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A Cell Attachment Peptide From Human C-Reactive Protein

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Abstract The serum acute phase reactant, C-reactive protein (CRP), is selectively deposited at sites of tissue damage and degraded by neutrophils into biologically active peptides. A synthetic peptide corresponding to residues 27–38 present in each of the five identical subunits of CRP mediated cell attachment activity in vitro. Although the CRP-derived peptide contains a Tuftsin (TKPR)-like sequence at its amino-terminus, the Tuftsin tetrapeptide itself, as well as several synthetic peptides of CRP, failed to inhibit the cell-attachment activity to the CRP-derived peptide. Peptides containing the sequences responsible for the cell attachment activity of the extracellular matrix proteins, fibronectin (Fn) and laminin, failed to inhibit the CRP-derived peptide cell attachment activity. However, the addition of the RGDS and RGDSPASSLP cell-binding peptides of Fn to cells enhanced attachment to the active peptide from CRP. In the converse experiment, the cell-binding peptide of CRP did not influence cell attachment to Fn or laminin. A peptide corresponding to the same stretch of amino acid residues within the homologous Pentraxin, serum amyloid P-component (SAP), displayed nearly identical cell-attachment activity. Several monoclonal antibodies (mAb) specific for the CRP-derived cell-binding peptide neutralized its cell-attachment activity. These mAbs reacted with intact CRP and neutralized the cell-binding activity of CRP itself. The findings suggest that a peptide with cell-binding activity could be generated from the breakdown of CRP and then contribute directly to cellular events leading to tissue repair.

Key words: acute phase reactant, serum amyloid P-component, tissue repair, extracellular matrix proteins

One of the earliest systemic changes in response to tissue damage and inflammation caused by acute insults such as trauma or infection is the rapid synthesis by hepatocytes of a diverse group of blood proteins termed *acute phase reactants* [reviewed in Ballou and Kushner, 1992]. In humans and most mammals, the prototype acute phase reactant is C-reactive protein (CRP), since its concentration may increase by several thousandfold [Pepys and Baltz, 1983]. CRP gene expression is triggered by the inflammatory cytokines IL-6 and IL-1 [Gantner et al., 1989; Li et al., 1990]; CRP production is regulated at the post-transcriptional level by transforming growth factor-β [Taylor et al., 1990]. CRP is a member of the Pentraxin family of proteins and is composed of five identical, nonglycosylated subunits of 206 amino acids each that are noncovalently bound to each other in a pentameric discoid arrangement [Osmand et al., 1977; Woo et al., 1985; Lei, et al., 1985]. CRP displays a lectin-like Ca^{++}-dependent binding reactivity directed primarily toward phosphorylcholine (PC) [Volanakis and Narkates, 1981] and several other phosphate monoesters [Soelter and Uhlenbruck, 1986]. CRP also mediates a variety of host defense biological activities that include phagocytosis [Mortensen and Duskiewicz, 1977], leukocyte activation [Zahedi and Mortensen, 1986; Zeller et al., 1986], and triggering of the classical complement cascade [Kaplan and Volanakis, 1974; Siegel et al., 1974].

The only other known Pentraxin is serum amyloid P-component (SAP), a protein consisting of two planar pentraxin discs arranged face to face [Osmand et al., 1977]. SAP is an acute phase reactant only in the mouse [Pepys and Baltz, 1983]. SAP has extensive sequence homology with CRP and also displays Ca^{++}-dependent binding reactivities; however, its binding specificity is directed toward a few glycosylated-

Abbreviations used: BSA, bovine serum albumin; CRP, C-reactive protein; Fn, fibronectin; KLH, Keyhole Limpet hemocyanin; mAb, monoclonal antibody(ies); PC, phosphorylcholine; Pep, peptide; SAP, serum amyloid P-component

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proteins and polysaccharides, especially the galactan polymer present in agarose [Hind et al., 1984; Kubak et al., 1988]. The genes for human SAP and CRP are nearly identical in their organization, and are located on the long arm of chromosome 1 [Woo et al., 1985; Floyd Smith et al., 1986].

CRP is selectively deposited at sites of damaged tissue [Kushner et al., 1963] where it presumably binds to any of a variety of physiological substrates such as fibronectin (Fn) [Salonen et al., 1984; Tseng and Mortensen, 1988], laminin [Swanson et al., 1989], phospholipids on damaged membranes [Volanakis and Narkates, 1981], and chromatin [Robey et al., 1984; DuClos et al., 1988], especially certain histones [DuClos et al., 1991]. All of these binding interactions occur via the single PC-binding site that is present on each CRP subunit [Roux et al., 1983]. CRP also undergoes proteolysis by neutrophils, releasing peptides that elicit activation responses of monocytes and neutrophils such as chemotaxis and superoxide production [Robey et al., 1987; Shephard et al., 1990]. The leukocyte responses are associated with peptides containing any one of the three tuftsin tetrapeptide (Thr-Lys-Pro-Arg)-like sequences that are present in each subunit of CRP [Robey et al., 1987]. During an analysis of one of the tuftsin-bearing synthetic peptides from CRP for its interaction with the monocyte CRP-receptor [Tebo and Mortensen, 1990], we found that it mediated the cell-attachment activity as described herein. The findings suggest that CRP-derived peptides may contribute to wound repair processes via an interaction with cellular receptors that appear to be different from the integrin receptors for the extracellular matrix proteins, Fn and laminin.

