PATHOGENETIC SIGNIFICANCE OF HYPERSENSITIVITY REACTIONS OF IMMEDIATE AND DELAYED TYPES IN THE COURSE OF NEPHRITIS

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UDC 616.61-002-092:612.017.3

A combination of an immunologic study of rats from the first days after receiving injection of nephrotoxic serum until the development of chronic renal failure and determination of the functional and morphological characteristics of the disease confirmed the view that the nephritis develops in stages and that hypersensitivity reactions of immediate and delayed types play different pathogenetic roles in the changing course of the disease. In the early periods the predominant features are those of hypersensitivity of immediate type, evidence of which is given by the liberation of mediators of the pathochemical phase and changes in kidney function. The dominant factor in the formation of chronic nephritis is the development of hypersensitivity reactions of delayed type.

KEY WORDS: nephritis; hypersensitivity of immediate type; hypersensitivity of delayed type.

The character and pathogenetic significance of immunologic changes during nephritis have received insufficient study. Aside from papers in which the role of humoral and cellular responses in the pathogenesis of this disease are compared [9, 10, 12, 13], the results of a few investigations have yielded evidence of differences in the intensity of hypersensitivity of immediate (HIT) and delayed (HDT) types in the course of nephritis [3, 5, 6].

The object of the present investigation was to test the hypothesis put forward previously [7] according to which reactions of HIT and HDT have different relative roles in the pathogenesis of nephritis and to correlate them with changes in renal activity and the severity of the course of the pathological process in this disease.

EXPERIMENTAL METHOD

The intensity of the HIT and HDT reactions was determined on the 5th, 10th, 15th, 25th, 45th, and 60th days after the development of Masugi's nephritis in male Wistar rats kept in metabolic cages. To judge the intensity of the individual phases of HIT, the titer of antikidney antibodies in the complement fixation test (CFT) and the plasma-cell response of the bone marrow, lymph nodes, and spleen, reflecting the immune phase, were studied, the serotonin concentration in the kidneys and blood was determined by a biological method [11], and the number and functional state of the platelets and mast cells in the kidneys and mesentery (reflecting the development of the pathochemical phase of these reactions) were investigated. The HDT reactions were judged from the presence of lymphocytolysis, by intradermal labial tests, and from the ability of the lymphocytes to produce a factor inhibiting migration of macrophages from fragments of the spleen in vitro in the presence of kidney antigen [15]. Data obtained with myocardial and hepatic antigens and also the results of tests on intact rats served as the control for all the experiments. The pathophysiological phase was studied with respect to the formation of the basic syndromes of nephritis. Daily for 45 days after injection of the nephrotoxic serum and in the control group for 6-8 days the quantity of water drunk in the 24-h period, together with the diuresis and excretion of protein, creatinine, sodium, and potassium, were determined. At the end of the experiments the animals were killed, the blood levels of creatinine, nonprotein nitrogen and urea, sodium and potassium, and protein and protein fractions were studied; erythrocytes, leukocytes, and platelet counts were undertaken, the hemoglobin measured and the myelogram studied. The volume of the extracellular space was investigated by the sodium thiocyanate dilution method; the mean arterial blood pressure was measured manometrically in the aorta. Morphological changes in the kidneys were studied in histological preparations stained with hema-

Department of Pathological Physiology, Chernovtsy Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 3, pp. 264-267, March, 1979. Original article submitted January 16, 1978.

0007-4888/79/8703- 0259$07.50

TABLE 1. Changes in Excretory Function of Kidneys in Rats with Masugi Nephritis (M ± m)

Day of disease	No. of animals	Diuresis, ml/24 h	Filtration, ml/24 h	Reabsorption, % of filtration	Sodium excretion, μmoles/24 h	Sodium filtration, mmoles/24 h	Sodium reabsorption, % of filtration	Protein excretion, mg/24 h
Intact rats	30	5,3±0,31	367,6±18,37	98,5±0,31	13,2±0,37	49,9±2,05	99,7±0,01	1,3±0,03
5th	30	5,8±0,64	355±20,9	98,2±0,22	13,6±1,68	45,7±3,05	99,6±0,08	3,1±0,23*
15th	22	6,5±0,89	246,9±15,64**	96,9±0,5*	12,8±1,13	37,6±4,0*	99,5±0,07*	4,0±0,52**
25th	10	7,3±0,8*	216,4±12,53**	96,3±0,66**	13,8±0,69	29,5±1,79**	99,5±0,04**	8,9±1,02**
45th	10	3,4±0,61*	115,8±8,28**	97±0,48**	14,5±0,58*	15,4±1,19**	99±0,08**	13,2±0,94**

Legend. *) $P < 0.05$, **) $P < 0.001$ compared with data for intact rats.

