Characterization of Antigenic and Cytotropic Properties of C-Reactive Protein Subunits

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UDC 616.155.34-008.939.6-02:615.155.32/=07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 12, pp. 609-611, December, 1993
Original Article submitted June 23, 1993

Key Words: *C-reactive protein; subunits; lymphocytes; neutrophils*

A considerable body of evidence indicates that C-reactive protein (CRP), a classical acute phase reactant, plays a role in nonspecific defense and acts as an endogenous immunomodulating factor [1,7, 8]. The biological activity of CRP subunits has been studied since 1987. In some pathologies CRP circulates as free subunits [6], the biological effects of which may differ from those of native CRP [2,9]. The spectrum of CRP activity is far from fully investigated. The purpose of this study was to compare CRP and its subunits by the following parameters: antigenicity, mitogenicity towards lymphocytes, and the ability to activate oxidative burst in human neutrophils.

MATERIALS AND METHODS

Electrophoretically homogeneous CRP isolated by ion-exchange chromatography on DE-32 and DE-52 cellulose (Whatman, Great Britain) from the ascitic fluid of cancer patients was used. The preparation of native CRP was immunochemically pure judging from the following findings: it was reactive with monospecific anti-CRP antiserum and unreactive with a wide range of other specific antisera, such as forensic medical antiserum to human serum proteins, antisera to IgA, IgM, IgG, α- and β-lipopolysaccharides, albumin, transferrin, C1q, and superoxide dismutase. The preparation purity was monitored by immunodiffusion, passive hemagglutination and its inhibition, Ovary, and rocket immunoelectrophoresis. In order to obtain soluble monomeric CRP (mCRP) native CRP, a pentamer (pCRP), was dialyzed against 0.14 M NaCl and treated for 1 min at room temperature with 0.36 M HCl (1/10 of the volume), which was neutralized with an equal volume of 0.36 M NaOH [10]. Antisera against pCRP and mCRP were prepared by rabbit immunization. Rabbits (2 for each preparation) were immunized with 400 μg of the proteins in complete Freund's adjuvant in the hind paw and boosted after 14 days by injection (200-250 μg protein without adjuvant) in the popliteal lymph nodes (0.2 ml in each node). Sera were prepared 14 days after the second injection and their specificities were estimated by passive

TABLE 1. Cross-Reactivity of Anti-pCRP and Anti-mCRP (Reverse Hemagglutination Titers)

Antiserum	Antigen to SE	
	mCRP	pCRP
Anti-mCRP (№ 911-1)	1920	320
Anti-mCRP (№ 911-2)	480	240
Anti-pCRP (№ 911-3)	15	960
Anti-pCRP (№ 911-4)	10	240
Anti-pCRP (№ 376)*	768	4096
Same, depleted with mCRP	64	2048
Same, depleted with pCRP	32	0

Note. * indicates commercial antiserum produced by the Institute of Vaccines and Sera, St. Petersburg.

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0007-4888/93/0012-1512$12.50

agglutination of sheep erythrocytes (SE) conjugated with mCRP or pCRP [11]. The conjugates were obtained by incubation (8 min at room temperature, all components prepared on normal saline) of sheep erythrocytes with CRP (about 200 μg/ml) and 2 mg/ml $CrCl_3 \times 6H_2O$ (Sigma, USA) followed by washing with phosphate-buffered saline (pH 7.4) containing 0.5% fetal calf serum (FCS, Institute of Vaccines and Sera, St. Petersburg).

Blast transformation was carried out in 96-well flat-bottomed plates (Medpolymer Plant, St. Petersburg) in RPMI-1640 medium (Flow, Great Britain) containing 10% FCS and gentamicin. The incorporation of ^{3}H-thymidine was evaluated by measuring radioactivity in a RackBeta counter (Pharmacia, Sweden). Oxygen metabolism in human neutrophils was assessed by reduction of nitro blue tetrazolium (NBT test) [2].

RESULTS

Splitting of CRP to monomers was performed by a modification of a method described elsewhere [10]. We replaced Tris-buffer with 0.14 M NaCl, which prevented the formation of insoluble precipitate during the exposure of CRP to pH 2.0. Transient opalescence of the solution was eliminated by neutralization. The subunit preparation contained no insoluble material. Monomeric and native CRP differed in electrophoretic mobility in 7% polyacrylamide gel (Reanal, Hungary) free of a reducing agent: mCRP migrated rapidly, while pCRP remained near the start zone. Both preparations produced a single band after gel staining with amido black 10B (Reanal).

Serological analysis showed that mCRP and pCRP have different antigenic properties (Table 1), which agrees with the finding that these proteins have only 16% antigenic homology [10]. The antiserum to mCRP reacted more actively with mCRP than with pCRP, and serum to pCRP was more reactive with the pentamer. Commercial donkey antiserum to CRP (Institute of Vaccines and Sera, St. Petersburg) contained predominantly anti-pCRP antibodies: its depletion with mCRP lowered the pCRP titer only 2-fold, while the mCRP titer was lowered more than 90% (Table 1).

Native CRP exhibited a mitogenic activity towards human lymphocytes, while its subunits were inactive in this respect, which may be related to their monovalency (Fig. 1). Monomeric CRP could bind to lymphocytes, judging from its ability to block the mitogenic effect of pCRP upon simultaneous inclusion with lymphocyte cultures. In the presence of PHA both mCRP and pCRP exhibited a comitogenic activity.

