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# Characteristics of the Binding of Human C-Reactive Protein (CRP) to Laminin

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Human CRP binds to the basement membrane protein laminin *in vitro* in a $Ca^{2+}$-dependent manner via the phosphorylcholine (PC) binding site of C-reactive protein (CRP). The binding was saturable at a molar ratio of 4 (CRP/laminin). The specificity of the binding was shown by inhibition of binding of labeled CRP to laminin by unlabeled CRP, but not by human IgG. Specific binding was optimal in the presence of 5 mM $Ca^{2+}$, but did not occur in the absence of $Ca^{2+}$ or in the presence of EDTA. The binding of $Ca^{2+}$ to CRP causes a conformational change in the molecule, which is required for binding to PC and to laminin. The PC binding site of CRP was implicated in the binding to laminin on the basis of inhibition by both soluble PC and anti-idiotypic mAbs directed to the TEPC-15 PC-binding idiotype found on mouse antibodies to PC. In addition, mouse mAbs specific for the CRP PC binding site displayed decreased reactivity with CRP already bound to laminin. The binding of CRP to laminin provides a possible explanation for selective deposition of CRP at inflamed sites. The CRP-laminin interaction may serve as a means of concentrating CRP at sites of tissue damage so that the CRP might function as a ligand for leukocytes, an event that will result in removal of necrotic tissue and cell debris.

**Key words: protein binding, basement membrane, PC idiotype, extracellular matrix glycoproteins, acute-phase proteins**

C-reactive protein (CRP) is the prototype acute-phase reactant of man, since blood levels increase several hundred-fold from 100 ng/ml within 24 h of tissue injury or acute infection [1,2]. Human CRP is a member of the pentraxin family of proteins and is composed of five identical nonglycosylated subunits each having an MW of 23,500 [3,4]. The amino acid sequence of human CRP has been determined [5], as well as the nucleotide sequence, revealing a subunit of 206 amino acids coded for by two exons and an intron within the codon for the third amino acid [6,7]. CRP was discovered on the basis of its lectin-like binding activities directed toward the C-poly-

Abbreviations used: CRP, C-reactive protein; Fn, fibronectin; ELISA, enzyme-linked immunosorbent assay; EIA, enzyme immunoassay; SAP, serum amyloid P-component; PC, phosphorylcholine; TBS, Tris-buffered saline; mAb(s), monoclonal antibody(ies); $EC_{50}$, effective concentration for 50% inhibition; KLH, Keyhole Limpet hemocyanin; B-, biotinylated-; HRP, horseradish peroxidase.

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saccharide of pneumococcal cell walls [8]. This binding is $Ca^{2+}$-dependent and is directed primarily toward the phosphorylcholine (PC) group that is present in C-polysaccharide [9], and the phospholipids, lecithin and sphingomyelin [10]. However, phosphate groups on DNA [11], some oligosaccharides [12], certain phospholipids [13], a variety of monophosphate esters [14], and very low-density lipoproteins [15,16] are also bound by CRP with an affinity that is significantly lower than the binding to PC [12–16]. The DNA binding of CRP has a greater affinity [11]. CRP also binds to various physiological polycations [17]. Several biological activities have been attributed to CRP, such as complement activation [18,19], opsonization [20], macrophage activation [21–23], platelet activation [24], and chemotactic activity [25,26]. These activities are in general proinflammatory and thus associated with enhanced nonspecific host resistance.

An earlier observation on the fate of CRP in vivo was that it was selectively deposited at sites of tissue destruction in close association with damaged or necrotic cells [27]. The mechanism of the deposition of CRP at inflammatory sites has not been explained. One partial explanation of the deposition of CRP may be related to the observations of the binding of CRP to fibronectin (Fn) in vitro [28,29]. The binding of CRP to plasma Fn was characterized by us as a high-affinity reaction that occurred via the $Ca^{2+}$-dependent PC-binding region of CRP [29]; CRP binding also inhibited the potentiation of cell attachment activity by Fn [30].