MATERIALS AND METHODS

Purification of CRP and SAP

CRP was purified from human ascites fluids by Ca^{++}-dependent affinity chromatography on PC-phenyl sepharose (Pierce Chemical Co., Rockford, IL) as described by us elsewhere [Zahedi and Mortensen, 1986; Tebo and Mortensen, 1990]. Human serum amyloid P-component (SAP) was purified on the basis of its Ca^{++}-dependent affinity for agarose as described by others, with minor modifications [Potempa et al., 1985]. Briefly, human plasma (American Red Cross) was allowed to clot by adding 10 mM $CaCl_2$; the serum was diluted 1:2 with TBS (20 mM Tris, 140 mM NaCl, and 2.5 mM $CaCl_2$, pH 7.4) and passed rapidly (30 ml/h) through a 1.0 × 10.0 cm bed of Bio-gel A 0.5 m agarose beads (Bio-Rad, Richmond CA) followed by extensive washing. The SAP was eluted from the column with TBS containing 10 mM EDTA. Fractions with A_{280} readings > 0.2 were pooled and a final purification was achieved by sizing through a 0.5 × 50 cm column of ACA-34 Ultrogel (LKB, Upsala, Sweden) using TBS containing 5 mM EDTA. The SAP was assessed for CRP, IgG, IgA, C1, and C3 by ELISA and found to be negative. The concentration of SAP was measured by rocket immunoelectrophoresis using a rabbit monospecific anti-human SAP (Calbiochem, San Diego, CA). The SAP appeared as a single band of approximately 31 kDa upon SDS-PAGE run under reducing conditions. The size corresponds to the 204 amino acid subunit of SAP that contains 11% carbohydrate by weight [Siripont et al., 1988]. Both SAP and CRP were stored at 4°C at 0.2–1.0 mg/ml and retained binding activity for at least 3 months and 12 months, respectively.

Synthetic CRP and SAP Peptides

Two peptides corresponding to the first of the three tuftsin-like sequences found in human CRP [Robey et al., 1987] and the corresponding sequence in human SAP were synthesized by the Ohio State University Biochemical Instrument Center by using *t-boc* synthesis on a model 9500 Peptide Synthesizer (Milligen/Millipore, Burlington, MA). For convenience the peptides were labeled as shown after cell-binding activity was documented:

CB-Pep (CRP) (r27)T-K-P-L-K-A-F-T-V-C-L-H (r38)
CB-Pep (SAP) (r27)E-K-P-L-Q-N-F-T-L-C-F-R (r38).

Both peptides were solubilized in 5% acetic acid at 5 mg/ml. The CB-Pep (CRP) was covalently linked to KLH and BSA with the heterobifunctional cross-linking reagent m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) (Pierce Chemical Co., Rockford, IL). Two milligrams of MBS were dissolved in 0.5 ml DMSO and added to 5 mg of the carrier protein to achieve a molar ratio of MBS to protein of 40:1. The mixture was stirred for 30 min and the free MBS removed by gel filtration on Sephadex G-50 (Pharmacia). The peptide (10 mg) was added to each of the activated carrier proteins for 3 h with stirring;

the free peptide was separated from the peptide carrier by gel filtration. The molar ratio of the CRP peptide to BSA was ~15; the ratio was ~90 for the peptide-KLH conjugate. The preparation of the other synthetic peptides of CRP is described by us elsewhere [Swanson and Mortensen, 1990; Swanson et al., 1991]. These peptides include the functional PC-binding peptide (PC-pep), the Ca^{++}-binding region peptide (Ca^{++}-bind-pep), and the N-terminal and C-terminal peptides listed below:

PC-pep:	(r47)R-G-Y-S-I-F-S-Y-A-T-K-R-Q-D-N-E-I(r63)
Ca^{++}-bind-pep:	(r134)I-L-G-Q-E-Q-D-S-F-G-G-N-F-E-G(r148)
CT-pep:	(r191)K-Y-E-V-Q-G-E-V-F-T-K-P-Q-L-W-P(r206)
NT-pep:	(r1)Q-T-D-M-S-R-K-A-F-V-F-P-K-E-S(r15).

All of these peptides were stored as solutions at 2–5 mg/ml in 5% acetic acid and diluted only at the time of their use.

Preparation of Antibodies to CB-Pep

A rabbit polyclonal antiserum to the CB-Pep was generated by immunizing rabbits with 0.5 mg of CB-Pep (CRP)-KLH in complete Freund's adjuvant intradermally at multiple sites, followed by two weekly s.c. immunizations one month later with 100 μg of the immunogen in incomplete adjuvant. The serum was obtained 5 weeks after the last challenge and possessed an ELISA titer of 1/3,200 vs. the CB-Pep-BSA conjugate. The antiserum did not react with BSA, human IgG, or the other CRP-derived peptides.

