reaction was developed for 15 min at room temperature. The plate was read at $A_{(414)}$ on a Titertek multiskan plate reader (Flow Laboratories, Helsinki, Finland).

Western blots

This assay was performed in SDS–PAGE using 13% polyacrylamide gels and the Laemmli buffer system as previously described,[21] and transferring to Immobilon-P membranes (Millipore Co., Bedford, MA) using the BioRad transblot system (BioRad, Richmond, CA). The membranes were blocked with 1% bovine serum albumin (BSA) and incubated with affinity-purified mAb at 20 μg/ml or polyclonal antibody at 1:100 dilution, except mAb 3A1 which was incubated at 40 μg/ml, for 30 min at room temperature followed by three washes with washing buffer. Samples were incubated with the secondary antibody, peroxidase-conjugated goat anti-mouse IgG or anti-rabbit IgG (Pel-Freez Biologicals, Rogers, AK), for 30 min at room temperature, and after washing, colour was developed with 4-chloro-1-naphthol as directed (BioRad Laboratories).

For testing binding to limulin, 5 μg/ml mAb (except 3A1 which was used at 40 μg/ml) was applied and the secondary antibody was conjugated with alkaline phosphatase (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD), using the BCIP/NBT (5-bromo-4-chloro-3-indolyl-phosphate/nitroblue tetrazolium) substrate system obtained from Gibco/BRL life technologies, Inc., Gaithersburg, MD.

Table 1. Cross-reactivity between pentraxins tested with anti-hCRP mAb by ELISA assays

mAb	hCRP*	limCRP†	raCRP*	rat CRP*	hSAP*	FP*
Anti-native CRP mAb						
1B1	**0·03**§	>20·0	**4·0**	>20·0	>20·0	>20·0
1D6	**0·08**	>20·0	**1·25**	>20·0	>20·0	>20·0
5A11	**0·80**	>20·0	>20·0	>20·0	>20·0	>20·0
8D8	**0·003**	>20·0	**0·03**	>20·0	>20·0	>20·0
Anti-native hCRP mAb which also react with modified CRP						
2C10	**0·009**	**2·0**	**2·50**	>20·0	>20·0	>20·0
3A1	**6·00**	**>20·0**	**13·0**	>20·0	**6·0**	>20·0
Anti-neo-CRP mAb						
4B10	**0·001**	>20·0	>20·0	>20·0	>20·0	>20·0
6A12	**0·003**	**20·0**‡	**3·0**	>20·0	>20·0	>20·0
8C10	**0·001**	>20·0	**0·001**	>20·0	>20·0	>20·0
7A10	**0·10**	>20·0	>20·0	>20·0	**2·0**	>20·0
6B7	**0·01**	>20·0	**0·01**	**0·01**	>20·0	>20·0
7A8	**0·005**	>20·0	**0·005**	>20·0	>20·0	>20·0
9H8	**0·02**	>20·0	>20·0	>20·0	>20·0	>20·0
3H12	**0·006**	>20·0	**0·006**	**0·006**	>20·0	>20·0

* Amount (μg/ml) of mAb required for 50% maximal reactivity.
† Amount (μg/ml) of mAb required for 10% maximal reactivity.
‡ Did not bind to limulin in Western blot assays.
§ 'Strong' cross-reactivities observed with ≤0·1 μg/ml are shown in bold and underlined; 'weak' cross-reactivities are shown in bold only.

RESULTS

Reactivity of anti-human CRP mAb with limulin

Fourteen mAb with specificities for different epitopes of hCRP were tested for the ability to react with limulin by both ELISA and Western blot assays. Two anti-native hCRP mAb which also react with modified CRP (2C10 and 3A1) bound to limulin by both ELISA and Western blot assays (Table 1; Fig. 1), although at least 1000-fold more 2C10 and 40-fold more 3A1 was required for binding to limulin than for binding to hCRP. A third anti-hCRP mAb (anti-neoCRP mAb 6A12) also bound weakly to limulin by ELISA assay but not by Western blot analysis. The reactivity of 2C10 and 3A1 with limulin in Western blot analysis in SDS–PAGE is shown in Fig. 1a; limulin migrated in three bands of about 29,000, 30,000 and 31,000 MW, respectively. 2C10 and 3A1 reacted with the 31,000 MW (most strongly) and 30,000 MW bands, but not with the band of 29,000 MW. Anti-native CRP mAb 1B1 directed to the PC-binding site[24] and anti-native CRP mAb 8D8 directed to the complement-binding site[27] of human CRP did not react with limulin by either ELISA or Western blot assay (Table 1).

