ENZYME ACTIVITY OF BLOOD LEUKOCYTES IN RATS WITH EXPERIMENTAL NEPHROTOXIC NEPHRITIS

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Enzyme activity of the blood leukocytes in Masugi's nephritis was studied in rats by a quantitative cytochemical method. Injection of the nephrotoxin during the first hours and days did not cause specific changes in enzyme activity. These changes were greatest on the 8th day, when, besides the severest histological changes in the kidneys, a decrease in the complement titer, and the presence of fixed nephrotoxin and autoglobulin in the glomeruli, increased dehydrogenase activity of the lymphocytes and acid phosphatase activity of the neutrophils were observed. In the later stages the histological changes became less marked and enzyme activity fell. In animals with proteinurea the acid phosphatase activity of the neutrophils was higher than in animals without proteinurea.

KEY WORDS: *Masugi's nephritis; leukocytes; enzyme activity*

Masugi's nephritis is one of the principal experimental models reproducing diseases of the kidneys in animals that closely resemble human glomerulonephritis in their pathogenetic and morphological picture. The hypothesis of the autoimmune nature of this disease has become almost generally accepted, and in many investigations into the pathogenesis of nephrotoxic nephritis attention has been concentrated on immunologic responses connected with the production of anti-organ antibodies. Relatively few studies have been made of the role of leukocytes in the development of kidney diseases [7, 8, 11, 12]. As a rule these investigations have been morphological in character and have not reflected the functional state of the cells participating in the pathological process. One parameter of this state is the enzyme activity of the cells.

The object of this investigation was to study enzyme activity of the blood lymphocytes and neutrophils during the development of Masugi's nephritis in rats.

EXPERIMENTAL METHOD

Masugi's nephritis was produced in 56 noninbred albino rats weighing 190-220 g by intravenous injection of nephrotoxic serum obtained by immunization of rabbits with a suspension of previously perfused rat kidneys. The titer of antikidney antibodies in the serum in the passive hemagglutination test (PHT) was 1:2560, and in the complement fixation test (CFT) 1:1280.

Blood was taken from the caudal vein before and at various times after injection

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TABLE 1. Cytochemical Indices of Enzyme Activity in Lymphocytes and Neutrophils after Injection of Nephrotoxic Serum in Rats with and without Proteinurea (M ± m)

Day after injection of serum	Rats without proteinurea					Rats with proteinurea				
	number of animals	lymphocytes			neutrophils	number of animals	lymphocytes			neutrophils
		SD	α-GPD	AP	AP		SD	α-GPD	AP	AP
Before injection	9	13,4±0,79	5,58±0,69	47,5±5,03	139,0±22,1	14	12,3±0,64	5,05±0,36	42,0±3,84	128,0±23,2
8th	4	15,5±2,32	5,85±1,26	42,2±13,2	225,5±28,1	6	16,4±0,61	5,64±0,49	39,2±3,52	266±43,0
10th	7	7,73±0,83	3,03±0,49	60,1±8,19	183,1±31,6	9	9,84±1,14	3,89±0,75	42,0±7,28	191,0±37,8
13th	7	13,9±0,94	5,11±0,28	45,4±7,7	105,0±19,6	9	14,5±0,73	5,47±0,74	59,4±6,80	142,0±14,8
20th	8	11,0±0,63	6,37±1,08	43,6±6,5	59,0±11,2	11	12,2±0,46	6,01±0,47	39,0±8,3	161,0±33,0
28th	6	11,1±2,92	4,14±1,34	43,6±10,5	105,0±20,5	9	11,5±1,11	3,15±0,34	51,6±8,8	158,0±15,1

of the nephrotoxin. Some animals were killed at the same times for histological and immunofluorescence analysis. On the 14th-16th day the urine of the surviving animals was tested for the presence of proteinurea. The complement titer and circulating antibodies against nephrotoxin and against rat kidney tissue were determined in the blood serum (PHT and CFT).