Mouse monoclonal Ab (mAb) were also prepared against the CB-Pep (CRP). BALB/c mice were immunized with 100 μg of the peptide-KLH suspended in Ribi synthetic adjuvant (Ribi Immunochemicals Inc., Hamilton, MT) by injecting 0.2 ml i.p. Mice were boosted with 100 μg of the peptide-KLH by the i.p. route every 2 weeks until a serum titer of > 1:5000 was obtained. An injection of 50 μg peptide-KLH was given i.v. 3 days prior to fusion of the spleen cells with P3x63.AG853 myeloma cells (Ig nonsecreting variant). The initial screening of the clones for IgG antibody against the CB-Pep was done by direct ELISA. Briefly, the peptide (500 ng/well) or peptide-BSA was coated onto Immulon II (Dynatech, Chantily, VA) plates in 20 mM carbonate/bicarbonate buffer (pH 9.6) and blocked with 1% BSA in 0.15 M NaCl buffered with 20 mM Tris, ph 7.4, containing 2 mM $CaCl_2$. The presence of bound mAb was detected with a peroxidase-conjugated goat anti-mouse γ-chain specific antibody followed by the peroxidase TMB substrate (Kirkegaard & Perry Labs, Inc., Gaithersburg, MD). Clones producing IgG mAb were subcloned once by limiting dilution and adapted to grow in Dulbecco's MEM containing 10% horse serum. Approximately 5×10^6 cells were injected i.p. into pristane-treated BALB/c mice to generate mAb containing ascitic fluid. The IgG mAb were purified from the ascites using a fast-flow protein-G column (Pharmacia, Piscataway, NJ).

Cell Binding Assay

Cell adhesion was assayed by coating polystyrene microplates or 35 mm plates with proteins or peptides and then allowing NRK-49F cells (normal rat kidney fibroblasts) (ATCC, Rockville, MD) or other adherent cell lines to attach during a 45 min interval at 37°C. The peptides at various concentrations and Fn or laminin at 1 μg/microplate well were diluted in carbonate-bicarbonate buffer (pH 9.6) and allowed to dry overnight. The coated wells were then washed twice with PBS and blocked with 1% BSA for 30 min at 37°C. The NRK-49F cells were obtained from confluent 25 cm^2 flasks by trypsin-EDTA, washed 3× in DMEM (M.A. Bio-Products, Walkersville, MD) plus 1% BSA and resuspended to $3–4 \times 10^4$/ml in the same medium. The cells at $\sim 7 \times 10^3$/well in a total volume of 200 μl were incubated for 45 min at 37°C in a 5% CO_2 incubator. The plates were washed 3× with PBS to remove unattached cells. The attached cells were fixed with 1% paraformaldehyde and stained with 1% toluidine-blue. The cells attached to the microplates were read microscopically by examining a wedge section corresponding to ⅛ of the well. The % cells attached was calculated on the basis of the number of attached cells/total number of cells added. Other cell lines tested for adherence were handled in the same manner.

To test for inhibition of attachment by various peptides to the CRP-derived peptide, or to Fn, the fibroblasts were incubated with a peptide for 90 min before testing the cells for attachment. Fn was routinely used as a control to determine if the incubation altered cell-attachment activity. The CB-Pep of CRP was coated at either 10 μg or 20 μg/microplate well in the

inhibition assays. In some assays mAb to the CB-pep were evaluated for their ability to alter cell attachment by adding 10 μg/well of purified mAb. Each peptide was tested for inhibition in at least 3 separate experiments in triplicate for each concentration.

RESULTS

Cell Attachment to CRP-Derived Synthetic Peptides

Synthetic peptides corresponding to the PC-binding and Ca^{++}-binding functional domains of CRP, as well as the peptide composed of residues 27 through 38, were assayed for cell attachment activity. The latter peptide was chosen because it contains the Tuftsin-(TKPR)-like sequence at its N-terminus. The cell attachment assay employed was essentially the same as that originally used to measure the activity of the extracellular matrix protein Fn [Piersbacher and Ruoslahti, 1984]. The CRP peptide corresponding to residues 27–38 was as active in the cell attachment assay as the equivalent peptide from SAP (Fig. 1). A dose-response relationship existed between the extent of cell attachment and the amount of coated peptide, with 50% cell attachment occurring at 20–30 μg/well when using a microplate assay. Fn at 1 μg/well was included in all the assays and always resulted in 60–80% cell attachment. The other CRP-derived peptides, including the 15 amino acid N-terminal and C-terminal peptides that are both relatively hydrophilic, failed to promote cell attachment (Fig. 1). Since the cell-attachment activity was very reproducible we designated the two active peptides: cell-binding peptide of CRP, or CB-Pep (CRP), and cell-binding peptide of SAP, or CB-Pep (SAP). One feature of the cell attachment activity of the CB-Pep (CRP) was that the fibroblasts did not "spread" after attachment as they characteristically do following attachment to Fn [Piersbacher and Ruoslahti, 1984].