Reactivity of anti-human SAP mAb with limulin

Eleven anti-hSAP mAb with specificities for different epitopes[25] were used to study further the cross-reactivity between human pentraxins and limulin. Two mAb (anti-native mAb 8H5 and 8D2; 8D2 also reacts with modified hSAP) reacted with limulin by ELISA, but more than 1000-fold greater amounts were required than for reactivity with hSAP (Table 2). Western blots showed that both mAb were reactive with the 31,000 and 30,000

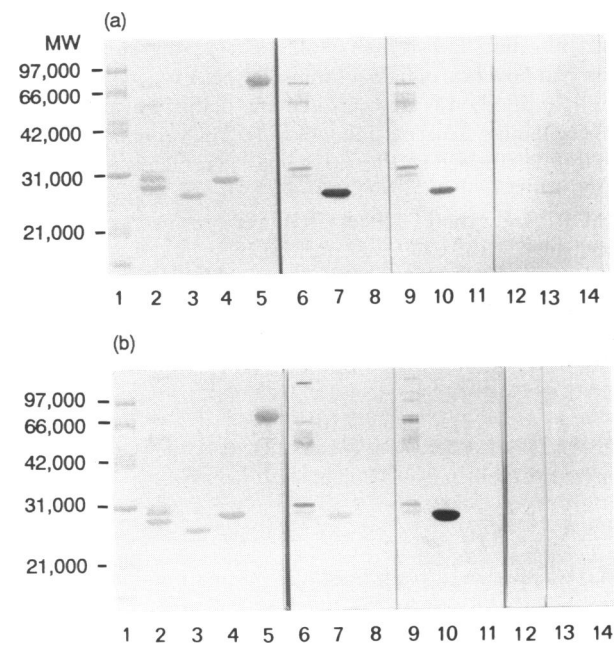

Figure 1. Western blot reactivity of anti-hCRP (a) and anti-hSAP mAb (b). Lanes 1–5 in both panels show the protein standards, limulin, hCRP, hSAP and transferrin, respectively, stained with Coomassie blue. In (a) limulin, hCRP and transferrin were blotted with mAb 2C10 in lanes 6–8, mAb 3A1 in lates 9–11, and with control anti-C1q mAb in lanes 12–14. In (b) limulin, hSAP and transferrin were blotted with mAb 8H5 in lanes 6–8, mAb 8D2 in lanes 9–11 and with control anti-C1q mAb in lanes 12–14.

Table 2. Cross-reactivity between pentraxins tested with anti-hSAP mAb by ELISA assays

mAb	hSAP*	limCRP†	FP*	hCRP*	raCRP*	rat CRP*
Anti-native hSAP mAb						
3H7	**0·08**‡	>20·0	>20·0	**0·4**	>20·0	>20·0
9H3	**0·001**	>20·0	>20·0	>20·0	>20·0	>20·0
8H5	**0·015**	**10·0**	>20·0	**2·0**	**5·0**	>20·0
Anti-native hSAP mAb which also react with modified SAP						
8D2	**0·03**	**20·0**	>20·0	**5·0**	>20·0	>20·0
2D8	**0·06**	>20·0	>20·0	>20·0	>20·0	>20·0
Anti-neo-hSAP mAb						
6D3	**0·02**	>20·0	>20·0	>20·0	>20·0	>20·0
5D5	**0·007**	>20·0	**0·1**	**0·7**	**1·25**	>20·0
4C11	**0·313**	>20·0	>20·0	>20·0	>20·0	>20·0
2E3	**0·015**	>20·0	>20·0	>20·0	>20·0	>20·0
7F5	**0·013**	>20·0	>20·0	>20·0	>20·0	>20·0
2A6	**0·002**	>20·0	**0·007**	**9·0**	**0·002**	**0·052**

* Amount (μg/ml) of mAb required for 50% maximal reactivity.
† Amount (μg/ml) of mAb required for 10% maximal reactivity.
‡ 'Strong' cross-reactivities observed with ≤0·1 μg/ml are shown in bold and underlined; 'weak' cross-reactivities are shown in bold only.

