MASUGI'S NEPHRITIS FROM THE AGE ASPECT

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Masugi's nephritis was induced in Wistar rats aged 3 months and 6 and 3 weeks. Identity of the intensity and duration of fixation of nephrotoxic antibodies in the renal glomeruli was established irrespective of the animals' age, and fixation was accompanied by a lowering of the serum complement level. The disease followed the severest course in the rats aged 6 weeks. The proliferative-membranous glomerulitis which they developed after 48 h increased in severity and was accompanied by the development of autoimmune processes. The mortality among the experimental rats was 35%. The disease followed the mildest course in the rats aged 3 weeks. In these animals, unlike the other groups, the morphological changes in the kidneys were not accompanied by increased proteinuria and they had no tendency to progress. In the course of the disease a steady increase in the complement level was observed. Toward the end of the experiment all the animals were still alive. The difference in the course of the disease in the young rats was evidently connected with differences in their immunological reactivity: an extremely low initial serum complement level, high initial acid phosphatase activity of the blood neutrophils, and the appearance of foci of infiltrating lymphocytes and plasma cells in the interstices of the renal cortex.
KEY WORDS: Masugi's nephritis; age differences; complement; neutrophils; acid phosphatase.

Clinical [1, 5] and experimental [9, 15, 17] studies have revealed age differences in the course of acute glomerulonephritis. In the investigations cited no analysis was made of the concrete data from which the cause of differences in the course of the disease connected with age could be established.

The object of this investigation was to study Masugi's nephritis from the age aspect and to make a parallel study of various indices determining or reflecting the severity of the disease.

EXPERIMENTAL METHOD

Masugi's nephritis was produced in Wistar rats by injection of the globulin fraction of nephrotoxic serum in a dose of 0.03 ml/10 g body weight into the caudal vein. The serum was obtained by intraperitoneal immunization of rabbits with a 20% suspension of perfused rats' kidneys [7]. Choice of the age groups of rats was based on features of their physiological development [8, 10]. Three groups of animals were used: young rats aged 3 weeks and weighing 30-40 g (54 animals), rats aged 6 weeks weighing 70-90 g (25 animals), and rats aged 3 months weighing 130-150 g (25 animals). The following indices were studied during the course of the disease.

1. The intensity of fixation of nephrotoxic antibodies and autoglobulins in the renal glomeruli of the rats was determined by fluorescence microscopy using labeled donkey serum against rabbit globulin and labeled rabbit serum against rat globulin.

2. The complement-fixing activity of the blood serum was measured by the usual method of titration of the hemolytic activity of the complement.

3. Acid phosphatase (AP; orthophosphoric acid monoester phosphohydrolase; 3.1.3.2) activity of the blood leukocytes was determined by the azo-coupling method [12] and the enzyme activity was expressed by Kaplow's index [13].

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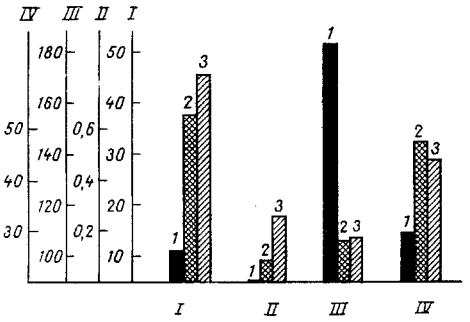

Fig. 1

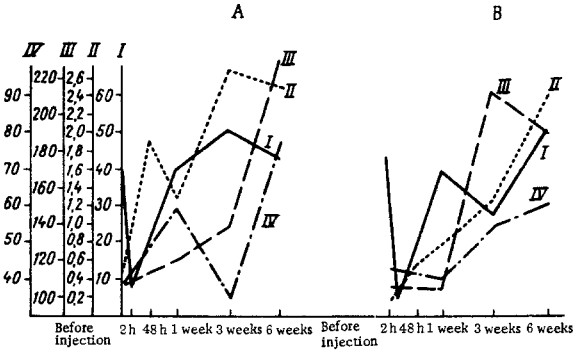

Fig. 2

Fig. 1. Age differences in indices studied in three groups of rats before injection of nephrotoxic globulin. Here in Figs. 2 and 3: I) serum complement titer, in hemolytic units; II) proteinuria, in mg/24 h; III and IV) AP activity of neutrophils and lymphocytes respectively, expressed as index of activity. 1) Rats aged 3 weeks, 2) 6 weeks, 3) 3 months.

