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ELIMINATION OF DEPOSITS OF IMMUNE COMPLEXES FROM THE KIDNEYS OF AUTOIMMUNE NZB/N MICE BY INJECTION OF PERFUSATE OF A HETEROLOGOUS SPLEEN

A. B. Tsypin, V. A. Nasonova,
L. A. Vedernikova, L. N. Pleskovskaya,
L. V. Beletskaya, T. A. Ryazantseva,
and B. M. Manuilov

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The treatment of nephritis accompanying systemic lupus erythematosus (SLE), despite the extensive use of immunosuppressants, still remains a difficult problem because of the unremitting progression of the kidney lesion. New Zealand mice (NZB/N), which spontaneously develop an autoimmune disease similar to human SLE [7, 10], constitute an irreplaceable model for the development of new methods of its treatment. Methods of treatment of NZB/N mice, including a special diet or administration of corticosteroids, cytostatics, monoclonal antibodies, and so on [6, 8, 9, 11], can arrest the development of the disease only in the preclinical stage and are ineffective if the autoimmune process is well advanced.

The aim of this investigation was to study the possibility of influencing the late stage of development of the autoimmune process by intravenous injection of a solution obtained during perfusion of an isolated hog spleen (perfusate) intravenously into NZB/N mice in which the disease was of long duration. The starting point of the research was the fact that the mechanism of phagocytosis, responsible for the elimination of circulating immune complexes (CIC) from the body, is inhibited in New Zealand mice [7, 10], just as it is in patients with SLE [2, 4]. According to the results of the writers' previous investigations [3, 5], splenic perfusate can considerably increase phagocytosis by increasing the metabolic activity of neutrophils and macrophages.

EXPERIMENTAL METHODS

Experiments were carried out on 103 male NZB/N mice aged 6 and 10 months. The experimental group consisted of 54 and the control group of 49 mice. Animals of the experimental group were given an intravenous injection of 0.2 ml of splenic perfusate every 2-3 days for eight injections. Mice of the control group received 0.2 ml of isotonic buffer solution (pH 7.4) by the same schedule. All procedures connected with obtaining the perfusate were carried out under sterile conditions. The spleen was taken from a healthy animal (pig), the artery and vein were cannulated, after which the vascular bed of the spleen was washed free from blood with physiological saline. The spleen was then perfused in a closed circuit with 100 ml of isotonic buffer solution (pH 7.4) for 45 min, with the temperature of the perfusate 37°C. The perfusate was oxygenated under a pressure of 2 atm, and its pH was corrected to normal values with Tris-buffer. The rate of perfusion was 20-25 ml/min. The necessary conditions were maintained for normal viability of the spleen, as shown by the arteriovenous

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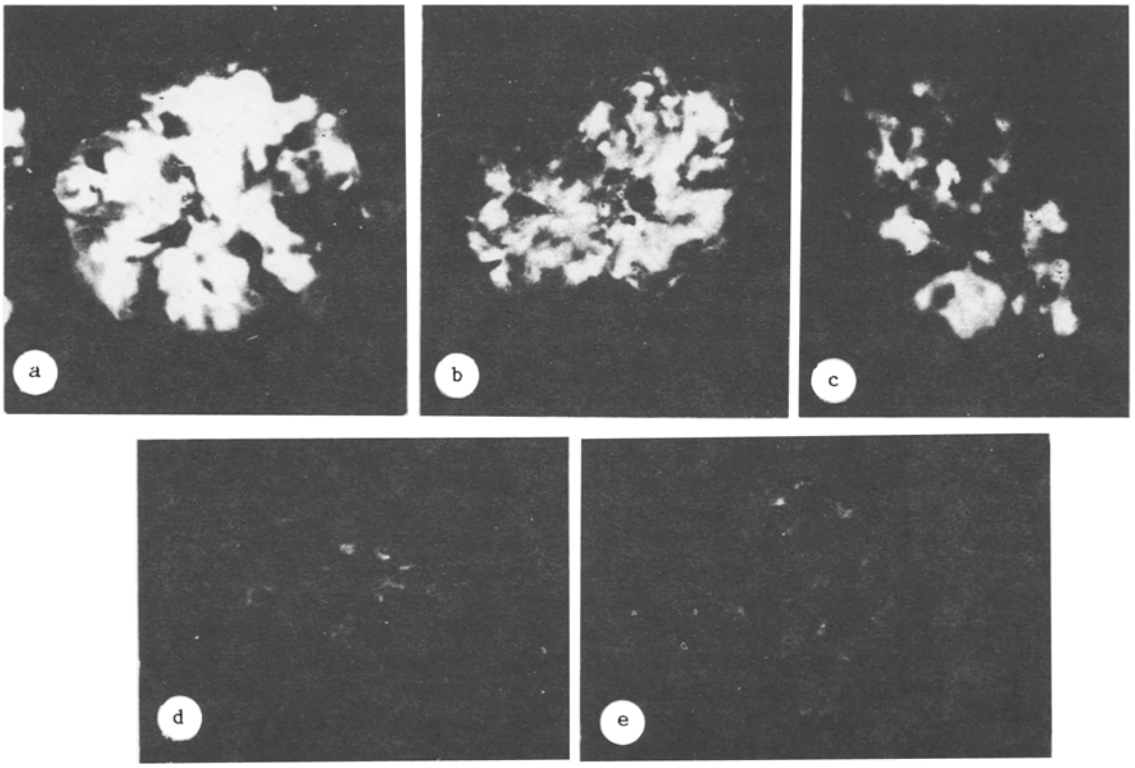

