ACTION OF HETEROGENEIC ANTILYMPHOCYTIC SERUM ON THE DEVELOPMENT OF NEPHROTOXIC NEPHRITIS IN RATS

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UDC 616.61-099-092.9-085.365.018.53-039.71

A persistent proteinuria developed in August rats 24 h after the administration of nephrotoxic serum. Injection of heterogeneic antilymphocytic serum 2 h before the nephrotoxic serum prevented or significantly reduced the level of proteinuria in the initial stage of nephrotoxic nephritis in the rats. On the 5th-7th day after injection of nephrotoxic serum, the level of the proteinuria rose sharply in the animals receiving a preliminary injection of antilymphocytic serum, and reached its level in the animals of the control group. Treatment of rats with antilymphocytic serum in the second phase of nephrotoxic nephritis did not lower the level of proteinuria.

The ability of heterogeneic antilymphocytic serum (ALS) to suppress cellular immune responses and the primary formation of humoral antibodies has been demonstrated by many investigators and is well discussed in the surveys by James [17, 18]. ALS inhibits the development of experimental and spontaneous diseases in the pathogenesis of which a leading role is played by immune disorders: adjuvant autoallergic encephalitis [10, 16], adjuvant arthritis [4], and adjuvant thyroiditis in rats [8] and spontaneous Coombs-positive hemolytic anemia in New Zealand mice of line NZB [5].

The pathogenesis of nephrotoxic nephritis (NTN) has not been completely explained. Both the mechanism of damage to the kidney by nephrotoxic serum (NTS) and the role of autoimmune processes in the development of the pathology require close examination. The possible role of a delayed hypersensitivity reaction due to lymphocytes in the pathogenesis of NTN cannot be ruled out, as shown by work indicating the possibility of transfer of NTN from affected to healthy animals by means of blood lymphocytes and lymph [6, 11].

For these reasons it was decided to study the action of heterogeneic ALS on the development of NTN in rats.

EXPERIMENTAL METHOD

August rats weighing 150-180 g were used for the production of nephrotoxic nephritis. Protein in the urine was determined by the Roberts–Stol'nikov method. The protein concentration in the urine of healthy rats did not exceed 0.3 ‰. Chinchilla rabbits weighing 1.5-2 kg were used to prepare the ALS and NTS.

To obtain NTS the rabbits were immunized by Maslakov's method [1] with antigen prepared from the cortex of rat kidneys, thoroughly washed with physiological saline, and perfused through the renal artery. Sera causing persistent proteinuria in 100% of rats on the day after two separate intravenous injections, each of 1 ml/150 g body weight, at intervals of 24 h, were used in the experiments. ALS was prepared by immunizing rabbits with lymphocytes from the lymph gland of the rats. Inguinal, axillary, submandibular, and mesenteric lymph glands of the rat were freed from connective tissue and ground in a loosely fitting

Department of Internal Medicine and Occupational Diseases, Faculty of Hygiene, I. M. Sechenov First Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR E. M. Tareev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 72, No. 8, pp. 34-37, August, 1971. Original article submitted November 26, 1970.

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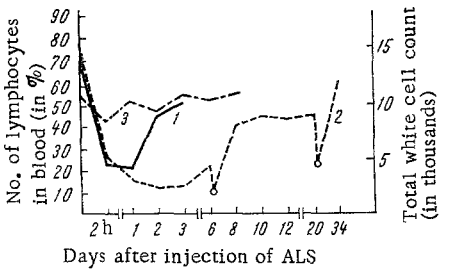

Fig. 1

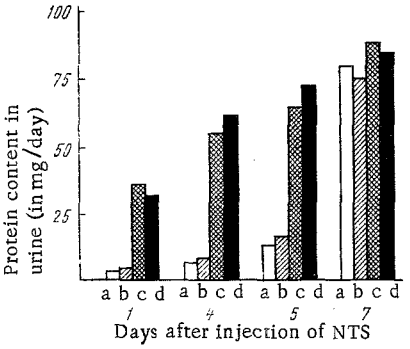

Fig. 2

Fig. 1. Changes in number (in %) of circulating lymphocytes in blood of rats receiving ALS: 1) single injection of ALS; 2) repeated injections of ALS; 3) total white cell count. Arrows indicate times of injection of ALS. Circles on curve 2 denote percentage of lymphocytes 2 h after injection of ALS.