MW bands, but not with the 29,000 MW band, of limulin (Fig. 1b).

Reactivity of anti-hCRP mAb with other pentraxins

The two anti-hCRP mAb reactive with limulin by both ELISA and Western blot assays were tested for reactivity with the other pentraxins (raCRP, rat CRP, hSAP and FP). mAb 3A1 was comparably reactive with raCRP and hSAP as with hCRP, and mAb 2C10 reacted weakly with raCRP; however, both mAb failed to react with the other pentraxins (Table 1). Of the other 12 anti-hCRP mAb, three of four anti-native CRP (one reacted strongly) and five of eight anti-neo-hCRP (four reacted strongly) reacted with raCRP (Table 1 and Fig. 2a); two of the latter also reacted strongly with rat CRP (Table 1 and Fig. 2b). One anti-neo-hCRP mAb which failed to react with either limulin or raCRP reacted weakly with hSAP. mAb 1B1 directed to the PC-binding site of hCRP[24] showed only weak reactivity with raCRP (about 100-fold less than with hCRP) and no reactivity at all with rat CRP and FP, while mAb 8D8 directed to the C1q-binding site of hCRP[27] showed strong reactivity with raCRP but failed to react with rat CRP. None of anti-hCRP mAb reacted with FP.

Reactivity of anti-hSAP mAb with other pentraxins

The two anti-hSAP mAb reactive with limulin also were tested for reactivity with the other pentraxins; both mAb reacted weakly with hCRP and one also reacted weakly with raCRP. Three of the other nine anti-hSAP mAb reacted weakly with hCRP, and three of the five anti-hSAP mAb which cross-reacted with hCRP also cross-reacted with raCRP. Indeed, one of these cross-reactive mAb reacted with all four other vertebrate pentraxins tested, and even though this mAb (2A6) was raised to and bound strongly with human SAP, its reactivity with rabbit and rat CRP was particularly strong; however, it did not react with limulin (Table 2 and Fig. 2). Only two of 11 anti-hSAP mAb reacted with FP (Table 2). No other cross-reactivities were observed.