Inhibition of Cell Attachment to CB-Pep (CRP)

To examine the specificity of the binding to the active peptide from CRP, we performed inhibition experiments by simply allowing the competing peptides to bind to the cells prior to adding them to CB-Pep (CRP) coated wells. The homologous CB-Pep (CRP) inhibited cell attachment with 50% inhibition occurring at ~300 μg/ml (Fig. 2). The CB-Pep (SAP) displayed very similar inhibitory activity to both CB-Pep (CRP) and CB-Pep (SAP) coated wells (data not shown). The tuftsin tetrapeptide TKPR that has a sequence similar to the N-terminus of the CB-Pep (TKPL) failed to alter cell attachment to

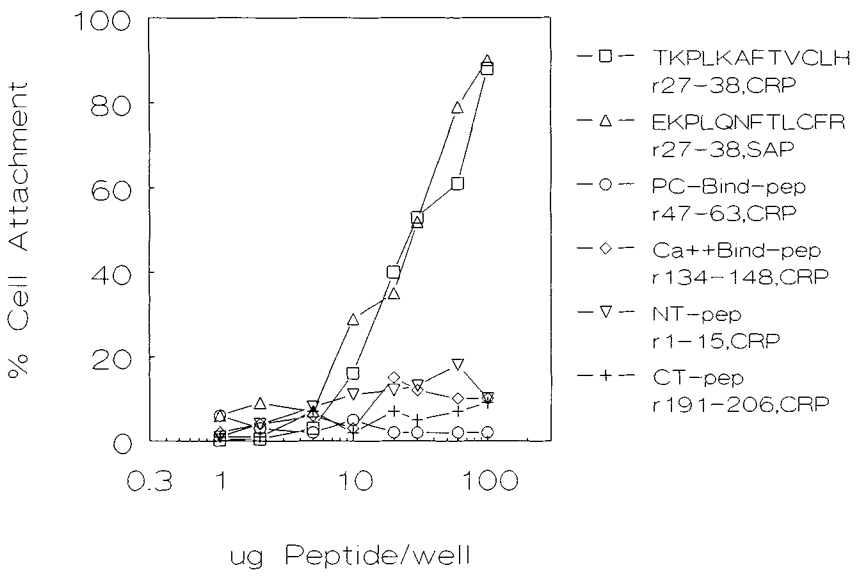

Fig. 1. Cell attachment to synthetic peptides from CRP. Peptides corresponding to regions of relative hydrophilicity and with a high surface probability in the intact protein were coated onto polystyrene microplates and assessed for their ability to mediate attachment of normal rat kidney fibroblasts (NRK-49F). Unattached cells were removed by washing; the attached cells were fixed, stained, and counted. The % cell attachment was calculated as number of attached cells/total number of cells added.

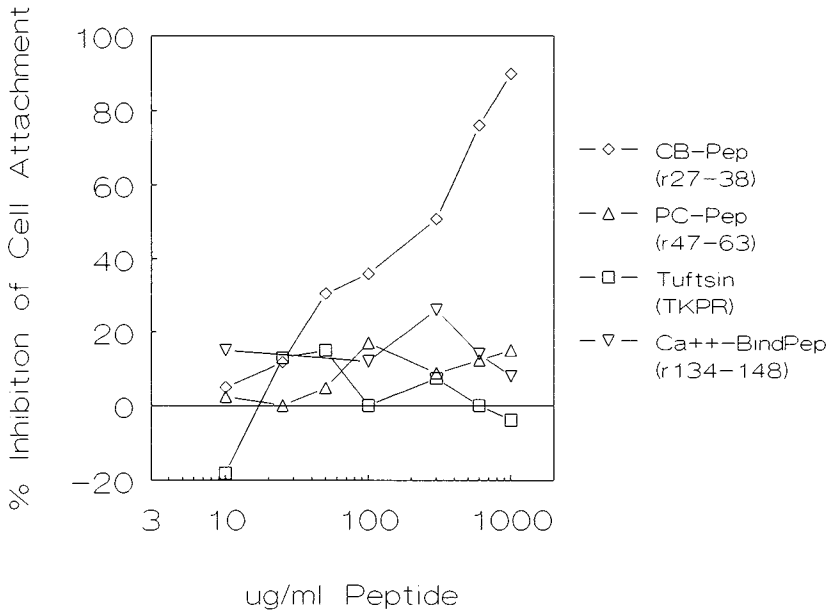

Fig. 2. Inhibition of cell attachment to the cell binding peptide of CRP [CB-Pep (CRP)]. Synthetic peptides derived from CRP and SAP were incubated at 37°C with NRK fibroblasts in medium prior to adding the cells to 20 μg/well of CB-Pep (CRP). Data represent the mean values from 4–6 experiments with each peptide.

the CRP-derived peptide (Fig. 2). In the same experiments the PC-binding peptide of CRP, as well as the peptide from the Ca^{++}-binding region, failed to significantly influence the CB-pep (CRP) mediated attachment activity.