Laminin is a major glycoprotein component of the basement membrane with diverse biological activities [for review, see 31–33). Laminin (~850,000 MW) has a cruciform shape and is composed of three short chains and one long chain, each chain containing rod-like segments and globular domains, indicating that laminin is a multidomain glycoprotein [32,33]. Laminin binds to other structural proteins of the extracellular matrix such as collagen type IV, heparan sulfate proteoglycan, and entactin/nidogen and is thought to play an essential role in basement membrane assembly [31,32]. Laminin is also an important mediator of cellular events, including adhesion, growth, morphology, differentiation, and migration [31,32]. All of these properties have led to the suggestion that laminin may participate in wound repair [32]. Laminin has been shown to selectively bind a few serum proteins such as C1 [34] and C3d [35] and plasminogen and tissue-type plasminogen activator [36].

During our studies on CRP binding to Fn, we also observed binding of CRP to laminin, which did not alter the cell attachment-promoting activity of laminin [30]. The purpose of these studies was to characterize the binding interaction of CRP to immobilized laminin. We report here that the binding involves the PC binding site of CRP and that the conformation of CRP is altered by this binding reaction on the basis of the expression of epitopes on CRP. The implications of the findings are that CRP binding to laminin at sites of tissue damage may play a role in the early stages of tissue repair.

## MATERIALS AND METHODS

### Purification and Measurement of CRP

CRP purification was accomplished by affinity chromatography, as described elsewhere, with slight modifications [37]. Briefly, pleural or ascites fluids from cancer patients were passed through a 2 × 10 cm column of 10 ml agarose beads (A-5m, Bio-Rad, Richmond, CA) in the presence of 5 mM $Ca^{2+}$ to remove agarose binding

proteins such as serum amyloid P-component (SAP). The effluent from the agarose beads was immediately passed through a column of p-aminophenyl phosphoryl choline-derivatized agarose beads (Pierce Chemical, Rockford, IL) at 30 ml/h in 0.01 M Tris buffered (pH 7.8) 0.15 M saline (TBS) containing 5 mM $CaCl_2$. The bound protein was eluted with a $Ca^{2+}$ chelator (10 mM sodium EDTA in TBS), and the fractions containing protein were pooled, recalcified to 2.5 mM, and allowed to bind to the PC-affinity matrix again. The column was washed with $Ca^{2+}$-containing TBS and the CRP eluted by competing with 2.0 mM PC-chloride in TBS containing 1 mM $Ca^{2+}$. The eluted protein was extensively dialyzed against 0.15 M NaCl with 0.02 M HEPES and 5 mM EDTA to dissociate bound PC. The CRP concentration was determined by radial immunodiffusion using a goat anti-human CRP or by a competitive ELISA using a rabbit IgG anti-CRP. The ELISA had a sensitivity of 10 ng/ml of CRP. The purity of CRP was assessed by SDS-PAGE under reducing conditions using a 4.0% stacking gel and either a 10% or a 12% resolving gel. The protein in these preparations was >97% CRP based on reactivity with antibody in the competitive ELISA. A single band of approximately 23 kD was observed on a reduced SDS-PAGE using the silver staining technique (Pierce Chemical). The CRP was stored as a sterile filtered solution of 1–2 mg/ml at 4°C.

### Monoclonal Antibodies (mAb) to CRP

Thirteen mAbs to CRP were generated; their specificity for different epitopes on CRP is described elsewhere [38]. The HD2-4 mAb to CRP was obtained from the American Type Culture Collection (ATCC). The HD2-4 mAb recognizes an epitope on that plane of the CRP molecule opposite the PC binding site [39]. The PC-binding mouse myeloma protein TEPC-15 (IgA) was purchased from Sigma. The HB-33 hybridoma line, which produced mAb AB1-2 against the idiotypic determinant of the PC-binding myeloma protein HOPC8 [40], was obtained from the ATCC. Two mAbs to the PC-specific TEPC15 (T-15) idiotope, 4C11 and F6, were a generous gift from Drs. Mary McNamara Ward and Ronald E. Ward [41,42]. The 4C11 mAb is PC-site specific, and its binding is inhibited by PC, whereas F6 is near-site-specific and its binding to T-15 is not inhibited by PC [41]. Both 4C11 and F6 were affinity-purified on a T-15 agarose column [42]. All of the hybridomas were adapted to growth as ascites tumors, and the ascitic fluid was used as a source of mAbs. The mAbs were purified using staphylococcal protein A-agarose beads (MAPS II, Bio-Rad). The purified IgG mAbs were stored as sterile solutions of 1–4 mg/ml in TBS, pH 7.4, at −20°C or 4°C. A single rat mAb ($IgG_{2b}$) to laminin was purchased from Chemicon, Int. (El Segundo, CA).