Fig. 2. Dynamics of indices studied in rats aged 6 weeks and adult rats after injection of nephrotoxic globulin. A) Rats aged 3 months, B) aged 6 weeks. Here and in Fig. 3: horizontal axis, time of taking samples before and at various intervals after injection of nephrotoxic globulin.

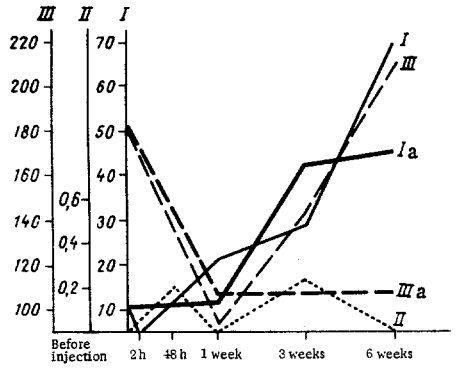

Fig. 3. Dynamics of indices studied in rats aged 3 weeks, I, II, III) experimental groups, Ia, IIIa) control groups.

4. Morphological changes in the kidney tissue were revealed by staining by Van Gieson's method, with hematoxylin–eosin, fuchsin, and the PAS reaction.

5. Proteinuria (in mg/24 h).

Blood and urine for the study of the above-mentioned indices were collected before injection of the nephrotoxic globulin and during the first 2 days and 1, 3, and 6 weeks after its injection. Some of the animals were killed at these times for immunofluorescence and histological investigations.

EXPERIMENTAL RESULTS

The investigations before injection of nephrotoxic globulin into the animals showed (Fig. 1) that the serum complement-fixing activity in the rats aged 3 weeks was considerably lower than in the animals of the other age groups ($P < 0.001$). They differed from the older animals also in the AP activity of their blood leukocytes: Whereas activity of the enzyme in the lymphocytes was low (the difference between rats aged 3 and 6 weeks was significant, $P = 0.02$), very high AP activity was found in the neutrophils (compared with other groups $P < 0.001$). No difference was found between the initial indices in the rats aged 6 weeks and the adults. Before the beginning of the experiment protein was absent from the urine only of the rats aged 3 weeks. The increase in proteinuria, both as regards the frequency of its detection and the protein concentration, followed a parallel course to the animal's age, and in the adult rats it varied between the limits of 0.48 ± 0.06 mg/24 h, significantly higher than in the animals aged 6 weeks ($P = 0.01$). The proteinuria was unaccompanied by any pathological changes

in the kidney tissue. The presence of protein in the urine of the rats, with no evidence of kidney lesions, has also been reported by other workers [15, 16]. Classical studies involving the reproduction of Masugi's nephritis showed that the intensity of the clinical and pathological disturbances caused by injection of nephrotoxic serum is determined primarily by the quantity of nephrotoxins fixed in the recipient's kidney; a rapid decline in the serum complement level is an indication of the beginning of the immune process between kidney tissue antigen and the nephrotoxic antibodies [14, 17]. Different views are held on the question of whether the age of the animals affects the intensity of fixation of nephrotoxins in the glomeruli. Experiments on rabbits have shown that the renal glomeruli of young animals, because of their antigenic immaturity, are unable to fix nephrotoxic antibodies and, consequently, the heterologous phase of nephritis, determining the subsequent course of the disease, does not develop in these animals [11, 17].

Experiments on young rats have demonstrated equally intensive fixation of nephrotoxic antibodies in the glomeruli of animals aged between 1 day and 6 weeks [9].

The present experiments established the identical intensity and duration of fixation of nephrotoxins in the renal glomeruli of animals of all groups irrespective of age, with maximal intensity after 48 h and with some decrease in intensity by the 6th week of the experiments; fixation was accompanied by a sharp fall in the serum complementary activity, which could be detected as early as 2 h after injection of the globulin (in all groups $P < 0.001$).