Effect of Cell-Binding Peptides From Extracellular Matrix Proteins on Cell Attachment to the CB-Pep of CRP

Although the RGD or RGDS sequence is not present within CRP, the sequence is embedded in a variety of proteins and mediates the cell-binding activity of several extracellular matrix proteins [Ruoslahti, 1988]. Therefore, we tested whether RGD-bearing peptides influenced binding to the CB-Pep (CRP). Experiments in which the Fn cell attachment tetrapeptide RGDS, or the longer decapeptide, RGDSPASSLP, were added to the indicator cells prior to adding them to plates coated with the CB-Pep (CRP), resulted in significant enhancement of cell attachment activity (Fig. 3). By contrast, preincubation with the cell attachment peptide from laminin, YIGSR [Graf et al., 1987], did not influence cell attachment to the CB-Pep (CRP) (Fig. 3). The results suggest that an interaction occurs between the integrin receptor for Fn and the receptor that recognizes the CRP-derived peptide [Ruoslahti, 1991].

Effect of CRP-Derived Peptides on Cell Attachment to Fn

Although the RGD(S) sequence of Fn is not present in CRP, the activity of other cell attachment regions within Fn might be influenced by the CRP attachment peptide [Ruoslahti, 1988]. Therefore, the synthetic peptides from both CRP and SAP were tested for inhibition of cell attachment mediated by Fn alone. The CRP and SAP-derived peptides failed to significantly inhibit the cell binding activity of Fn when tested over the same range of concentrations of the inhibitory RGDS peptide (Fig. 4). Two other soluble CRP peptides, the PC-binding peptide and the C-terminal peptide, also failed to inhibit Fn-mediated cell attachment (Fig. 4). The "spreading activity" displayed by adherent cells that occurs subsequent to attachment to Fn was also not altered by incubating the cells with the peptides. The results suggest that the receptor for the CB-Pep of CRP or SAP is distinct from the integrin receptor for Fn [Albelda and Buck, 1990].

Cell Binding to Intact CRP and SAP Proteins

Cell-attachment activity was also evaluated with intact, purified pentameric human CRP and SAP to determine if the derived peptide was active in the context of the parent protein. Al-

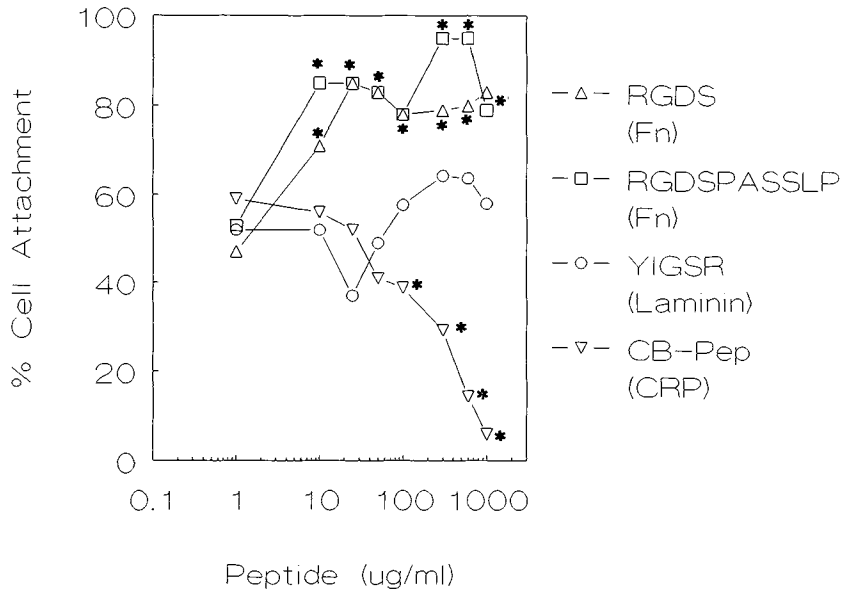

Fig. 3. Effect of cell-attachment peptides from the extracellular matrix proteins Fn and laminin on cell-binding activity to the CB-Pep of CRP. NRK fibroblasts were incubated with the competing peptide prior to allowing the cells to interact with CB-Pep (CRP). The * denotes significant inhibition or enhancement vs. the controls (no competing peptide).