### CRP Binding to Laminin

Laminin was purchased from Sigma (St. Louis) and had been purified from the mouse EHS tumor [43] and stored at 1.0 mg/ml. Laminin was directly coated onto Immulon I (Dynatech) microtiter plates by adding 100 μl of a 5 μg/ml solution in 0.02 M $NaCO_3$ buffer (pH 9.6) and allowing the binding to occur overnight at 4°C. Binding of this amount was essentially quantitative as measured by enzyme immunoassay (EIA) using the rat mAb (Chemicon International) to laminin. Plates were washed four times with 0.02 M TBS, pH 7.4, containing 5 mM $CaCl_2$ and 0.05% BSA. The plates were blocked with 1 mg/ml BSA in TBS. Purified CRP diluted in TBS + 5 mM $Ca^{2+}$ + 1 mg/ml BSA was allowed to bind to the immobilized laminin for 3 h at

37°C. The bound CRP was measured by an EIA with 1.4 μg of biotinylated IgG (B-IgG) rabbit anti-human CRP as the primary antibody followed by peroxidase-conjugated strepavidin as the secondary reagent (1:2,000 for 1 h, room temperature). The TMB substrate (Kirkegard and Perry) for horseradish peroxidase was used in the colorimetric reaction, and the product was measured by recording $Abs_{450}$ on an EIA microplate reader (Dynatech). The effect of $Ca^{2+}$ concentration on CRP binding to laminin was determined by adding a constant amount of CRP (5.0 μg) to the immobilized laminin and varying the amount of $CaCl_2$ from 0.5 mM to 10 mM. Trace $Ca^{2+}$ was removed by the addition of 5.0 mM EDTA. To test the effects of PC on CRP binding to laminin, optimal amounts of CRP (5.0 μg) and $CaCl_2$ (5.0 mM) were used, and the PC concentration varied from 0.08 μM to 3.1 mM.

The effect of various mAbs on the binding of CRP to laminin was examined by a simple modification of the above procedure. A constant amount of CRP (5.0 μg) was mixed with fivefold dilutions within the range of 1,000–0.064 ng for each of the mAbs: AB1-2, BB9-2, RL1-28, RL1-38, and RL3-52. The mixture was allowed to bind to the immobilized laminin for 3 h at 37°C and the bound CRP detected as described. The binding of the mAbs to laminin was investigated by allowing 1,000, 100, or 10 ng of the mAb to bind to the immobilized laminin for 3 h. Bound mAb was detected by a peroxidase-conjugated, affinity-purified goat anti-mouse IgG, followed by the addition of TMB substrate.

### Titers of mAbs to CRP Immobilized via Various Substrates

An EIA was developed for determining the 50% titer of the various mAbs toward CRP bound to laminin, PC-KLH, rabbit IgG anti-CRP, and Fn. The PC-derivatized keyhole limpet hemocyanin (PC-KLH) was a generous gift from Dr. Joan Siegel (Rush Medical Center, Chicago, IL) and has 31 moles PC per $10^5$ MW of KLH; the binding of CRP to this preparation of PC-KLH has been described elsewhere [44]. Laminin was immobilized onto an Immulon I microtiter plate. Both PC-KLH at 1 μg/ml, IgG anti-CRP (0.5 μg/well), and Fn (0.5 μg/well) were immobilized onto an Immulon II (Dynatech) microtiter plate in 0.02 M $NaCO_3$ (pH 9.6) overnight at 4°C. The plates were washed and blocked with 1 mg/ml BSA diluted in TBS + $Ca^{2+}$. CRP was allowed to bind to the substrates at concentrations resulting in equivalent amounts of bound CRP:50 μg/ml for laminin, 5 μg/ml to IgG anti-CRP and Fn, and 0.31 μg/ml to PC-KLH; all mixtures were incubated for 1 h at 37°C except CRP with laminin, which was allowed to bind for 3 h at 37°C. The biotinylated mAbs (B-mAb) to CRP were generated and their specificity determined as described elsewhere [38]. The B-mAbs were allowed to bind to the immobilized CRP at amounts ranging from 2,000 ng/well to 0.16 ng/well for 1 h at 37°C. The mAbs were detected with HRP-conjugated strepavidin followed by TMB substrate; the reaction was stopped with 1N HCl and the $Abs_{450}$ recorded. The 50% titer was determined as the amount of mAb necessary to achieve an $Abs_{450}$ reading of one-half the maximum value. The strepavidin-HRP complex displayed slight binding to laminin, which was subtracted from the absorbance obtained with the B-mAb.