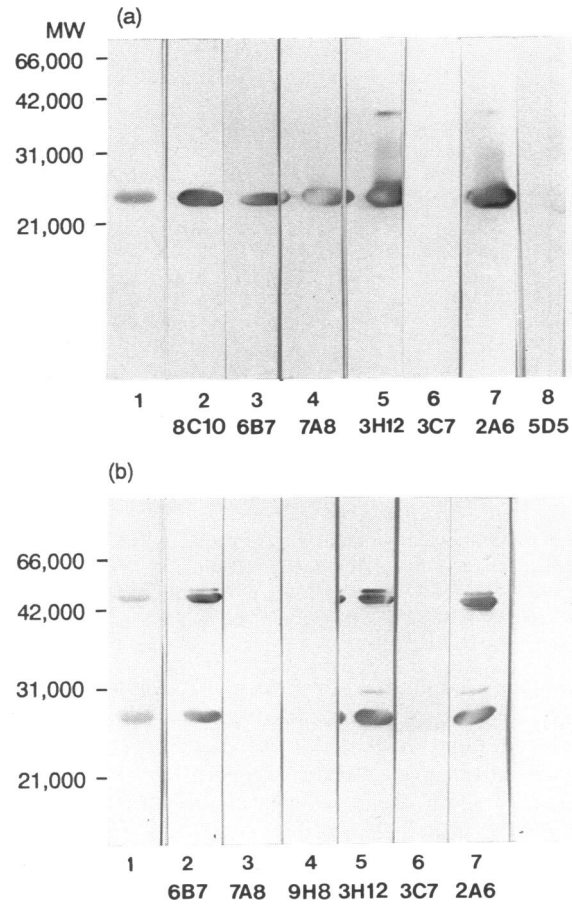

Figure 2. Western blot reactivity of mAb against hCRP and hSAP with rabbit (a) and rat (b) CRP under non-reduced conditions. In (a) lane 1 was stained with Coomassie blue; lanes 2–5 were blotted with anti-hCRP mAb 8C10, 6B7, 7A8 and 3H12, respectively; lane 6 with anti-C1q mAb 3C7 as a control; and lanes 7 and 8 with anti-hSAP mAb 2A6 and 5D5. In (b) lane 1 was stained with Coomassie blue; lanes 2–5 were blotted with anti-CRP mAb 6B7, 7A8, 9H8 and 3H12, respectively; lane 6 with anti-C1q mAb 3C7; and lane 7 with anti-hSAP mAb 2A6.

Reactivity of anti-limulin peptide antibody with limulin and other pentraxins

A polyclonal antibody was raised in rabbit against limulin peptide 141-156, a highly conserved region homologous to hCRP residues 135-150 (11/16 residues identical to limulin) and hSAP residues 133-148 (12/16 residues identical to limulin). The antibody bound strongly to limulin and weakly to hCRP and raCRP but not to rat CRP, hSAP or FP when tested by ELISA assays involving immobilization of the pentraxin on polystyrene microtitre plates, conditions known to bring about expression of neoantigens in the human pentraxins[20,24,25] (Fig. 3). This antibody also bound to the 29,000 MW band but not to 30,000 or 31,000 MW bands of limulus CRP on Western blots (data not shown), indicating a specificity distinct from the cross-reactive epitopes recognized by the anti-hCRP and anti-hSAP mAb.

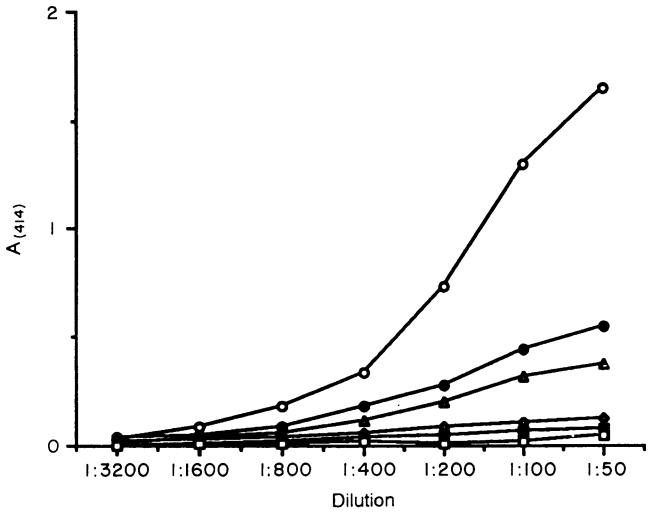

Figure 3. ELISA reactivity of anti-limulin peptide serum with pentraxins. Pentraxins (5 μg/ml) were directly immobilized on the polystrene plates and incubated with serial dilutions of antiserum. limCRP (○); RaCRP (●); hCRP (△); hSAP (◇); FP (■); rat CRP (□).

This antibody did not bind to either hCRP captured by KLH-PC or hSAP captured by solubilized zymosan extract (data not shown), conditions which lead to expression only of native and not of neoantigens in the human pentraxins.[20,24,25] Thus, the anti-peptide antibody was reactive with modified ('neo-') and not native CRP epitopes, comparable to anti-hCRP mAb specific for various synthetic CRP peptides which reacted with neo-CRP but not native CRP determinants.[20,24]