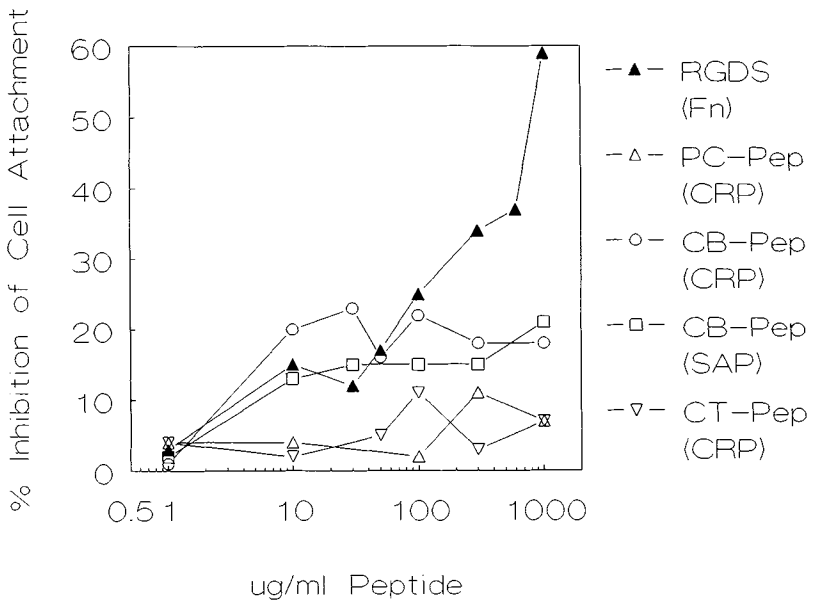

Fig. 4. Effect of CRP-derived peptides on the cell-attachment activity to Fn. Various synthetic peptides corresponding to surface regions of CRP were allowed to bind to NRK fibroblasts prior to testing them for attachment to Fn at 5 μg/well. The active peptide from Fn (RGDS) served as a control. The mean values from 3 experiments are shown. The SEM was < 15% for each of the mean values.

though plasma Fn mediated extensive cell attachment when used as a control, CRP also displayed cell-attachment activity by itself (Fig. 5). A comparison of the Fn (m.w. of 550,000) activity vs. that of CRP (m.w. of 120,000) on a molar basis reveals that the activity of CRP is approximately 1/20 of that displayed by Fn (Fig. 5). SAP mediated very little cell-binding activity under the same conditions. Since the purified CRP may have undergone some proteolysis and con-

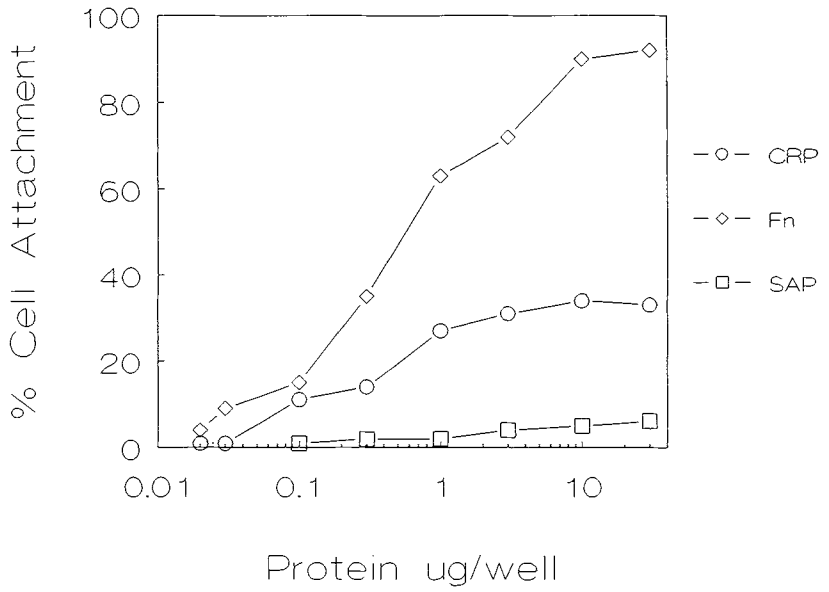

Fig. 5. Cell attachment activity to the purified Pentraxins, CRP and SAP. Various amounts of CRP and SAP were coated onto microplates and the cell-binding activity compared to that mediated by Fn under the same conditions. Values represent the mean from 4 identical experiments.

tain the active peptide, a recently isolated preparation of CRP obtained by PC-affinity chromatography was used. All CRP preparations consistently promoted cell-binding activity in a dose-dependent manner; however, the maximum binding activity was only 35% vs. >90% for Fn (Fig. 5).

Activity of the Cell-Binding Peptide of CRP Against Various Cell Lines

Several different established cell lines from different tissues and species were evaluated for their attachment activity to the CB-Pep (CRP). All of the adherent cell lines, independent of their origin, were capable of binding to the peptide (Table I). Nonadherent monocytic, granulocytic, T-cell, and B-cell lines failed to bind to the CB-Pep (CRP) coated wells; however, these nonadherent cells also failed to bind to Fn.