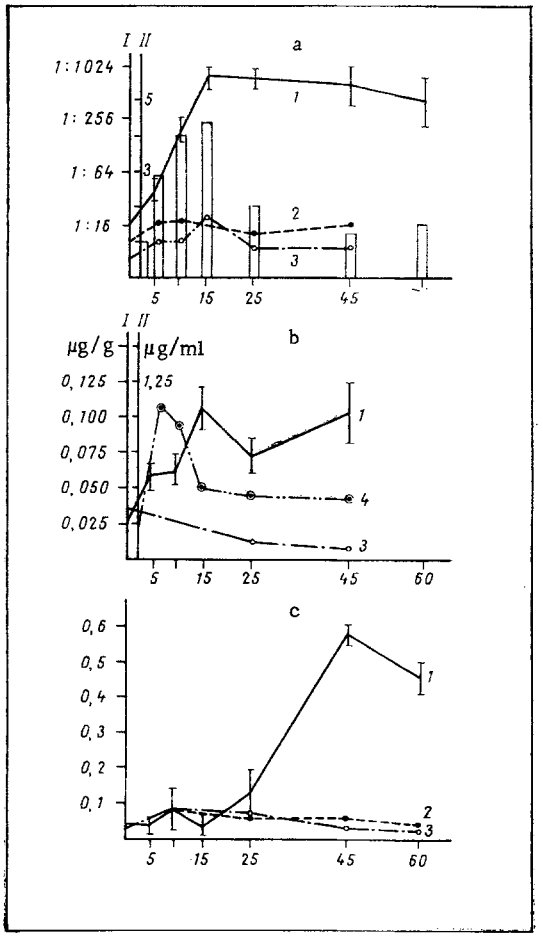

Fig. 1. Indices of HIT (a, b) and HDT (c) reactions in the course of Masugi nephritis. Abscissa, days of disease; ordinate: a) antibody titer in CFT (I) and relative percentage of plasma cells (II); b) serotonin concentration, in μg/g tissue (I) and in μg/ml blood (II); c) intensity of inhibition of macrophage migration. 1) Reaction with kidney antigen (a, c); kidneys (b); 2) reaction with liver antigen; 3) reaction with myocardial antigen (a, c); myocardium (b); 4) blood. Unshaded columns represent plasma cells in lymph nodes.

toxylin–eosin and by the Jones–Mowry and MacManus methods. The numerical data were subjected to statistical analysis by parametric methods and the results of the serologic investigations were analyzed by the method described in [8].

EXPERIMENTAL RESULTS

Since nephritis is characterized by a combination of urinary, edematous, and hypertensive syndromes and also by blood changes, the development and intensity of these changes were studied in the experimental animals at different periods of the disease: before the 15th day (the period of acute nephritis), on the 25th-30th day (the period of subacute nephritis, changing to chronic nephritis), and on the 45th-60th days (chronic nephritis, development of chronic renal failure – CRF).

As the nephritis progressed the hypercreatininemia of the rats increased; retention uremia was aggravated by the production variety, and hypo- and dysproteinemia, hypochromic anemia, leukocytosis, and thrombocytopenia developed against the background of definite disturbances of medullary hematopoiesis.

The dynamics of the changes in some indices of renal activity is illustrated in Table 1. Slight changes of diuresis and sodium excretion before the 25th day of the disease developed as a result of a decrease of both filtration and (mainly) reabsorption in the functioning nephrons. In the stage of chronic nephritis the changes observed progressed, in agreement with the results of clinical observations.

The gradual increase in the total extracellular space indicated the formation of a syndrome of edema; by the 45th day of nephritis the rats had developed a hypertensive syndrome.