### Specificity of CRP Binding to Laminin

Specific binding of CRP to laminin was measured by allowing unlabeled CRP and biotinylated CRP (B-CRP) to compete for immobilized laminin. A constant amount (300 ng) of B-CRP was mixed with different amounts of unlabeled CRP, and

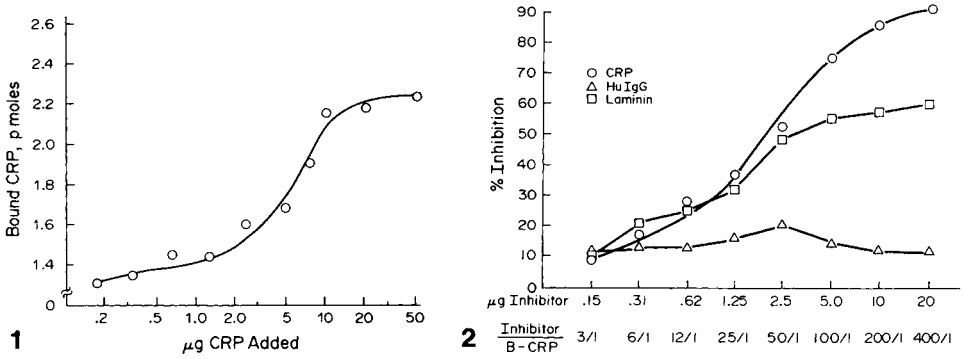


Fig. 1. Saturable binding of purified human CRP to laminin. Various amounts of CRP were allowed to bind to 500 ng laminin immobilized directly onto a polystyrene microplate. Bound CRP was determined by EIA as described in Materials and Methods. Saturation binding occurred at a molar ratio of 4 (CRP/laminin).

Fig. 2. Specificity of CRP binding to laminin. The percent inhibition of the binding of 50 ng biotinylated-CRP to 25 ng of immobilized laminin was measured by EIA. The competing proteins were: laminin (□), CRP (○), and human IgG (△). The amount of inhibitor is expressed in both μg and as a ratio of inhibitor/B-CRP. Data are the mean values from three experiments.

the mixture was allowed to bind to the immobilized laminin for 3 h at 37°C. The bound B-CRP was determined by adding HRP-strepavidin followed by TMB substrate.

## RESULTS

### Binding of Human CRP to Laminin

Addition of purified human CRP to immobilized laminin results in saturable binding (Fig. 1). Laminin, a basement membrane glycoprotein, (500 ng/well) was allowed to attach to a polystyrene microtiter plate. The attachment of the laminin was quantitative. Saturable binding of CRP to the immobilized laminin was obtained with the addition of 10 μg of CRP, which resulted in specific binding of approximately 2.2 pmoles of CRP (Fig. 1). At saturation, the molar ratio of CRP/laminin is calculated at approximately 4, using an MW of 117,500 for CRP and 900,000 for laminin. The amount of CRP bound to the laminin was detected by a direct EIA with a polyclonal rabbit (IgG) anti-human CRP. The saturable binding of CRP to laminin was reproducible for each of three purified human CRP preparations examined and therefore was further characterized.

Since immobilized CRP can be regarded as an equivalent to CRP deposited at sites of inflammation, an attempt was also made to measure binding of laminin to CRP. CRP was captured on microtiter plates by any one of the following methods: direct coating, $Ca^{2+}$-dependent binding to immobilized PC-KLH, or binding to rabbit IgG anti-CRP coated plates. In attempts to determine whether laminin could bind to these immobilized forms of CRP, extensive nonspecific binding of laminin was encountered in blocked wells not containing CRP, making the determination of specific laminin binding to CRP impossible. Laminin was detected with a rat mAb to laminin by EIA. Since laminin is not found in the circulation, it is unlikely to bind to