Effect of Antibodies to the Cell-Binding Peptide of CRP

A polyclonal rabbit antibody (IgG preparation) to the CB-Pep was not capable of neutralizing the cell-binding activity of the CB-Pep-BSA. The polyclonal antibody recognized the CB-Pep only when it was attached to the BSA carrier and therefore we generated several mouse mAb to the CB-Pep. The mAb were initially screened for the presence of the IgG isotype and reactivity with the CB-Pep (CRP) coated directly onto ELISA plates. Specific reactivity of the mAb with purified CRP, SAP, and the CB-Pep (CRP), but not with Fn or IgG, was used to select 5 IgG

TABLE I. Cell Attachment Activity of the CRP-Derived Cell-Binding Peptide Against Various Adherent Cell Types

Cell line/ cell type	Source/tissue	Cell attachment to CB-Pep (CRP) (%)[a]
NRK-49F, fibroblasts	Kidney	40
CHO, epithelial	Ovary	31
Primary fibroblasts	Human skin	60
Hep3B.2, epithelial	Human hepatoma	30
++/Li, epithelial	Mouse hepatoma	45
J774.2	Mouse macrophage	46
U-937	Human monocytic	< 1
HL-60	Human myelocytic	< 1
Jurkat	Human T-cell	< 1
Ramos	Human B-Cell	< 1

[a]CB-Pep (CRP) was tested at 20 μg/well in the microplate assay. Data are mean values from 2 experiments with each cell line. Cell lines obtained from ATCC, except for the diploid human fibroblasts.

TABLE II. Reactivity of Mouse Monoclonal Antibodies (mAb) to the Cell-Binding Peptide of CRP With Peptides and Serum Proteins

		ELISA reactivity (absorbance)[a]					
mAb	Isotype	CB-Pep (CRP)	CB-Pep (SAP)	CRP	SAP	Fn	IgG
D7.1	IgG_1	0.30	0.12	0.37	0.10	0.20	0.01
C11.1	IgG_{2a}	0.85	0.50	0.36	0.29	0.23	0.01
H4.1	IgG_{2b}	1.34	1.05	1.40	0.89	0.08	0.43
D4.1	IgG_{2a}	1.02	0.70	0.90	0.60	0.18	0.13
D12.1	IgG_1	0.75	0.46	0.56	0.28	0.09	0.72

[a]Absorbance at 550 nm using 10 μg/ml of purified mAb vs. 500 ng/well of peptide or 100 ng/well of protein.

mAb that were subsequently tested for their effect on cell attachment. All 5 of the mAb reacted not only with the CP-Pep (SAP), but also intact CRP and SAP; none of the mAb reacted with Fn, although H4.1 and D12.1 displayed weak reactivity with IgG (Table II). The mAbs designated H4.1 and D4.1 were able to inhibit the cell-attachment activity to the CB-Pep, whereas mAb D12.1, D7.1, and C11.1 did not (Table III). Additional testing of the 5 mAb capable of neutralizing CB-Pep mediated cell attachment against CRP-mediated cell attachment revealed that the 2 mAb active against the peptide were also capable of inhibiting cell-binding activity of CRP itself (Table III). The findings suggest that the CP-Pep sequence is on the surface of intact CRP and is responsible for at least some of its cell attachment activity.

TABLE III. Effect of Monoclonal Antibodies (mAb) to the Cell-Binding Peptide of CRP on Cell Attachment Activity*

	Cell attachment (%); mean (SD)[a]	
mAb	CB-Pep	CRP
None	38.1 (7.0)	32.8 (4.3)
H4.1	19.6 (2.1)	12.0 (5.7)
D4.1	24.0 (4.0)	15.5 (3.2)
D12.1	33.6 (8.1)	35.5 (4.9)
D7.1	48.8 (8.9)	38.3 (6.0)
C11.1	38.0 (3.5)	37.1 (6.9)

*CB-Pep was used at 10 ug/microplate well and CRP was coated at 10 ug/well. The mAb were tested at 10 ug/ml.
[a]Data are the mean (SD) values from 4 experiments. Significant inhibition ($P < 0.01$) occurred with the mAb:H4.1 and D4.1.

DISCUSSION

CRP is an acute phase reactant that becomes greatly elevated in the blood and is selectively deposited at sites of tissue destruction. Presumably, the CRP deposited at such sites is degraded to biologically active peptides since in vitro studies using neutrophils or neutrophil-derived proteases document cleavage of CRP into biologically active peptides [Robey et al., 1987; Shephard et al., 1990]. During the course of studies examining the specificity of the opsonic CRP-receptor on human monocytic cell lines [Tebo and Mortensen, 1991] we observed that the peptide bearing the first of three tuftsin-like sequences present in the CRP subunit had cell attachment activity by itself, that was not dependent on binding of the peptide to Fn or laminin. We previously reported binding of CRP itself to Fn and laminin [Tseng and Mortensen, 1988; Swanson et al., 1989]; however, the relative inefficient cell-binding activity of CRP when compared to Fn may explain the lack of any previous report of cell attachment activity for CRP. During the course of these experiments a report by Dhawan et al. [1990] was made that clearly demonstrated cell-attachment activity to a synthetic peptide from human SAP that possesses a similar, but not an identical, sequence. We show here that the 12 residue peptides from the same relative position in both the CRP and SAP molecules mediate nearly identical activity and are recognized by mAb generated against the CRP-derived peptide.