Throughout the period of observation the animals showed signs of development of both HIT and HDT, but their intensity differed in the different periods of nephritis. After the first few days of the disease the titer of antikidney antibodies in the blood rose and the number of plasma cells in the immunocompetent organs increased (Fig. 1a). That this activation of HIT was connected with the developing disturbances of renal activity was shown by positive correlation between the blood creatinine level, on the one hand, and the titer of antikidney antibodies ($r = +0.343$, $P < 0.02$) and the plasma-cell reaction of the bone marrow ($r = +0.366$, $P < 0.01$) on the other hand. This correlation also was confirmed by the progressive rise in the titer of antikidney antibodies, whereas the titers of antimyocardial and antiliver antibodies showed no significant change.

Since the mechanism of the third, pathophysiological stage of HIT reactions involves both antibodies directly and also biologically active substances liberated in the pathochemical phase of the reactions themselves, the participation of humoral reactions in a pathogenetic role was indicated by an increase in the concentration of the mediator of these reactions (serotonin) in the kidneys and blood (Fig. 1b). Similar results have been obtained during an investigation of the concentrations of both serotonin and of another bioamine, namely histamine, in the kidneys and blood during the course of nephritis [4]. The absence of correlation between the increase in the serotonin concentration in the kidneys and blood ($r = +0.08$) and also the absence of significant changes in the serotonin concentration in the myocardium and liver were evidence of the renal origin of the serotonin detected, possibly as a result of increased local liberation of serotonin from its depots in the platelets and mast cells. This is a fact of considerable interest, for bioamines participating in the regulation of activity of many organs including the kidneys [1, 14] could be the connecting link between disturbances of glomerular activity (antibodies interact with antigens of the target organ in the glomeruli) and the changes in tubular activity appearing early during acute nephritis, and also with disturbances of energy formation in the kidney [2]. Positive correlation in acute nephritis between the serotonin concentration in the kidneys and the blood nonprotein nitrogen level ($r = +0.448$, $P < 0.05$) confirms the pathological role of the pathochemical phase of the HIT reactions in this period of the disease.

Although the titer of antikidney antibodies still remained relatively high after the 15th day of the disease (until the end of observation), the decrease in plasmatization of the immunocompetent organs with some decrease in the serotonin concentration in the kidneys and blood suggested a diminution of the pathogenetic role of HIT reactions in progression of the nephritis.

The intensity of the HDT reactions, like that of the HIT, was phasic in character in the course of experimental nephritis. Spontaneous lymphocytolysis in the control experiments (incubation with 0.9% sodium chloride solution) did not exceed 10%; the same results were obtained when blood was incubated with kidney, myocardial, and liver antigens from intact animals and, with the last two also, in the course of nephritis. An increase in the intensity of lymphocytolysis was observed after the 25th day of the disease; on the 45th and 60th days of nephritis the percentage of damaged lymphocytes differed significantly from the initial value ($P < 0.001$). The results of the labial tests and of investigation of the ability of the spleen cells to produce macrophage migration inhibition factor in the presence of kidney antigen were similar (Fig. 1c); in all cases the macrophage migration inhibition index was the same when myocardial and hepatic antigens were used as in intact rats.

Consequently, the study of the state of HDT revealed definite activation of this type of hypersensitivity during the period of transition to the chronic stage of nephritis; at this same time (25th-45th day) definite correlation was found between the macrophage migration inhibition index and the blood creatinine level ($r = -0.858$, $P < 0.001$), the development of infiltration of the kidneys with lymphocytes and macrophages, and the progressive decrease in the mass of active nephrons, characteristic of chronic nephritis. The results indicating activation of HDT during the period of development of chronic nephritis agree with the results of clinical investigations [6]. Meanwhile the formation of CRF (60th day of the disease) was accompanied by some decrease in the activity of the cellular reactions.

The combined study of the immunologic state of rats during the first few days after injection of nephrotoxic serum into the animals and until the development of CRF together with the functional and morphological characteristics of the disease thus confirmed the view that the disease develops in stages and that HIT and HDT reactions play different pathogenetic roles in the dynamics of nephritis. In the initial periods of the disease the predominant features are those of HIT reactions, evidence of which is given by the liberation of mediators of the pathochemical phase and changes in renal activity. Development of HDT reactions is the predominant feature of the pathogenesis of development of chronic nephritis.

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