DISCUSSION

There is marked amino acid sequence as well as ultrastructural homology between the CRP and SAP of various species.[1,3,6,7,9,16,28,29] Previous studies utilizing polyclonal antibodies have indicated distinct cross-reactivities between the C-reactive proteins of different species,[8,17,18] including at least one shared epitope between hCRP and limulin,[14,19] but these cross-reactivities have been relatively limited.[8] Further, cross-reactivity between CRP and SAP had not been observed.[6,8] The generation of well-defined panels of mAb against hCRP and hSAP[20,24,25] allowed reinvestigation of antigenic similarity between pentraxins of representative species, utilizing CRP from man, rabbit, rat and the horseshoe crab and SAP of man and hamster to help identify conserved regions of the molecules. In addition, a polyclonal antibody to limulin peptide 141–156, a highly conserved region containing 12 residues identical with hSAP 133-148 and 11 identical residues with hCRP 135-150, was generated for use in this study.

None of the anti-human pentraxin mAb showed strong cross-reactivity with limulin. Definite, although weak, cross-reactivity with limulin was observed with only three of 14 anti-hCRP mAb and only two of 11 anti-hSAP mAb. This limited cross-reactivity using mAb is consistent with the weak cross-reactivity between mammalian CRP and limulin reported by Robey and Liu;[14] the definite but weak cross-reactivity between hCRP and limulin reported by Vasta *et al.*[19] and the comparatively rare cross-reactions between vertebrate CRP from different species reported by Maudsley and Pepys[8] using polyclonal antibodies. Limulin has been considered the horseshoe crab CRP based upon immunological cross-reactivity, sequence homology and shared PC-binding activity with hCRP.[14,16] However, limulin has comparable, and perhaps somewhat greater, sequence homology with hSAP than with hCRP (e.g. 33·3% as compared to 31·7% when limulin encoded by the 1·4 gene is considered) using the GCG Protein Analysis Module of the Sequence Analysis Software program and also shares lectin-binding properties with this pentraxin.[10,11,29] Since in the present study limulin also was shown to have comparable cross-reactivity with hSAP as with hCRP, it seems reasonable to consider this molecule the limulus SAP as well as the limulus CRP.

Calcium-dependent binding to PC is one of the defining features of the CRP molecule.[1–3] Surprisingly, mAb 1B1 which reacts with the PC-binding site of huCRP,[24] did not react with limulin or rat CRP and was 100-fold less reactive with rabbit than with huCRP. The region of the putative PC binding sites of huCRP, residues 51-66 in the study of Nguyen *et al.*[15] and residues 47-63 in the study of Swanson and Mortensen,[30] shows strong homology with rabbit CRP (13/15 and 15/17 identical residues, respectively). Nonetheless, it has previously been shown that the PC-binding sites of human and rabbit CRP are functionally and structurally different.[31] The relatively limited cross-reactivity of this anti-PC-binding site mAb supports the existence of significant conformational differences in the PC-binding sites of various species. It is of interest that hSAP also shows partial homology to hCRP in this region (9/15 and 10/17 identical residues, respectively), even though it lacks PC-binding activity.

Nguyen *et al.*[16] and Liu *et al.*[7] pointed out identity in nine of 15 positions between hCRP residues 133-147 and limulin residues 139-153 in the region thought to be associated with the Ca^{2+} binding of CRP.[32,33] The polyclonal antibody was raised against limulin peptide 141-156 from this region and reacted strongly with limulin by ELISA and Western blot assays. Alignment of this 'common CRP peptide' with the homologous regions of hCRP and raCRP, as well as with hSAP and FP, is as in Table 3[17] where * indicates residues similar in charge or hydrophobicity. The anti-peptide antibody bound only weakly to hCRP and raCRP and did not bind to rat CRP, hSAP or FP at all. The lack of more extensive immunological cross-reactivity even in this structurally highly homologous region further emphasizes the immunological distinctiveness between the pentraxins generally, and is similar to observations with other proteins such as myoglobins of different species.[34]

Most strong cross-reactivities of anti-hCRP mAb (4/8 anti-neo- and 1/6 anti-native-determinants) and anti-hSAP mAb (2/6 anti-neo- and 0/5 anti-native-determinants) with pentraxins of other species involve specificities recognizing sequence-determined epitopes (i.e. neo-determinants). The stronger reactivities between linear than between conformational pentraxin determinants are consistent with the results of comparable investigations with other proteins.[35]