On the basis of the facts described above it might be expected that the course of induced nephritis would be similar in the rats of all age groups. However, analysis of the later observations led to the conclusion that the course of the disease was severer in the animals aged 6 weeks (Fig. 2). As early as after 48 h they developed proliferative-membranous glomerulitis, which increased in severity up to a maximum by the 6th week of the experiments. Starting from the first week, degeneration of the tubular epithelium was observed. The development of glomerulonephritis was accompanied by a sharp increase in the proteinuria, which was significant at all stages of the investigation (after 48 h $P = 0.05$, at other times $P < 0.001$). A second decrease in the serum complement level ($P = 0.05$) and an increase of intensity (up to ++) of fixation of autoglobulins in the glomeruli were observed only in this group of animals (in the 3rd week of the experiment), evidence of the activation of autoimmune mechanisms [6, 17]. A marked increase in AP activity of the neutrophils (after the 3rd week of the experiment, $P < 0.001$) and, to a lesser degree, of the lymphocytes was observed. By the end of the experiment 35% of the animals had died.

In the adult rats (Fig. 2) proliferative-membranous glomerulitis did not develop until the 3rd week of the experiment, it was less severe than in the animals of the previous group, and it had no tendency to progress: By the 6th week proliferation was absent and the glomerulitis was membranous in character. No second decrease in the complement level and increase in the intensity of fixation of autoglobulins was observed. However, in these rats, just as in the rats of the previous group, there was a steady increase in proteinuria ($P < 0.001$, $P = 0.02$, $P < 0.001$, and $P < 0.001$, respectively). The development of the disease was accompanied by an increase in AP activity of the leukocytes, which reached a maximum later than in the animals aged 6 weeks (increase significant only by the 6th week of the experiment, $P < 0.001$). By the end of the experiment 25% of the rats had died.

The disease followed the mildest course in the rats aged 3 weeks (Fig. 3). These animals developed membranous-proliferative glomerulitis by the 3rd week of the experiment, it had no tendency to increase in severity, and despite the fact that the histological changes in this case were similar to those observed in the adult rats, the disease was not accompanied by increasing proteinuria. In the animals of this group the only distinguishing feature was a steady rise in the complementary activity of the serum: Toward the end of the experiments the complement titer was higher than in the control animals of the same age ($P < 0.001$). Fixation of autoglobulins was slight or absent. The third feature, peculiar only to the rats aged 3 weeks, was the appearance of infiltration with lymphocytes and plasma cells in the interstices of the renal cortex by the 6th week of the experiments. High AP activity of neutrophils, observed in the young rats before injection of nephrotoxin, fell by the end of the 1st week, and a similar decrease also was found in the control rats of the same age. By the 3rd and in particular, the 6th week a sharp increase in AP activity of the neutrophils was observed in the experimental young rats (compared with the 1st week of the experiment $P < 0.001$), at a time when its activity in the group of control animals still remained low (the difference between the experiment and control was significant, $P < 0.001$). Fluctuations in lymphocyte activity, whether due to age or induced by injection of nephrotoxin, were not significant in this group. All the animals were still alive at the end of the experiments.

These observations can be summed up as follows. In rats of three age groups in which Masugi's nephritis was produced under identical experimental conditions, the disease followed the severest course in animals in

the period of sexual maturation. In these animals not only was the onset of the disease more acute (the heterologous phase of nephritis), but its subsequent course also was more severe (the autologous phase of nephritis). The causes of this severe course of the disease in the rats of this age group could not be established by these experiments and further study of the problem is required.

The mildest course of nephritis in the rats aged 3 weeks was due in all probability to the character of their immunological reactivity. For example, the low serum complement concentration characteristic of animals of this age reduces the possibility of induction of immunological responses which play the leading role in the development of Masugi's nephritis. The high AP activity of the neutrophils observed in young rats before the injection of nephrotoxic globulin is an indirect indication of the increased phagocytic activity of these cells [2-4], and this could facilitate phagocytosis of antigens with a nephrotoxic action (especially before their fixation in the kidney tissue), and thus prevents the development of the disease. Finally, the formation of foci of infiltration of lymphocytes and plasma cells in the interstices of the renal cortex must not be forgotten. Their accumulation close to places characterized by the strongest immunological reaction, associated with the aggressive action of the nephrotoxic antibodies, immune complexes, and autoantibodies against damaged kidney tissue, can hardly be accidental. The diversity of the functions performed by these cells is well known, but their appearance only in the 3-week-old animals, which develops the disease in its mildest form, between the 3rd and 6th weeks of the disease – at a time of the greatest increase in serum complementary activity and decrease in proteinuria down to its total disappearance – suggests that these cells are responsible for local reactions of a protective character.

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