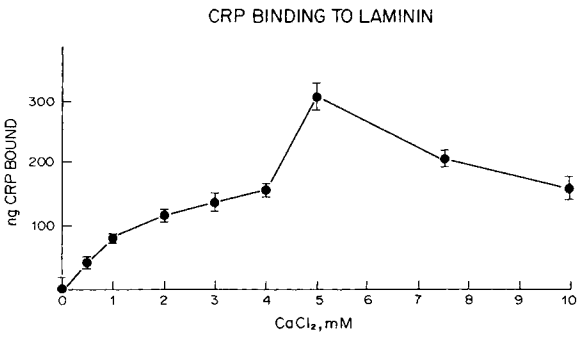


Fig. 3. The effect of $Ca^{2+}$ concentration on binding of CRP to laminin. The amount of specifically bound CRP was measured when 5 $\mu$g of CRP was allowed to bind to 500 ng of laminin in the presence of various concentrations of $Ca^{2+}$. EDTA at 5.0 mM was added to chelate trace $Ca^{2+}$ to obtain the data at 0 mM $CaCl_2$. Data obtained from two experiments.

CRP under pathophysiological conditions. Therefore, only the binding of soluble CRP to immobilized laminin was further examined.

## Specificity of CRP Binding to Laminin

To show that the binding of CRP to laminin was specific, we tested whether unconjugated CRP could compete vs. biotinylated CRP (B-CRP) for binding sites on laminin and whether the addition of soluble laminin could inhibit the binding of B-CRP to immobilized laminin. Since the use of B-CRP increased the sensitivity of the binding assay, only 25 ng of laminin per well was used. The results clearly indicated that the binding sites on laminin could be readily competed for by the unconjugated CRP and that soluble laminin could inhibit B-CRP binding to immobilized laminin (Fig. 2). Addition of purified human IgG (polyclonal) to the laminin failed to inhibit binding of CRP to the laminin (Fig. 2). Addition of a single rat mAb to laminin also failed to alter CRP binding (data not shown).

## Calcium Dependence of CRP Binding to Laminin

Since the binding reactions to the PC binding site of CRP are $Ca^{2+}$ dependent, we tested the effects of $Ca^{2+}$ concentration on CRP binding to the immobilized laminin. As shown in Figure 3, 5 mM $CaCl_2$ promotes the optimal binding of CRP to laminin. When 5 mM EDTA was added to the assay reaction to chelate any trace calcium ions, CRP binding to laminin was completely inhibited. This experiment shows that the binding of CRP to immobilized laminin is $Ca^{2+}$-dependent.

## Phosphorylcholine (PC) Inhibition of CRP Binding to Laminin

Since the PC binding site on CRP is important for the attachment of CRP to various substrates, including C-polysaccharide, chromatin, lecithin, and fibronectin, the possibility that soluble PC mediated CRP binding to laminin was investigated. A competitive ELISA was performed to determine whether varying concentrations of free PC would interfere with the binding of CRP to laminin. As seen in Figure 4, PC concentrations $\geq$ 20 $\mu$M inhibited CRP binding to laminin; however, PC concentrations $<$ 1 $\mu$M were not inhibitory. Addition of similar concentrations of the choline

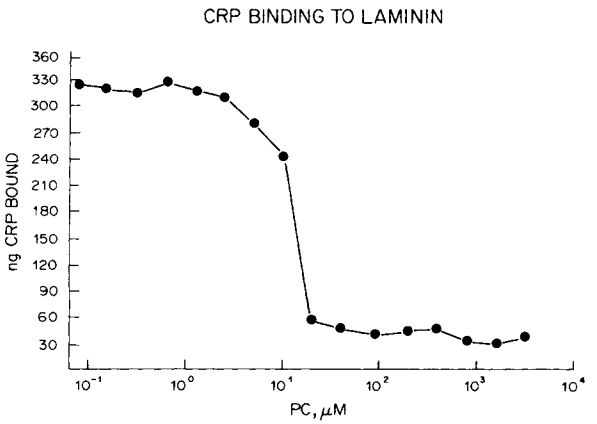


Fig. 4. Inhibition of the binding of CRP to laminin by phosphorylcholine (PC). Bound CRP was measured by EIA when 5 μg CRP was allowed to bind to 500 ng laminin in the presence of various concentrations of PC. Mean values for four experiments.

base of PC to the binding reaction failed to inhibit CRP binding. These data suggest that the PC binding site of CRP is involved in its binding to laminin.