The issue of whether cell binding in vitro to synthetic peptides from the 2 serum pentraxins has physiological relevance should be considered in terms of the reported activities of the proteins. Since CRP, but not SAP, was capable of mediating cell attachment that was partially neutralized by mAb to the CP-Pep of CRP, and CRP is known to be found at sites of tissue destruction [Ballou and Kushner, 1992], we think it is likely that the cell-binding activity may occur in the milieu of other tissue repair events. The concentration of peptide or CRP

needed for this activity could easily be achieved locally at sites where it accumulates. Robey et al. [1987] show that μM or μg/ml concentrations of peptide elicit superoxide production and chemotactic activity with monocytes. These levels translate into CRP concentrations of 50 μg/ml, blood levels often achieved during the acute phase [Ballou and Kushner, 1992]. In addition, limited cleavage of CRP with leukocyte elastase could yield a peptide beginning at residue 25 (Pro) containing the 27–38 fragment. Whether such a fragment is actually generated in vivo and contributes to the biological activities attributed to the CRP-derived peptides from neutrophil degradation remains to be shown.

Several reports document the existence of specific receptors on PMNs [Muller and Fehr, 1986] and monocytes [Tebo and Mortensen, 1990; Ballou and Cleveland, 1991; Crowell et al., 1991]; however, none of the studies identify the region on the CRP ligand that is recognized by the receptor. In preliminary studies we reported that the synthetic CB-Pep (CRP) partially blocks receptor-mediated specific binding of ^{125}I-CRP to U937 cells [Mortensen et al., 1991]. We are currently investigating whether other surface regions of CRP are recognized by the leukocyte opsonic receptor. It is conceivable that distinct receptors mediate endocytosis of intact CRP and attachment to the cell-binding peptide [Tebo and Mortensen, 1991].

The relationship of the CRP CB-Pep sequence to peptides from Fn and laminin displaying similar activity was considered [Yamada and Kennedy, 1987]. A computer search using the 12 residue CB-Pep sequence of CRP did not reveal any homologous sequences in any other protein except SAP. The YIGSR sequence from laminin resembles the YIGR sequence at residues 71–74 of SAP (a sequence not present in CRP); however, this sequence in SAP was not active in cell attachment assays as shown by Dhawan et al. [1990]. It is worth noting that Dhawan et al. [1990] found that most of the cell-attachment activity of the SAP-derived dodecapeptide was contained in the hexapeptide from the C-terminal half, not the Tuftsin-bearing N-terminal half. Our results are consistent with this finding since the Tuftsin tetrapeptide from the N-terminus did not inhibit cell attachment. A comparison of the derived sequences for residues 27–38 of the cloned genes for various pentraxins listed below clearly reveals a conserved region:

Pentraxin	Sequence (Residues 27–38)
Human CRP	T K P L K A F T V C L H
Human SAP	E K P L Q N F T L C F R
Rabbit CRP	K K P L K A F T V C L Y
Mouse CRP	K K P L N T F T V C L H
Mouse SAP	E K P L Q N F T L C F R
Hamster FP	D K P L Q N F T V C F R.

The critical residues within this sequence have yet to identified; however, the conserved residues in the C-terminal portion include cys-36 that is thought to be part of the single internal disulfide bond with cys-97. Since the reduced and oxidized (dipeptide) forms of the peptide, as well as the CB-Pep covalently linked via the cys-36 to BSA, promoted cell attachment, we conclude that the peptide does not become covalently attached to the cells.

One noteworthy finding was that the addition of the RGDS containing peptides to the cells significantly enhanced cell-binding to the CB-Pep (CRP) suggesting some form of membrane activation or receptor-receptor interaction. If new synthesis of a receptor for the CB-Pep (CRP) is involved, a cooperative effect between receptors might contribute substantially to wound repair. Since the molecular characteristics of the receptor for the CB-Pep (CRP) are still unknown, it is difficult to speculate further on the nature of receptor interactions. Based on our previous partial characterization of the monocyte CRP-receptor as a noncovalently linked heterodimer [Tebo and Mortensen, 1990] we anticipate that if the receptor for the CB-Pep of CRP is the same as the leukocyte CRP-receptor, then the receptor might have the molecular properties of members of the integrin-receptor family [Ruoslahti, 1991].

The very unusual presence of 3 evenly spaced Tuftsin-like sequences within each of the CRP subunits at residues 27–30, 113–116, and 200–203 was first noted by Robey et al. [1987] and served as the basis for several studies of biological activities of CRP-derived peptides. Activities associated with the Tuftsin-bearing CRP-derived peptides are neutrophil and monocyte activation [Robey et al., 1987; Shephard et al., 1990] and platelet aggregation-release reactions [Fiedel, 1988]. However, in the cell attachment assays we were unable to block or increase the activity with the Tuftsin tetrapeptide, suggesting that tuftsin-like N-terminus of the CRP CB-peptide

is not involved. This conclusion is consistent with the reported leukocyte activation activity of Tuftsin, and the lack of any report of cell attachment activity by this tetrapeptide [Najir and Fridkin, 1989]. Since both the CRP and SAP peptides have the same cell attachment activity but not an identical sequence, that is conserved within the Pentraxin family, it seems likely that the conformation of the peptide will be critical for activity. Even if the active peptide is generated in limited amounts, the intact CRP deposited at inflamed sites may be in a conformation suitable for cell attachment.

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