Despite structural similarities between hCRP and hSAP including within the purported ligand-binding region identified by Nguyen *et al.* (residues 51-66 for CRP)[15] and the region close to the calcium-binding site considered in this paper (residues 135-150 for CRP), which display amino acid identities of 53 and 75%, respectively, no strong and only a few weak cross-reactivities were observed between hCRP and hSAP. This again

Table 3. Alignment of the 'common CRP peptide' of limulin with homologous raCRP, hCRP, hSAP and FP

L	G	Q	D*	Q	D	S	V*	G	G	K	F	D*	A*	T*	Q	limCRP	(141-156)
L	G	Q	D*	Q	D	S	F*	G	G	S	F	E*	K*	Q*	Q	raCRP	(134-149)
L	G	Q	E*	Q	D	S	F	G	G	N	F	E*	G	S*	Q	hCRP	(135-150)
L	G	Q	E*	Q	D	S	Y	G	G	K	F	D*	R	S*	Q	hSAP	(133-148)
L	G	Q	E*	Q	D	N	Y	G	G	G	F	D*	N	Y*	Q	FP	(132-147)

* Residues similar in charge or hydrobicity.

indicates that despite their structural similarity, there is limited strong immunological cross-reactivity between the pentraxins detectable by mAb raised in higher vertebrates. Cross-reactivities between hCRP and hSAP were not previously reported when tested by dot-blot assays using anti-CRP mAb[24] or by immunodiffusion assays using polyclonal antibodies,[8] but neodeterminants were not assayed and the sensitivities of the assays were less than those used in the present study.

It is of interest that the more cross-reactive anti-native CRP mAb 8D8 was shown to react with the C1q-binding site of hCRP.[27] Similarly, two anti-neo-CRP mAb, 6B7 and 3H12, whose CRP-binding sites were localized to residues 114-121 and 199-206, respectively,[24] were relatively cross-reactive since they bound to rabbit and rat as well as to human CRP. The former region was reported to serve as a nuclear localization signal[36] and the latter region to be involved in the modulation of phagocytic leucocytes.[37] These results are consistent with the hypothesis that functionally significant regions of CRP molecules are phylogenetically conserved. Thus, even though only limited cross-reactivities were observed between limulin and the vertebrate pentraxins as well as within the vertebrate penetraxins, perhaps the cross-reactive mAb identified and cross-reactive epitopes localized in the present study will be useful in the further identification and investigation of these functionally important conserved areas of hCRP and other pentraxins.

ACKNOWLEDGMENTS

We appreciate the generous gift of hamster female protein from Dr J. E. Coe of the Rocky Mountain Laboratories of the National Institutes of Health. We also thank Dr D. E. Harris for helpful discussions, and Ms M. Rolfe-Shaw for helpful secretarial assistance. This paper was presented in part to the Annual Meeting of the American Association of Immunologists held in Atlanta GA in April 1991.[38] This research was supported in part by National Institutes of Health Grant GM-42437 to J.J.M. H.G. holds the Thomas J. Coogan Jr Chair in Immunology established by Majorie Lindheimer Everett.