## Effect of mAbs to CRP on the Binding of CRP to Laminin

Monoclonal antibodies directed against different epitopes of CRP were tested to determine whether they altered the binding of CRP to laminin. The mAbs BB9-2, RL1-28 and RL3-52, which recognize epitopes near the PC binding site based on inhibition of binding by PC, did not affect CRP binding to laminin (Fig. 5). However, the mAb AB1-2, which is an anti-idiotypic antibody against the PC-binding murine myeloma protein TEPC 15 and which also binds to the PC-binding site of CRP [40],

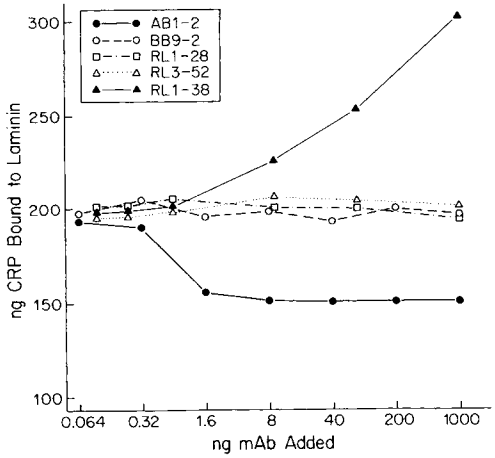


Fig. 5. The effect of various mAbs to CRP on the specific binding of CRP to laminin. Bound CRP was determined after CRP (5 μg) was allowed to bind to laminin (500 ng) in the presence of different amounts of the following mAbs: AB1-2 (●), BB9-2 (○), RL1-28 (□), RL3-52 (△), and RL1-38 (▲).

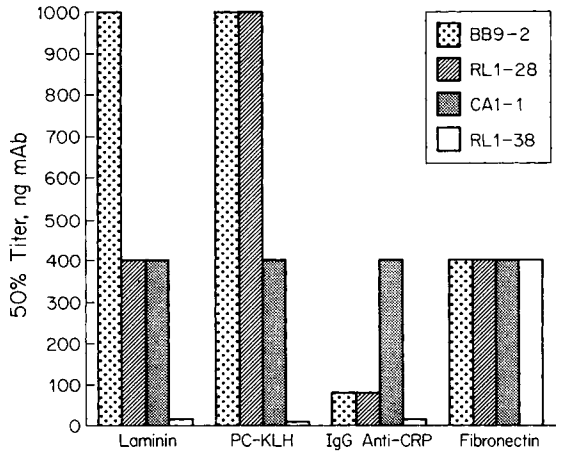


Fig. 6. Reactivity of anti-CRP mAbs with CRP bound to different substrates. The quantity (ng) of each of the mAbs [BB9-2 (▩), RL1-28 (▨), CA1-1 (▧), and RL1-38 (□)] required for 50% of maximum binding to a constant amount of CRP bound to laminin, PC-KLH, IgG anti-CRP, or fibronectin was measured. Data obtained from two experiments for each of the CRP ligands.

partially inhibits CRP binding to laminin. Addition of RL1-38, a mAb to an epitope unrelated to the PC-binding site, to CRP resulted in an increase in CRP binding to laminin. The RL1-38 mAb did not bind directly to laminin, and the increase in binding of mAb RL1-38 to laminin can be inhibited by the addition of 20 $\mu$M PC. Thus mAb RL1-38 did not increase CRP binding to laminin by first binding to laminin and then capturing the CRP or by altering the specificity of the CRP-laminin interaction.

## Titers of Various Monoclonal Antibodies for CRP Immobilized on Different Matrices

Since the mAbs to CRP generated by us [38] recognize different epitopes on CRP, the 50% titers of these mAbs to CRP that had been immobilized by laminin, PC-KLH, IgG anti-CRP, or fibronectin were compared to detect whether the same epitopes of CRP are expressed after binding to these different matrices. The mAbs to the PC binding site of CRP, BB9-2 and RL1-28, required a higher concentration to achieve a 50% titer when CRP was bound to laminin, PC-KLH, and fibronectin than when the CRP was randomly bound by IgG anti-CRP (Fig. 6). However, the non-PC site-specific mAbs, CA1-1 and RL1-38, required the same concentration to achieve a 50% titer when CRP was bound via any one of the three ligands. The results suggest that CRP binding to laminin is similar to CRP binding to PC-KLH and fibronectin, but different from the binding to IgG anti-CRP.