Fig. 2. Action of ALS on development of proteinuria in rats in first phase of NTN: a) ALS injected daily, 3 days before NTS; b) ALS injected 2 h before NTS; c) NTS injected; d) NRS injected 2 h before NTS.

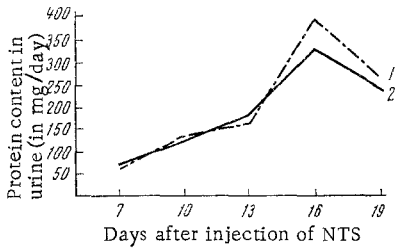

Fig. 3. Action of ALS on proteinuria in rats in the second phase of NTN: 1) animals receiving NTS only; 2) animals receiving ALS on 7th, 9th, 11th, and 15th days after injection of NTS.

Potter's homogenizer, and the resulting cell suspension was washed three times and resuspended in cold medium No. 199. The suspension usually contained 85-90% of living cells. From $3 \cdot 10^8$ to $8 \cdot 10^8$ lymphocytes were injected intravenously and subcutaneously into a rabbit once a week for three weeks, and two further injections were given after an interval of two weeks. Ten days after the last injection the rabbits were exsanguinated. The sera were pooled and adsorbed with three portions of rat's erythrocytes for 1 h at 37°C, and the serum was allowed to stand overnight with the last portion of erythrocytes at 4°C. To each 100 ml of serum, 30 ml of erythrocyte residue was used. In this way, the hemagglutinating and hemolytic activity of the ALS was suppressed. The serum was then inactivated at 56°C for 30 min. The resulting serum had strong antilymphocytic action. After intraperitoneal injection of 0.5 ml of the serum thus obtained into rats weighing 150 g, within 2 h the percentage of lymphocytes in the blood of the animals had fallen to 20-25 from the normal level of 70-80. The percentage of polymorphs showed a corresponding increase, so that the total white cell count fell very slightly. After 24 h, the total white cell count had returned to normal, whereas the percentage of lymphocytes still remained low. During daily injections of ALS the percentage of lymphocytes in the blood remained low (10-15), while the total white cell count varied within normal limits (Fig. 1).

NTN was produced in rats by injecting NTS into the femoral vein on two successive days in a dose of 1 ml/150 g body weight. Damage to the kidneys was assessed from the appearance of a marked and persistent proteinuria, which, if the NTS was of high activity, reached 3.3 ‰ 18 h after the second injection, and also from the results of histological investigation of the kidneys.

ALS was injected intraperitoneally into the rats in a dose of 0.5 ml/100 g body weight either once or repeatedly at various times before and after injection of NTS.

EXPERIMENTAL RESULTS AND DISCUSSION

The experiments were divided into two series. In series I the development of NTN was studied in rats receiving ALS before injection of NTS. The rats used in these experiments were divided into four

groups: animals (7) of group 1 received injections of ALS daily for three days before NTS, and three of them received two further injections after NTS at intervals of 24 h. The 15 animals of group 2 received ALS 2 h before injection of NTS, and 6 of them received a further two injections of ALS after the NTS at intervals of 24 h. The rats of group 3 received normal rabbits serum (NRS) in the same dose as ALS (0.5 ml/100 g body weight) 2 h before injection of NTS. The animals of group 4 (control) received NTS only.

Preliminary treatment of the animals with ALS delayed the appearance of proteinuria or reduced its level substantially in the first days after injection of NTS. The times of appearance and the intensity of the proteinuria in the animals of group 1 receiving ALS on three successive days before NTS, and in the animals of group 2 receiving ALS 2 h before NTS, did not differ significantly. From 5-6 days after the injection of NTS, the proteinuria level in the animals of both groups rose sharply and corresponded to the level of the proteinuria in the animals of the control group receiving NTS only. Repeated injections of ALS into the animals of groups 1 and 2 on the first days after the injection of NTS (on the 2nd and 4th days thereafter) did not increase the period of delay of proteinuria and did not reduce its level. Injection of normal rabbit serum before NTS (animals of group 3) had no effect on the development of the proteinuria by comparison with the animals of group 4 (Fig. 2).

In the experiments of series II the action of ALS on the course of NTN was studied in rats when the ALS was injected in the second phase of NTN, after the development of marked proteinuria. The group of six animals received injections of ALS 7, 9, 11, and 15 days after the injection of NTS.