During cytochemical analysis of the blood cells acid phosphatase (AP; phosphohydrolase of orthophosphoric acid monoesters, 3.1.3.2) was studied in the lymphocytes and neutrophils by the azo-coupling method [9], and the succinate dehydrogenase [SD; succinate: (acceptor)-oxidoreductase, 1.3.99.1] and α-glycerophosphate dehydrogenase [α-GPD; α-glycerol-3-phosphate: (acceptor)-oxidoreductase, 1.1.2.1] activity of the lymphocytes was determined by a quantitative cytochemical method [4]. AP activity was expressed by Kaplow's index [10]. Activity of the dehydrogenases was expressed as the number of formazan granules per lymphocyte.

EXPERIMENTAL RESULTS

Morphological investigation of the kidneys of the animals revealed the development of focal or diffuse proliferative-membranous glomerulonephritis 24 h after injection of the nephrotoxic serum. At the same time, groups of lymphocytes and histiocytes were found around the dilated lymphatics. The most marked changes, consisting of diffuse proliferative-membranous glomerulitis and tubular degeneration, were found on the 8th-10th day after injection of the nephrotoxin. A gradual decrease in severity of the changes in the kidneys was observed later.

At all times of the investigation starting from 24 h intensive fluorescence of the capillary membranes of the glomeruli was observed in the kidneys of the experimental rats by the use of labeled antiserum revealing nephrotoxins. With the course of time the intensity of fluorescence decreased. Fluorescence of the glomeruli of the kidneys with labeled rabbit antiglobulin (rat) serum was found only on the 8th-9th day after injection of the nephrotoxic serum. Meanwhile the complementary activity of the animals' blood serum decreased at this time.

Circulating antibodies against nephrotic serum and against rat kidney antigen (PHT and CFT) were not found at any time of the investigation (starting from the 1st week after injection of the nephrotoxin).

Cytochemical analysis of the blood cells during the first few hours and days after injection of the nephrotoxin revealed synchronous changes in the activity of all enzymes studied: after 2 h their activity was lowered, it was increased after 24 h, and was particularly high after 48 h. The AP activity of the neutrophils and lymphocytes at this time was higher than initially, but the dehydrogenase activity was back to

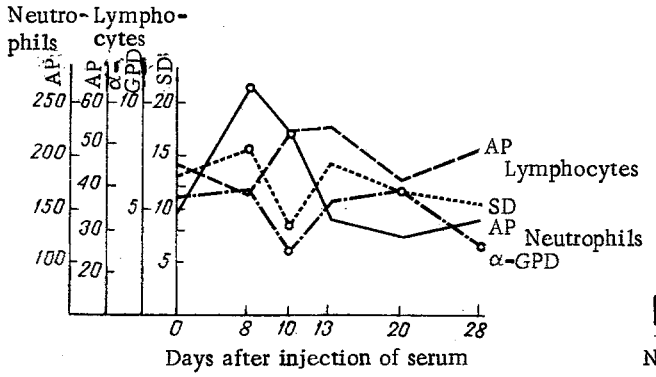

Fig. 1

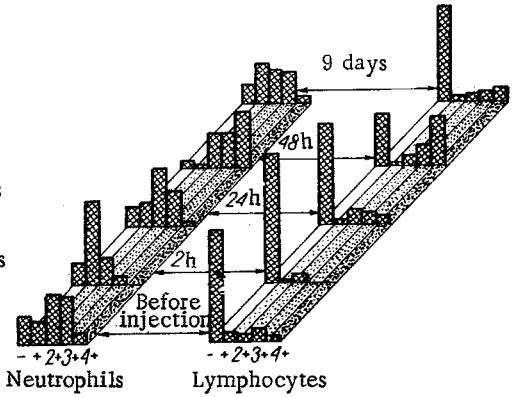

Fig. 2

Fig. 1. Activity of SD, α-GPD, and AP in lymphocytes and neutrophils of rats after injection of nephrotoxic serum (general group). Statistically significant changes in enzyme activity ($P < 0.05$) marked by circles.

Fig. 2. Histograms of distribution of neutrophils and lymphocytes by AP activity at various times after injection of nephrotoxic serum. Here and in Fig. 3, different intensities of shading correspond to intensities of reaction for acid phosphatase.