## Inhibition of CRP Binding to Laminin by PC-Binding Site Anti-Idiotypic mAbs

Since the binding of CRP to laminin may involve the PC binding site (Fig. 4), the effect of the anti-T15 idiotype mAbs AB1-2, 4C11, and F6 on the binding of CRP to laminin was examined by allowing them to react with CRP before adding it to laminin. All three of the anti-idiotypic mAbs partially inhibited the binding of CRP to

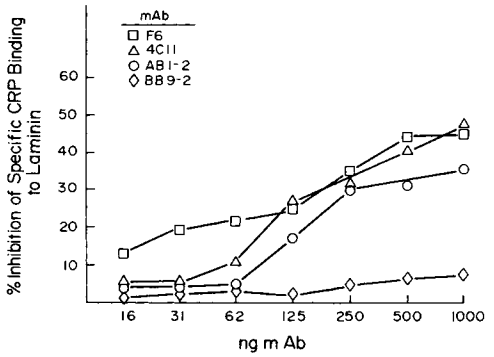


Fig. 7. Effect of anti-idiotypic (PC) mAbs on the binding of CRP to laminin. The percent inhibition of specific binding of CRP (5 μg) to 500 ng of laminin was measured by EIA. CRP was allowed to react with different amounts of the anti-idiotypic mAbs AB1-2 (○), F6 (□), and 4C11 (△) before addition to the laminin. The anti-CRP mAb BB9-2 ( ◇ ) was used as a control.

laminin, whereas the mAb BB9-2 was not inhibitory under similar conditions (Fig. 7). The mAbs did not bind to laminin. At low concentration of the anti-idiotype mAbs, F6 inhibits CRP binding more efficiently than the other mAbs. The observation that these anti-idiotypic mAbs, which recognize the PC binding region of both mouse Igs and CRP, with 4C11 representing an internal image of the PC binding site of the T-15 myeloma, and also inhibiting CRP binding to laminin, suggest that CRP attachment to laminin involves the PC binding site.

## DISCUSSION

These experiments show that CRP binds to laminin in a specific, saturable manner in vitro and that binding occurs via the $Ca^{2+}$-dependent, PC-binding site of CRP. Although laminin is able to bind to a few other blood proteins [34–36], the binding of CRP to laminin is relatively specific, as shown by competition between labeled CRP and unlabeled CRP for a limited number of binding sites on immobilized laminin. Saturable binding occurred at a molar ratio of 4 CRP molecules per laminin molecule. In addition, soluble laminin inhibited CRP binding to immobilized laminin, whereas human IgG, which is similar in size to CRP, was unable to inhibit binding of CRP. A rat mAb to laminin was also unable to inhibit the CRP-laminin interaction. Since both Fn and laminin effectively bind CRP only when immobilized, competition by soluble Fn for CRP binding to laminin cannot be tested directly [29]. The nature or location of the CRP-binding sites on laminin has not yet been determined, as the studies reported herein focused on characterization of the binding site on CRP.

The specific binding of CRP to laminin depends on the presence of physiological levels of $Ca^{2+}$, as shown by the complete lack of binding when trace amounts of $Ca^{2+}$ are chelated by EDTA. It has been shown that each of the CRP subunits is able to bind up to two $Ca^{2+}$ ions [3,45] and that $Ca^{2+}$ binding alters the conformation of CRP, as detected by a change in the circular dichroism spectrum of CRP [45]. The change in circular dichroism is thought to involve $Ca^{2+}$ interaction with the tyrosine-40 residue of CRP [45], although histidine residues at positions 38 and 76 have also

been implicated [46]. A requirement for $Ca^{2+}$ is not unique for CRP binding to laminin, since CRP binding to Fn [29] and a variety of PC-bearing substrates is also dependent on $Ca^{2+}$ [2,9,10,12–14]. The conformational change of CRP upon $Ca^{2+}$ binding is thought to facilitate binding at the PC binding site [44–46], and therefore a similar conformational change may be a prerequisite for specific CRP binding to laminin.