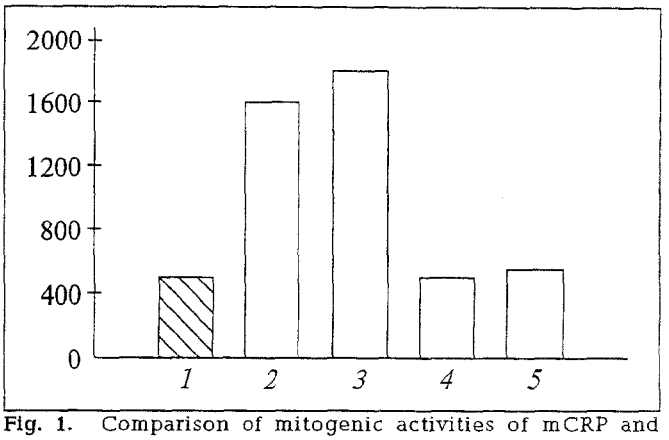

Fig. 1. Comparison of mitogenic activities of mCRP and pCRP towards human lymphocytes. Abscissa: *1*) native cells (control); *2*) pCRP, 100 μg/ml; *3*) pCRP, 200 μg/ml; *4*) mCRP, 100 μg/ml; *5*) pCRP+mCRP, 100 μg/ml each. Each bar represents the results of 9 experiments. Ordinate: ^{3}H-thymidine incorporation, cpm per 2×10^5 mononuclears.

At the same time, mCRP proved to be an active stimulator of neutrophils (Fig. 2). In the NBT test its stimulatory effect was higher than that of pCRP. Monomeric CRP was more active towards prodigiosan-stimulated and native neutrophils, its effective concentrations being an order of magnitude lower than those of PCRP.

Our results indicate that dissociation of native CRP to subunits leads to changes in the antigenic and biological activities of the protein. In contrast to native CRP, which stimulates both lymphocytes and neutrophils, mCRP cannot activate lympho-

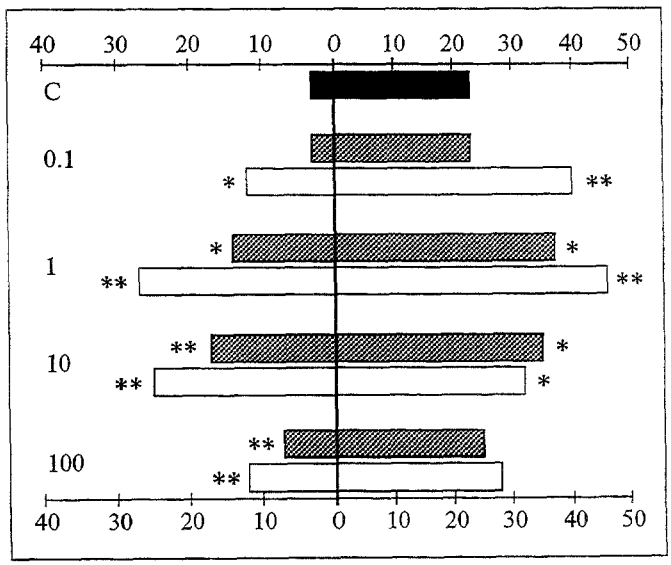

Fig. 2. Effects of mCRP and pCRP on the oxygen metabolism of neutrophils. *a*) native neutrophils; *b*) neutrophils stimulated with 12.5 μg/ml progidiosan. Horizontal line, left and right from zero: percentage of NBT-positive cells. C: control; numbers at left indicate CRP concentration in μg/ml. Each bar represents the results of 8 experiments. Shaded bars indicate pCRP, white bars indicate mCRP. One and two asterisks indicate statistically significant differences at $p<0.05$ and $p<0.01$, respectively.

cytes, while its activity towards neutrophils increases considerably. It can be concluded that the catabolism of CRP in the focus of inflammation gives rise to products with new immunoregulatory properties. Free subunits can be regarded as endogenous pH-dependent immunoregulatory agents, which differ from native CRP in a greater selectivity towards phagocytic reactions upon inflammation [3,4,5]. Presumably, CRP subunits are one of the factors that determine the alternation of phases in the inflammatory response.

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Effect of β-Carotene on the Development of Adjuvant-Induced Arthritis and Production of Interleukin-1 in Rats

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UDC 615.356:612.017

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 12, pp. 611-613, December 1993
Original article submitted June 25, 1993

Key Words: *β-carotene; interleukin-1; adjuvant arthritis*

Adjuvant-induced arthritis (AA) of rats, an autoimmune disease caused by subcutaneous injection of mycobacteria, accurately reflects the pathological changes observed in human rheumatoid arthritis. Although the role of immunological factors in the development of AA has been studied intensively, numerous aspects of this problem still remain unclear. The findings that mycobacterial heat-shock protein and rat proteoglycan (a constituent protein of articular cartilage) have identical amino acid sequences [14], on the one hand, and the discovery of a heat-shock protein reactivity of arthritic patients [13], on the other, confirm that the AA model is a valuable tool in the search for anti-inflammatory and antiarthritic drugs. At the present time, application not only of immunodepressive, but also of immunostimulatory agents seems quite reasonable [2]. This approach is based on the fact that an immunological dysregulation is developed in autoimmune diseases, i.e., despite the hyperreactiv-

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0007-4888/93/0012-1514 $12.50