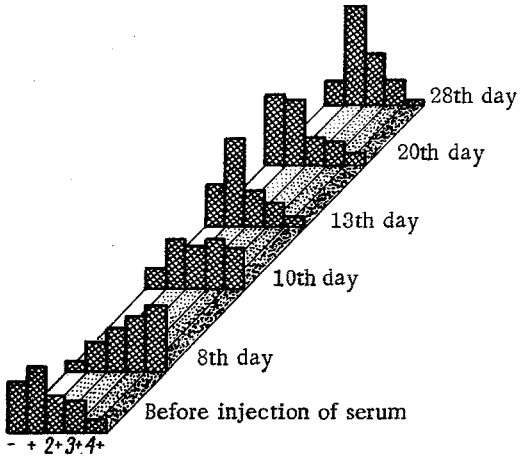

Fig. 3. Histograms of distribution of neutrophils by AP activity at late stages after injection of nephrotoxic serum.

its initial level. To determine the possible specificity of these changes, the indices obtained were compared with those for control animals receiving physiological saline. The results showed that the dynamics of enzyme activity of the blood cells was similar for the experimental and control animals. However, curves reflecting these changes in the rats of the experimental group gave larger variations and these were observed at later periods than in the control group.

Injection of nephrotoxin thus caused no specific change of enzyme activity in the white blood cells during the first hours or days. The dynamics of enzyme activity revealed was evidently the result of the generalized adaptation syndrome (GAS) accompanying any considerable procedure acting on the organism [5, 6]. The identity of the physiological changes taking place during the period of GAS during exposure of the organism to antigenic and nonantigenic stimuli has been demonstrated on several occasions [1-3].

Analysis of enzyme activity at later periods (Fig. 1) showed that after the rise in SD activity on the 8th day it fell, especially on the 10th day. Similar changes were observed in the activity of α-GPD. At no time of observation was a significant change found in the AP activity of the lymphocytes. More profound changes characterized the AP activity of the neutrophils: on the 8th and 10th days after injection of the nephrotoxic serum it rose sharply and then fell.

Injection of the nephrotoxic serum caused proteinurea in not all the animals. Since proteinurea is an indicator of a more severe lesion of the kidneys, it was interesting to analyze the enzyme activity separately in animals with and without proteinurea. The results showed a tendency for a lower initial activity of all the en-

zymes studied in the animals with proteinurea than in rats in whose urine no protein was found (Wilcoxon's criterion; $P < 0.05$).

No significant differences in SD and α-GPD activity could be found at any time after injection of the nephrotoxin between the groups compared. In rats with proteinurea a higher rise of AP activity in the lymphocytes could be seen on the 13th day. Compared with the group of rats without proteinurea, a higher level of AP activity in the neutrophils was found in these same animals (the difference was statistically significant on the 20th day after injection of nephrotoxin).

The increase in total AP activity of the neutrophils in the cell population took place as a result both of an increase in the number of cells containing the enzyme and of a change in the enzyme activity of those cells. As Fig. 2 shows, 2 h and 9 days after injection of nephrotoxin the total number of neutrophils with active AP was virtually the same (84 and 85% respectively), but the percentage of cells with high activity (3+ and 4+) differed sharply at these times: 5.9% after 2 h and 30.6% after 9 days. As will be clear from Fig. 3, by the 8th day there was a significant redistribution of cells by AP activity and the greatest number of neutrophils belonged to the group with high phosphatase activity. The appearance of the histogram on this day was a mirror image of that before the injection of nephrotoxin. Later a gradual return of the population structure to normal was observed, but even by the 28th day, despite restoration of normal enzyme activity, the distribution of cells with reactions of different intensities still differed from its initial pattern. However, the question remains open whether any connection exists between the level of hydrolase activity of the neutrophils and the degree of damage to the kidney tissue. On the basis of the results of analysis of correlation between these indices, no unambiguous answer can be given because of the small number of observations and the absence of significance of the correlation coefficient. Nevertheless, the much higher phosphatase activity of the neutrophils in rats with proteinurea (i.e., in animals with more severe kidney damage) can be considered to be the result of a definite role of these cells in the development of nephrotoxic nephritis. This fact has been established by a series of studies, consisting of morphological investigations of the damaged kidney and experiments in which nephrotoxic nephritis was induced in animals receiving antipolymorphonuclear serum [7, 8, 11, 12]. The present cytochemical analysis confirms this hypothesis to some extent.

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