Several different experimental approaches revealed that the PC binding site of CRP is involved. The observation that soluble PC at micromolar concentrations inhibits CRP binding to laminin provides direct evidence that the PC binding site of CRP is involved. The choline base is unable to inhibit the binding and has been shown to not bind to the PC-binding site [9]; thus the monophosphate group is responsible for the inhibition. CRP recognizes and binds a variety of monophosphate esters, albeit with lower affinity than PC itself [9,14]. The PC binding site is involved in CRP binding to several other macromolecules, including CPS [8], lecithin and sphingomyelin [13], galactan [12], chromatin [11], and fibronectin [28,29]. Binding of CRP to these compounds appears to be directed toward a primary phosphate group in the form of a monophosphate ester. It is not clear which domain(s) or residues within the laminin molecule contains primary phosphates as sugars or as phosphorylated amino acid residues [31,32]. Further evidence that CRP binds to laminin via the PC binding site is that mAbs that recognize epitopes at or near the PC binding site require more Ab to achieve a 50% titer by ELISA when the CRP is immobilized by laminin, PC-KLH, and fibronectin than when CRP is immobilized by polyclonal IgG anti-CRP. This suggests that CRP immobilized via laminin, PC-KLH, or fibronectin has fewer epitopes available for these mAbs and thus the binding to laminin probably occurs through the PC binding site. The epitopes recognized by the CRP PC-site specific mAbs would not be expected to be available for binding. Since CRP exists as a pentamer and each monomer contains a single PC-binding site, it seems likely that when CRP is immobilized via the PC binding site, not all of the monomers within the molecule have the PC site occupied, explaining why binding by these mAbs is not completely inhibited. The increase in the amount of mAb required for a 50% titer simply reflects the decrease in available PC binding sites on CRP. In contrast to the mAbs recognizing epitopes at or near the PC binding site, the mAbs recognizing epitopes not in the PC binding region do not require more antibody to obtain a 50% titer for CRP bound to laminin or PC-KLH, than for CRP bound to polyclonal IgG anti-CRP. Thus the epitopes recognized by the non-PC binding site-specific mAbs are not involved in CRP binding to laminin.

In testing the effect of various mAbs on the binding of CRP to laminin, it was observed that those mAbs specific for epitopes at or near the PC binding site, as determined by inhibition of binding to CRP in the presence of PC [38], did not inhibit CRP binding to laminin. Since all of the other data indicated that CRP binding to laminin is mediated through the PC binding site, this observation appears to be contradictory. However, since PC binding by CRP in the presence of $Ca^{2+}$ alters its conformation [47], the epitopes may not occur in the PC binding region itself. An alternative explanation is that the affinity of the mAb for CRP is less than the affinity of CRP for laminin. We also observed increased amounts of CRP bound to laminin in the presence of RL1-38, a mAb recognizing an epitope on CRP distinct from the PC binding site. The RL1-38 mAb may be altering the conformation of CRP so as to increase its binding to laminin. Since RL1-38 does not bind laminin, this mAb does not increase

binding by altering the conformation of laminin. Since PC inhibits CRP binding even in the presence of bound RL1-38, the mAb does not cause CRP to bind via a different mechanism, but rather augments the binding via the PC binding site, probably altering the conformation of CRP.

The anti-T15 idiotype mAb AB1-2, which has been shown by Volanakis and Kearney [40] to bind to CRP, partially inhibited the binding of CRP to laminin. Since this mAb recognizes an idiotope in the TEPC-15 mouse myeloma PC binding region, inhibition of CRP binding to laminin by AB1-2 provides additional evidence that the PC binding region of CRP is involved in laminin binding. Two other TEPC-15 anti-idiotype mAbs, 4C11 and F6, which have previously been shown to bind to CRP [48], also inhibited CRP binding to laminin. These two anti-idiotype mAbs are directed against distinct idiotopes within the PC-binding region: 4C11 is binding site-specific and inhibits PC binding by TEPC-15, whereas F6 is near-site-specific and does not [41]. The mAb AB1-2 also recognizes an idiotope near the PC binding site [49]. Because 4C11 is site-specific and represents an internal image of PC [42], its inhibition of CRP binding to laminin provides strong evidence that the CRP-laminin interaction occurs via the PC binding region of CRP.

The physiological significance of CRP binding to laminin is that it may provide a means for localizing CRP at sites of tissue injury at or near basement membranes where laminin would be exposed. The selective localization of CRP at sites of tissue damage was first documented by Kushner et al. [27]; however, confinement of CRP binding to any single structure or macromolecule has not been observed, but rather binding to several molecules liberated during tissue damage has been reported [50]. In a recent review, Martin and Timpl [31] suggest that laminin participates in wound repair. If laminin contributes to localizing CRP at sites of tissue damage, the bound CRP may be available for receptor-mediated binding of platelets [24], neutrophils [51,52], and monocytes [21–23]. All of these blood cells are thought to play a role in the initial stages of wound repair.

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