As Fig. 3 shows, injection of ALS into the animals in the second phase of NTN did not reduce their proteinuria compared with the control animals, but in some cases the proteinuria was higher in the animals receiving ALS than in the controls.

These results show that ALS prevents proteinuria in the first phase of development of NTN (in the first 5-6 days) and does not exhibit this action in the second phase of NTN. This action of ALS in temporarily delaying the appearance of the proteinuria is evidently nonspecific and it cannot be regarded as confirmation of the view that lymphocytes participate in causing damage to the kidneys in NTN.

According to data in the literature, renal damage and proteinuria on the first days after injection of NTS are the result of the direct toxic action of NTS on the basement membrane of the glomeruli, whereas in the second phase of development of NTN the leading role is played by immune complexes formed by heterogeneic NTS fixed to the basement membrane, and by antibodies produced against it [2, 9, 12, 13, 15]. These views regarding the development of NTN suggest that the action of ALS, when injected before NTS, in preventing proteinuria is connected with the immunodepressive action of the ALS. However, the action of ALS cannot be explained by depression of the formation of humoral antibodies against heterogeneic protein of the NTS, because the effect of ALS in delaying the proteinuria appears only in the first phase of NTN, whereas in the second phase, in which the development of the pathology is connected with the production of antibodies against the foreign protein, and the production of antirenal autoantibodies, ALS was ineffective.

Cochrance et al. [3] has shown that during the first few hours after injection of NTS, polymorphs accumulate in the glomeruli; these agglomerations of polymorphs cause considerable changes in the glomeruli and they play an important role in the development of NTN after the injection of moderate doses of NTS. These workers emphasize the role of complement, the accumulation of which has been observed where deposition of NTS takes place. They consider that at least one of the functions of the complement is to attract polymorphs into the zone of the immunological inflammatory focus. Results obtained by other workers [3, 7, 14] show that artificial lowering of the serum complement level in rats prevented the appearance of proteinuria in the animals immediately after the injection of NTS. In face of the foregoing facts, the action of ALS in delaying proteinuria in the first 5-6 days after injection of NTS can evidently be explained by the fixation of serum complement by the antigen–antibody complex formed by interaction of ALS with antigens of the lymphocytes.

LITERATURE CITED

1. D. A. Maslakov, Clinical and Morphological Changes in Experimental Cytotoxic Nephritis, Candidate's Dissertation, Moscow (1954).
2. V. V. Serov, Morphological Bases of Immunopathology of the Kidneys [in Russian], Moscow (1968), p. 134.

3. C. G. Cochrance, E. R. Unanue, and F. J. Dixon, J. Exp. Med., 122, 1 (1965).
4. N. L. F. Currey and M. Liff, Lancet, 2, 889 (1966).
5. A. M. Denman, E. J. Denman, and E. I. Holbrow, Lancet, 1, 1085 (1967).
6. R. Federlin, W. Geinweber, and E. F. Pfeiffer, Nature, 211, 1199 (1966).
7. D. K. Hammer and F. J. Dixon, J. Exp. Med., 117, 1019 (1963).
8. J. Kalden, K. James, W. G. Williamson, et al., Clin. Exp. Immunol., 3, 973 (1968).
9. C. F. Kay, J. Exp. Med., 72, 559 (1940).
10. S. Leibourtz, L. A. Kennedy, and M. H. Lessof, Lancet, 2, 529 (1968).
11. E. F. Pfeiffer, W. M. Rucholtz, and K. Federlin, Nature, 195, 718 (1962).
12. K. Rother, Arch. Exp. Path. Pharmak., 220, 448 (1953).
13. O. Spühler, H. U. Lollinger, and M. Enderlin, Schweiz. Med. Wschr., 81, 904 (1951).
14. E. R. Unanue and F. J. Dixon, J. Exp. Med., 119, 965 (1964).
15. A. Vogt and H. Kochem, Am. J. Path., 39, 379 (1961).
16. B. H. Waksman, S. Arbouys, and B. G. Arnason, J. Exp. Med., 114, 997 (1961).
17. K. James, Clin. Exp. Immunol., 2, 615 (1967).
18. K. James, Clin. Exp. Immunol., 3, 189 (1968).