Inhibitory Effect of C-Reactive Protein on Streptolysin O-Mediated Hemolytic Activity. Comparison of Conformational Variants

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It is shown that C-reactive protein binds to streptolysin O, an exotoxin of hemolytic streptococcus, and neutralizes its hemolytic activity. Incubation of C-reactive protein with the working dose of streptolysin O for 15-20 min at 37°C abolished the hemolysis of subsequently added erythrocytes. The concentration of C-reactive protein that reduced hemolysis by 50% was on average equal to 2.28±0.19 µg/ml. C-reactive protein antihemolytic activity was not affected by blocking of its phosphorylcholine-specific sites with free phosphorylcholine, but decreased as a result of blocking with pneumococcal C-polysaccharide and, particularly, with L-α-phosphatidylcholine. This indicates a hydrophobic nature of C-reactive protein-streptolysin O interaction. C-reactive protein subunits retained antihemolytic activity, while the aggregated C-reactive protein lost part of it.

**Key Words:** C-reactive protein; streptolysin O; erythrocytes

C-reactive protein (CRP) belongs to a group of hepatocyte-produced serum proteins which are induced by interleukin-6 during the acute phase of inflammation. CRP possesses opsonizing activity vis-a-vis certain microorganisms and injured autologous cells, and can exert various cytotoxic effects on neutrophils, macrophages, lymphocytes, and platelets [6]. Its main function is to eliminate foreign and autologous necrotized substances from the organism. Streptolysin O (SLO) is an exotoxin of β-hemolytic streptococcus. SLO can exert hemolytic, cytotoxic, and cardiotoxic effects [2]. It is the best studied representative of a group of related bacterial hemolysins that lose their activity after oxidation and restore it after treatment with thiol reagents [10]. SLO toxicity is realized via the formation of plasma membrane pores, through which cytoplasmic material from the target cell leaks [13]. SLO is neutralized by specific antibodies that are present in the serum of healthy donors and many animals; increased antibody titers are found in patients with acute rheumatism [1]. SLO is also inhibited by such plasma components as cholesterol [9], low density lipoproteins [14], and lysozyme [8]. Circulating immune complexes from patients with rheumatism contain CRP, along with SLO polypeptides [11], pointing to possible SLO-CRP interaction. However, this aspect has not been investigated, and the role of CRP in the composition of the circulating immune complexes remains unclear. In view of this the goal of the present work was an experimental study of CRP-SLO interaction and of the effect of the acute-phase reactant on the toxin-mediated hemolytic activity.

**MATERIALS AND METHODS**

CRP was obtained from ascitic fluid of cancer patients. The methods of CRP isolation and puri-
fication, control of homogeneity, and immunochemical purity, as well as of dissociation into subunits have been described earlier [3]. Thermoadmixed CRP was obtained by heating the native product at 63°C for 5 min. Commercial SLO preparation was used according to the manufacturers’ directions (Research Institute of Vaccines and Antisera, St. Petersburg); the titration of anti-SLO activities of the substances under study was performed in the same way. The only modification was diminishment of the reagent volumes due to the use of 96-well microplates (Medpolymer, St. Petersburg). Erythrocytes were obtained from the venous blood of healthy donors. Interaction between SLO and CRP was recorded in a solid-phase immunoenzyme assay. SLO was adsorbed in polystyrene flat-bottom 96-well plates that were precoated with cholesterol (Serva) placed in the wells as 0.1 mM ethanol solution and dried, or in untreated polystyrene plates. Wells were washed with phosphate-buffered saline, pH 7.2, followed by blocking of plastic surface with 2% bovine serum albumin solution (Gibco). SLO-coated wells were filled consecutively with native CRP solution (300 ng/ml) in buffered saline with subsequent incubation at 37°C for 2 hours, antiserum to human CRP (Research Institute of Vaccines and Antisera, St. Petersburg), and protein A-peroxidase conjugate (Research Institute of Experimental Medicine, St. Petersburg) with intervening washings before the addition of each new reagent. O-phenylenediamine mixed with H₂O₂ served as a substrate for the reaction development. The results were recorded on a Linkey comparator (Ladoga Research and Manufacturing Conglomerate) in a single-wave regime at 405 nm. We also used human anti-influenza γ-globulin (10% solution in ampoules, Research Institute of Experimental Medicine, St. Petersburg), lysozyme (Reanal), phosphorylcholine chloride (Sigma), egg L-α-phosphatidylcholine (EPC; Serva), and pneumococcal C-polysaccharide (Reanal).

RESULTS

The results of an immunoenzyme assay of SLO-CRP interaction (Fig. 1) attest to the binding of acute-phase protein with the toxin. The interaction is dose-dependent and proceeds more efficiently in the uncoated wells. In the cholesterol-precoated wells the specific binding is lower, probably due to the blocking of SLO hydrophobic sites that are also necessary for the interaction with CRP.

The effect of pure CRP preparation on the hemolytic properties of SLO was studied using routine assays of anti-SLO activity in patient sera. As can be seen in Fig. 2, preincubation of the standard dose of SLO with CRP (37°C, 15 min) led to an abolition of the hemolytic effect, i.e., to toxin neutralization. The CRP concentration reducing the hemolytic effect of the standard dose of