STATE OF SOME INDICES OF NONSPECIFIC IMMUNITY IN RATS WITH NEPHROTOXIC GLOMERULONEPHRITIS

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Changes in the interferon response of leukocytes, the bactericidal properties of the blood serum against Escherichia coli and Streptococcus, and activity of β-lysins were studied in experimental nephrotoxic glomerulonephritis produced in noninbred male rats by means of heterologous nephrotoxic serum. With this, experimental model changes in the nonspecific defense mechanisms depending on the pathological process in the kidneys could be studied in isolation. In rats with nephrotoxic glomerulonephritis the most marked changes occurred in the interferon response of the leukocytes. It was reduced by up to 90% of the normal level depending on the duration of the disease. By contrast, a change in the indices of antibacterial immunity was observed during the period of marked activity of the nephritic process.

KEY WORDS: experimental nephrotoxic glomerulonephritis; interferon response of leukocytes; bactericidal properties of blood serum; β-lysins.

Indices of nonspecific defense of the body play an important role in the development and course of infectious diseases [4-6, 13]. In glomerulonephrities, in the etiology of which both infectious and allergic factors play an important role, the state of nonspecific defense has been inadequately studied. Many workers [1, 3, 7-10] studying this pathological entity, especially in its nephrotic and mixed forms, have observed a decrease in the bactericidal and lysozyme activity of the blood serum, in the phagocytic activity of the leukocytes, and the properdin and complement titers and a raised β-lysin level.

No results of the investigation of the interferon response of the leukocytes in glomerulonephritis could be found in the accessible literature. The writers' previous investigation of 95 children with glomerulonephritis revealed a reduction in the interferon response of the leukocytes and antibacterial activity of the blood serum which was particularly well marked in the nephrotic and mixed forms of the disease.

To understand the immunological genesis of nephritis, the study of the indices of nonspecific immunity in experimental glomerulonephritis produced with the aid of heterologous nephrotoxic serum without the involvement of the infectious factor is of undisputed interest. In experiments on rats with this model the changes in the state of nonspecific immunity in nephritis depending on the pathological process in the kidneys can be studied in isolation. The investigation described below was carried out for this purpose.

EXPERIMENTAL METHOD

Noninbred male rats (71) were used. Nephrotoxic glomerulonephritis was produced in 41 of them by Masugi's method with certain modifications [14], and the remaining 30 rats acted as the control. The nephrotoxic serum was obtained by repeated immunization of rabbits with a homogenate of the cortical layer of perfused rats' kidneys. To produced glomerulonephritis, the serum (titer 1:1200) was injected repeatedly intravenously and subcutaneously into rats in doses of 0.9-1.0 ml/100 g body weight for 3-5 days.

During the course of the experiments, systematic observations were made on the protein excretion by the kidneys, the diuresis, and the pH and residue of the urine. Blood nonprotein nitrogen and proteins were studied after death. The general state of the animals, their behavior, body weight, and edema were taken into consideration.

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TABLE 1. Results of Laboratory Biochemical Tests in Experimental Nephrotoxic Glomerulonephritis in Rats (M ± m)

Duration of nephritis	Number of rats	Protein in urine (in %)	Nonprotein nitrogen (in mg %)
10 days	25	17,11±3,6	39,0±2,5
P		<0,001	<0,05
20 days	6	2,5±0,25	35,0±4,06
P		<0,05	>0,05
3 months	10	1,46±0,33	34,0±3,9
P		<0,05	>0,05
Control	30	0,45±0,05	29,3±0,41

Among the indices of nonspecific immunity, the interferon response of the leukocytes (IRL) was determined by titrating interferon in primary cultures of rat embryonic fibroblasts [12] and the β-lysin level and bactericidal activity of the blood serum against Escherichia coli (strain F_{25}) and Streptococcus (strain 80) also were investigated. The values of these indices were determined by a nephelometric method [2, 11].

To study the dynamics of the indices of nonspecific immunity blood was taken from the subclavian vein of the rats under ether anesthesia on the 10th and 20th days and 3 months after the beginning of injection of the nephrotoxic serum. The kidneys of the killed animals were examined histologically.

EXPERIMENTAL RESULTS

The indices of nonspecific immunity during glomerulonephritis of up to 10 days in duration were studied in 25 rats. By this time the clinical symptoms of nephritis were clearly marked. Edema of the subcutaneous cellular tissue was observed in the rats and in 16 of the 25 animals anasarca was present. Hypo- and dysproteinemia, with corresponding changes in the A/G ratio, were found in the blood. The serum nonprotein nitrogen was not substantially altered except in a few rats in which it reached 43-50 mg % (Table 1). The urinary proteins ranged from 0.9 to 90 g/liter, over 9 g/liter in most of the rats, the diuresis fell to 0.13 ml/h from a normal value of 0.45 ± 0.05 ml/h, and large numbers of leukocytes and epithelial cells appeared in the residue of the urine.

The morphological picture of the changes in the kidneys corresponded to the acute stage of proliferative-membranous glomerulonephritis. Most of the glomeruli were enlarged and showed marked proliferation of the endothelial and mesangial cells. The basement membranes of the capillary loops of the glomeruli were loose in texture and uniformly thickened. Moderate cloudy swelling and vacuolar degeneration were visible in the epithelium of the proximal convoluted tubules.

The titer of leukocytic interferon varied between dilutions of below 4-64, with a geometric mean value 90.6% below the index for healthy rats (Table 2). The β-lysin level was raised a little (to 56.7 ±2.8%, normally 52.7%), but the antibacterial activity of the blood serum against E. coli and Streptococcus was reduced to 70.3 and 24.4%, respectively, compared with 90.5 and 50.3% in the control group.