REFERENCES

1. Gewurz H., Mold C., Siegel H. & Fiedel B. (1982) C-reactive protein and the acute phase response. *Adv. Int. Med.* **27,** 345.
2. Pepys M.B. & Baltz M.L. (1983) Acute phase proteins with special reference to C-reactive protein and related proteins (pentraxins) and serum amyloid A protein. *Adv. Immunol.* **34,** 141.
3. Kilpatrick J.M. & Volanakis J.E. (1991) Molecular genetics, structure and function of C-reactive protein. *Immunol. Res.* **10,** 43.
4. Coe J.E. & Ross M.J. (1990) Amyloidosis and female protein in the Syrian hamster. Concurrent regulation by sex hormones. *J. exp. Med.* **171,** 1257.
5. Pepys M.D., Fletcher T.C., Richardson N., Munn E.A. & Feinstein A. (1978) Analogues in other mammals and in fish of human plasma proteins, C-reactive protein and amyloid P component. *Nature*, **273,** 168.
6. Baltz M.L., de Beer F.C., Feinstein A., Munn E.A., Milstein C.P., Fletcher T.C. *et al.* (1982) Phylogenetic aspects of C-reactive protein and related proteins. *Ann. N.Y. Acad. Sci.* **389,** 49.
7. Liu T.-Y., Syin C., Nguyen N.Y., Suzuki A., Boykins R.A., Lei K.-J. & Goldman N. (1987) Comparison of protein structure and genomic structure of human, rabbit and limulus C-reactive proteins: possible implications for function and evolution. *J. Protein Chem.* **6,** 263.
8. Maudsley S. & Pepys M.B. (1987) Immunochemical cross-reactions between pentraxins of different species. *Immunology*, **62,** 17.
9. Osmand A.P., Friedenson B., Gewurz H., Painter R.H., Hoffman T. & Shelton E. (1977) Characterization of C-reactive protein and the complement subcomponent C1t as homologous proteins and displaying cyclic pentameric symmetry (pentraxins). *Proc. natl. Acad. Sci. U.S.A.* **74,** 739.
10. Hind C.R.K., Collins P.M., Renn K., Cook R.B., Caspi C., Baltz M.L. & Pepys M.B. (1984) Binding specificity of serum amyloid P-component for the pyruvate acetal of galactose. *J. exp. Med.* **159,** 1058.
11. Kubak B.M., Potempa L.A., Anderson B., Mahklouf S., venegas M., Gewurz H. & Gewurz A. (1988) Evidence that serum amyloid P component binds to mannose-terminated sequences of polysaccharides and glycoproteins. *Molec. Immuol.* **25,** 851.
12. Marchalonis J.J. & Edelman G.M. (1968) Isolation and characterization of a hemagglutinin from *Limulus polyphemus*. *J. mol. Biol.* **32,** 453.
13. Kaplan R., Li S.S.-L. & Kehoe J.M. (1977) Molecular characterization of limulin, a sialic acid binding lectin from the hemolymph of the horseshoe crab, *Limulus polyphemus*. *Biochemistry*, **16,** 4297.
14. Robey F.A. & Liu T.-Y. (1981) Limulin: a C-reactive protein from limulus polyphemus. *J. biol. Chem.* **256,** 969.
15. Nguyen N.Y., Suzuki A., Ching S.-M., Zon G. & Liu T.-Y. (1986) Isolation and characterization of limulus C-reactive protein genes. *J. biol. Chem.* **261,** 10450.
16. Nguyen N.Y., Suzuki A., Boykins R.A. & Liu T.-Y. (1986) The amino acid sequence of limulus C-reactive protein. Evidence of polymorphism. *J. biol. Chem.* **261,** 10456.
17. Gotschlich E.C. & Stetson C.A., Jr. (1960) Immunologic cross-reactions among mammalian acute phase proteins. *J. exp. Med.* **111,** 441.
18. Nilsson L.-A. (1967) Immunodiffusion analysis of human, monkey and rabbit acute phase proteins. *Int. Archs. Allergy appl. Immunol.* **32,** 545.
19. Vasta G.R., Marchalonis J.J. & Kohler H. (1984) Invertebrate recognition protein cross-reacts with an immunoglobulin idiotype. *J. exp. Med.* **159,** 1270.
20. Ying S.-C., Gewurz H., Kinoshita C.M., Potempa L.A. & Siegel J.N. (1989) Identification and partial characterization of multiple native and neoantigenic epitopes of human C-reactive proteins by using monoclonal antibodies. *J. Immunol.* **143,** 221.
21. Cabana V.G., Gewurz H. & Siegel J.N. (1982) Interaction of very

low density lipoproteins (VLDL) with rabbit C-reactive protein. *J. Immunol.* **128,** 2342.

