Structure of C-reactive Protein Excreted in Urine during Acute Rejection Episodes

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During acute rejection of renal allografts C-reactive protein is excreted in urine in a monomeric form. We developed an immunochemical method for detecting monomeric C-reactive protein in human physiological fluids, which is based on latex agglutination.

Key Words: *acute rejection episode; excretion in urine; C-reactive protein; monomeric C-reactive protein; latex agglutination*

The urinary concentration of C-reactive protein (CRP) sharply increases during acute rejection of renal allografts, which serves as a diagnostic criterion in clinical practice [8]. Our previous studies showed that urinary concentration of CRP during acute rejection episodes increases to 400-20,000 ng/ml within several hours (normal <50 ng/ml) [1]. It should be emphasized that serum concentration of CRP remains practically unchanged.

The mechanisms underlying this process and the structure of excreted CRP during acute rejection of renal allografts remain unclear. Previous studies showed that CRP exists in native (pentameric) and monomeric forms (neo-form or modified form, neo-CRP) differing by their structure and functional activity [3,9]. In humans native CRP containing 5 noncovalently bound subunits is soluble and present primarily in the serum. Modified CRP is characterized by lower solubility and is associated with peripheral blood lymphocytes, natural killer cells, and B cells [9] and, according to some recent reports, with vascular walls [4].

Glomerular membranes retain molecules with a diameter of >6.4 nm. As differentiated from pentameric CRP (10 nm), monomeric form of CRP with a diameter of 3.3 nm can enter the nephron [7].

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We hypothesized that CRP in the urine of patients with acute rejection of renal allografts is presented by the monomeric form. This work was designed to confirm this hypothesis.

MATERIALS AND METHODS

Native CRP (electrophoretic purity >98%, Imtek, Moscow) was used. Denatured CRP was obtained as described elsewhere [6]. The solution of CRP in phosphate buffered saline (0.1 mg/ml) was twice frozen and defrosted (neo-CRP), 450 μl 8 M urea and 0.005 M ethylenediaminetetraacetic acid were added to 50 μl 1 mg/ml CRP, and the mixture was incubated at 37°C for 2 h. Excess urea was removed by dialysis against phosphate buffered saline (neo-CRP*). Affinity sorbents for the isolation of antibodies against CRP and neo-CRP were obtained routinely by covalent binding to BrCN-activated Sepharose. The sorbent with native CRP was treated with 2.5% glutaraldehyde at 37°C for 1 h under mixing. Polyclonal antibodies against neo-CRP and native CRP were obtained from blood serum of immunized rabbits by affinity chromatography. Latex particles with antibodies against neo-CRP and native CRP (neo-CRP-latex and CRP-latex) were prepared as described previously [1]. Enzyme immunoassay (EIA) of CRP in the serum and urine was performed [2].

Serum and urine samples from patients with acute rejection episodes were obtained from the Department

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of Transplantation of the Kidney and Pancreas (Institute of Transplantation and Artificial Organs). We examined 12 patients with increased urinary concentrations of CRP (>400 ng/ml).

RESULTS

The sensitivity of latex reagents with antibodies against neo-CRP was determined using native and denatured CRP. A comparative study was performed using the latex reagent and included EIA with antibodies against native CRP. Minimum concentration of the preparation causing agglutination of latex particles reflected sensitivity of the latex reagent. Minimum concentration of the preparation whose optical density more than 1.5-fold surpassed the baseline level was taken as sensitivity of CRP-EIA. In our experiments the neo-CRP-latex reagent was 4-fold more potent in recognizing neo-CRP than native protein (Table 1), which is consistent with published data. Experiments with monoclonal antibodies showed that native pentameric molecule of CRP carries at least 5 surface antigenic epitopes, while neo-CRP carries only 3 epitopes. It should be emphasized that one epitope is common for both forms [10]. We revealed that neo-CRP* exhibits no antigenicity typical of CRP, which disagrees with published data. Probably, treatment with 8 M urea leads to pronounced denaturation of CRP molecules.

Latex reagents with antibodies against CRP and neo-CRP were used to estimate CRP titers in the serum and urine from patients with acute rejection of renal allografts. The mean CRP/neo-CRP ratios in the serum and urine were 3.6 and 0.5, respectively. Our results indicate in the urine of patients with acute rejection of renal allografts C-reactive protein in the monomeric form (Table 2).

TABLE 1. Sensitivity of Latex Method and EIA with Native and Denatured CRP (ng/ml)

Method	CRP	Denatured CRP	
		neo-CRP	neo-CRP*
neo-CRP-latex	200	50	—
CRP-latex	50	100	—
CRP-EIA	5	5	1500

CRP is selectively accumulated in the urine of these patients (compared to other serum proteins). Our observations showed that urinary concentration of CRP in patients with acute rejection episodes can reach the level observed in the serum. It should be emphasized that the total protein content in the urine rarely surpasses 20% of its serum concentration.

Selective accumulation of CRP in the urine during acute rejection episodes probably plays a certain physiological role. *In vitro* experiments showed that neo-CRP intensifies platelet aggregation and stimulates and/or modulates human monocytes and neutrophils [5,11].

The mechanism underlying urinary excretion of CRP during acute rejection of renal allografts remains unclear. Local inflammation of the glomeruli during acute rejection episodes is accompanied by deformation of the basal membrane and impairment of normal filtration. Native CRP can migrate from systemic circulation into the renal glomerulus and dissociate into subunits under the influence of local physical factors. In many patients acute rejection episodes are accompanied by anuria, when 24-h urine volume does not surpass 100-300 ml. Glomerular filtration practically does not occur, and urine appears like renal tissue effusion. These data and selective accumulation of

TABLE 2. CRP Titer in Serum Samples and Urine Estimated with Latex Reagents

Patient, code	Blood serum			Urine		
	CRP-latex	neo-CRP-latex	CRP/neo-CRP	CRP-latex	neo-CRP-latex	CRP/neo-CRP
01	1:400	1:100	4.0	1:200	1:380	0.5
02	1:800	1:200	4.0	1:128	1:256	0.5
03	1:100	1:32	3.1	1:4	1:8	0.5
04	1:400	1:100	4.0	1:200	1:256	0.8
05	1:100	1:32	3.1	1:2	1:4	0.5
06	1:1600	1:400	4.0	1:4	1:8	0.5
07	1:400	1:100	4.0	1:32	1:64	0.5
08	1:400	1:100	4.0	1:8	1:20	0.4
09	1:200	1:64	3.1	1:4	1:8	0.5
10	1:100	1:32	3.1	1:16	1:32	0.5
11	1:400	1:100	4.0	1:32	1:64	0.5
12	1:200	1:64	3.1	1:8	1:16	0.5

CRP in urine suggest that the appearance of CRP in the urine during acute rejection episodes is realized via the intrarenal mechanism. Modified CRP is probably synthesized by immunocompetent cells in the kidney during acute phase. Observations performed in the 1980s showed that although the liver is a major source of CRP, several subpopulations of peripheral blood lymphocytes can synthesize this protein [3,9].

Further investigations are required to evaluate the mechanism underlying urinary excretion of CRP during acute rejection of renal allografts. Immunohistochemical assay of slices from rejected renal allografts holds much promise in this respect.

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