The indices of nonspecific immunity in glomerulonephritis with a duration of up to 20 days was studied in 6 rats. By this time the intensity of the clinical manifestations of glomerulonephritis had subsided. In most rats the edema was slight or absent. The urinary protein concentration varied between 1.2 and 3 g/liter. The nonprotein nitrogen in some rats was 45 mg % (Table 1) and the hypo- and dysproteinemia were less severe.

In many glomeruli of the kidneys fibrous replacement of the tubular part of the nephrons was observed morphologically and some of the tubules were atrophied. The basement membranes of the capillary loops of the glomeruli were thickened. The arterioles had thickened, sclerotic walls with hyperplasia of their elastic components.

The titer of leukocytic interferon in all the rats of this series was at the minimal level below 4 units (Table 2). The β-lysin activity was very slightly raised, to 54.9%. The bactericidal activity of the blood serum against E. coli and Streptococcus was 81.4 and 29.6%, respectively, a reduction of 11 and 42% compared with the control.

TABLE 2. Indices of Interferon Response of Leukocytes in Rats with Experimental Nephrotoxic Glomerulonephritis

Duration of nephritis	Number of rats	Titers of leukocytic interferon (in units; M ± m)	Decrease in per cent
10 days	25	1,59±1,2	90,6
Control	20	168,7±1,0	
20 days	6	0	100
Control	4	315,0±0,2	
90 days	10	3,99±0,9	95,3
Control	6	84,4±1,3	

Legend. Differences between indices for experimental groups were not significant ($P > 0.05$); differences between experimental and control groups were significant ($P < 0.01$).

The indices of nonspecific immunity in glomerulonephritis with a duration of up to 3 months (90 days) were studied in 10 rats. Clinical manifestations of nephritis in these rats were absent or very slight. The urinary protein concentration was between 0.3 and 3 g/liter (Table 1). No changes in the other indices studied could be found. Morphologically, some degree of thickening of the basement membranes was visible in the glomeruli. Some of the vascular loops of the glomeruli were sclerosed. Most of the convoluted tubules were unchanged, but a few showed cystic dilatation.

The leukocytic interferon titer in the rats of this series also was on a low level, below 4 units in most cases, i.e., reduced by 95.9% compared with the control. The β-lysin activity was very slightly raised, to 54.2%; the bactericidal activity of the blood serum against E. coli and Streptococcus was 84.4 and 37.8%, a decrease of 7 and 25%, respectively, compared with the control.

In rats with nephrotoxic glomerulonephrities the indices of nonspecific immunity (the interferon response of the leukocytes, the bactericidal activity of the blood serum) were lowered. The most marked changes affected the interferon response of the leukocytes. This was lowered not only in the acute phase of the disease (10 days), but more especially on the 20th day, and it still remained low on the 90th day, when the specific morphological process in the kidneys and the clinical manifestations of glomerulonephritis had subsided. By contrast, a reduction in the indices of antibacterial immunity was observed during the period of marked activity of the nephritic process.

The results suggest that the lasting depression of the interferon response of the leukocytes may be the most characteristic feature of the change in the nonspecific immunological response during glomerulonephritis and attributable to the functional and morphological changes in the kidneys in this pathological state. The duration of the change in the interferon response of the leukocytes was much greater than the duration of the clinical manifestations of the disease.

In the light of these observations showing depression of the interferon response of the leukocytes in glomerulonephritis, it is possible to explain clinical observations of a high incidence of nephritis among children with intercurrent virus infections.

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CROSS-REACTING THYMUS AND BRAIN ANTIGENS IN THE CEREBRAL CORTEX

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Rabbit antisera against antigens of whole mouse, rabbit, guinea pig, and human brain were found to have a cross cytotoxic action on lymphocytes of the thymus, lymph nodes, and spleen of the animals of these species. Mouse thymus cells were the most sensitive (index of cytotoxicity 63-100%); the cells of other mouse lymphoid organs and lymphocytes of the other species of animals and man were more resistant. Bone marrow cells were not injured by any serum. Antigens responsible for the cytotoxic properties of the sera were found to be located in the human cerebral cortex and to be absent from the white matter and the brain stem.

KEY WORDS: thymus; cerebral cortex; cross-reacting antigens.

Species-specific O-antigenic correlations between the mouse brain and thymus were first described by Reif and Allen [12]. Later similar correlations were discovered in rats [13]. We now know that species-specific and cross-reacting antigens are present in the brain and thymus of many species: rats [4, 11, 13-15], mice [4, 6, 12, 14, 15], and birds [5]. The brain of most species of animals contains antigens in common with antigens of mouse thymocytes [7]. Meanwhile the problem of which brain components, i.e., the gray or white matter, are more closely connected with these antigens has so far received little study [3, 9].

The object of this investigation was to examine this problem.

EXPERIMENTAL METHOD

Antisera against whole brain tissue of CBA mice, guinea pigs, and man and also of the various parts (cortex, brain stem, white matter) of the adult human brain were obtained from rabbits weighing 2-2.5 kg after immunization with brain homogenates together with Freund's complete adjuvant [2]. Titers of antibrain antibodies were determined by the complement fixation test in the cold [1] with the antigens used for immunization. Sera with titers of 1/160-1/320 were used. The sera thus obtained were heated to 56°C for 30 min and absorbed with liver homogenates and erythrocytes [5] of the species of animal or man relative to which the cytotoxic activity of the sera was subsequently to be tested. The tests were carried out by the method of Niederhuber and Möller [10] against thymus, lymph node, spleen, and bone marrow cells of the corresponding species of animal or against human lymph nodes, spleen, and bone marrow cells. The cell population of human lymphocytes before testing was enriched with T cells by passage through a cotton wool column (1 g:200 mg), previously washed with medium No. 199. The viability of the cells was estimated with a 0.2% aqueous solution of Trypan Blue.

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