22. Siegel J.N. & Gewurz H. (1989) C-reactive protein in aging Lobund Wistar rats. In: *Dietary Restriction and Aging* (ed. D. L. Snyder), p. 127. Alan R. Liss, Inc., New York.
23. Coe J.E., Margossian S.S., Slayter H.S. & Sogn J.A. (1981) Hamster female protein. A new pentraxin structurally and functionally similar to C-reactive protein and amyloid P component. *J. exp. Med.* **153,** 977.
24. Ying S.-C., Shephard E., de Beer F.C., Siegel J.N., Harris D.E., Gewurz B.E., Fridkin M. & Gewurz H. (1992) Localization of sequence-determined neoepitopes and neutrophil digestion fragments of C-reactive protein utilizing monoclonal antibodies and synthetic peptides. *Molec. Immunol.* (in press).
25. Gewurz A., Siegel J.N., Ying S.-C., Rite F. & Gewurz H. (1990) Monoclonal antibodies reactive with human amyloid P component. *FASEB J.* **4,** A2198.
26. Schluter S.F., & Marchalonis J.J. (1986) Antibodies to synthetic joining segment peptide of the T-cell receptor B-chain: serological cross-reaction between products of T-cell receptor genes, antigen-binding T-cell receptors and immunoglobulins. *Proc. natl. Acad. Sci. U.S.A.* **83,** 1872.
27. Jiang H., Siegel J.N. & Gewurz H. (1991) Binding and complement activation by C-reactive protein via the collagen-like region of C1q and inhibition of these reactions by monoclonal antibodies to C-reactive protein and C1q. *J. Immunol.* **146,** 2324.
28. Woo P., Korenberg J.R. & Whitehead A.S. (1985) Characterization of genomic and complementary DNA sequence of human C-reactive protein, and comparison with the complementary DNA sequence of serum amyloid P component. *J. biol. Chem.* **260,** 13384.
29. Vasta G.R. (1990) Invertebrate lectins, C-reactive protein and serum amyloid P component. Structural relationships and evolution. In: *Defense Molecules* (eds J. J. Marchalonis and C. L. Reinisch), UCLA Seminars, Vol. 121, p.183. Alan R. Wiley-Liss Inc., New York.
30. Swanson S.J. & Mortensen R.F. (1990) Binding and immunological properties of a synthetic peptide corresponding to the phosphorylcholine-binding region of C-reactive protein. *Molec. Immunol.* **27,** 679.
31. Oliveira E.B., Gotschlich E.C. & Liu T.-Y. (1980) Comparative studies on the binding properties of human and rabbit C-reactive proteins. *J. Immunol.* **124,** 1396.
32. Kinoshita C.M., Ying S.-C., Hugli T.E., Siegel J.N., Potempa L.A., Jiang H., Houghten R.A. & Gewurz H. (1989) Elucidation of a protease-sensitive site involved in the binding of calcium to C-reactive protein. *Biochemistry*, **28,** 9840.
33. Swanson S.J., Mullenix M.C. & Mortensen R.F. (1991) Monoclonal antibodies to the calcium-binding region peptide of human C-reactive protein alter its conformation. *J. Immunol.* **147,** 2248.
34. Hurrell J.G.R., Smith J.A., Todd P.E. & Leach S.J. (1977) Cross-reactivity between mammalian myoglobins: linear vs spatial antigenic determinants. *Immunochemistry*, **14,** 283.
35. Arnon R. & Maron E. (1971) An immunological approach to the structural relationship between hen egg-white lysozyme and bovine a-lactoalbumin. *J. Molec. Biol.* **61,** 225.
36. Du Clos T.W., Mold C. & Stump R.T. (1990) Identification of a polypeptide sequence that mediates nuclear localization of the acute phase protein C-reactive protein. *J. Immunol.* **145,** 3869.
37. Robey F.A., Ohura K., Futaki S., Fujii N., Yajima H., Goldman N., Jones K. & Wahl S. (1987) Proteolysis of human C-reactive protein produces peptides with potent immunomodulating activity. *J. biol. Chem.* **262,** 7053.
38. Ying S.-C., Marchalonis, J.J., Gewurz A.T., Gewurz B.E., Jiang, H., Siegel J.N., Harris D.E. & Gewurz H. (1991) Antigenic comparison of limulus and human C-reactive proteins (CRP). *FASEB J.* **5,** A1527.