Fig. 1. Kidney of NZB/N mouse during elimination of IC. a) Control (glomerulus completely filled with IC granules (4 points); b) partial elimination of IC granules from glomerulus (3 points); c) single large and small granules (2 points); d) residual signs of IC deposition (1 point); e) glomerulus virtually free from IC (0 point). Direct immunofluorescence method. Objective 40 (water immersion), ocular homal 3.

oxygen difference of 150 ± 5 mm Hg. To remove contaminating cells the perfusate was centrifuged for 5 min at 1000 rpm.

The level of CIC and of antibodies to DNA was determined in 46 mice aged 10 months (21 experimental and 25 control animals). CIC were detected by laser nephelometry. The serum was treated with 0.2 M EDTA (pH 7.5) to prevent complex formation due to activation of complement. A solution of polyethyleneglycol (mol. wt. 6000) was added up to a final concentration of 3%. The concentration of CIC was determined on a laser nephelometer (Hoechst-Behring, West Germany). The concentration of CIC was calculated on a Hewlett-Packard 85 computer (USA), from the standard curve. The level of antibodies to native DNA was determined by a radioisotope method using standard kits from Amersham International (England). The results were expressed in units relative to a standard. Immune complexes (IC) in the kidneys were studied by the direct immunofluorescence method [1] in 33 10-month-old mice (17 experimental and 16 control animals) and in 24 6-month-old mice (12 experimental and 12 control). Luminescent serum against mouse immunoglobulins, labeled with fluorescein isothiocyanate, was obtained from the N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR). Pieces of mouse kidney tissue measuring 4 mm^3 were frozen at −20°C and kept under these conditions for not more than 1 month. Sections 5 μ thick were cut in a cryostat (−20°C), placed on a slide, dried for 30 min at room temperature, and used at once after keeping at 4°C for not more than 48 h. Before treatment, the sections were thoroughly washed for 30 min in 0.85% NaCl solution in phosphate buffer (PBS, pH 7.4) to remove proteins not bound with the tissues. Labeled antiserum was then applied and the slide was kept for 30 min in a humid chamber at room temperature, after which it was washed for 10 min with PBS and mounted under a coverslip in neutral glycerol. The preparation was examined in the ML-2 luminescence microscope, and photographed on RF-3 film with 20× and 40× (water immersion) objectives and homal-3 ocular. For the histological control the sections were fixed in ethanol (96%) for 30 min and stained with hematoxylin and eosin. Altogether 342 sections were prepared, and in each section from 20 to 70 glomeruli were studied (on average 50.3 ± 1.7). The area occupied by IC deposits in the vascular glomerulus of the kidney was expressed on a 5-point scale (from 0 to 4). The number of points for all the glomeruli was added together and the average value characterizing the whole section calculated.

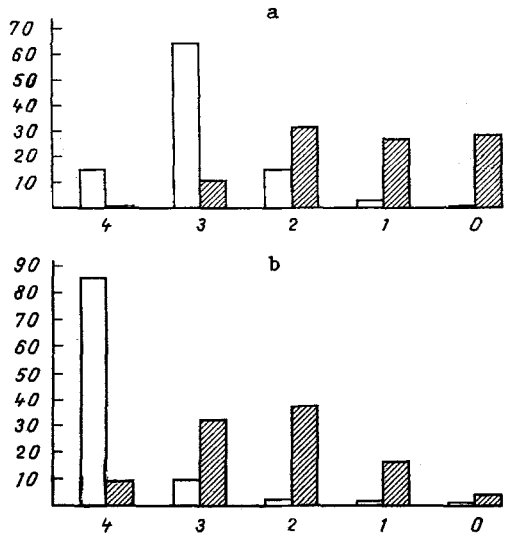

Fig. 2. Degree of filling of renal glomeruli with IC (in %) in NZB/N mice aged 6 (a) and 10 (b) months, after end of course of injection of perfusion fluid. Abscissa) points. Unshaded columns) control; shaded) experiment.

EXPERIMENTAL RESULTS

After eight injections of perfusion fluid the level of anti-DNA antibodies in animals of the experimental group (27.62 ± 1.74 U, n = 16) fell below that in the control (35.73 ± 1.89 U, n = 12; $p < 0.01$). The CIC level in the experimental group was 0.072 ± 0.029 mg/ml (n = 9), only one-fifth as high as in the control, which was 0.362 ± 0.133 mg/ml (n = 9; $p < 0.05$). These data are evidence of depression of the autoimmune response in animals of the experimental group to injection of heterologous splenic perfusion fluid.

The immunofluorescence investigation revealed a decrease in the quantity of deposits in the renal glomeruli of mice of the experimental groups during injection of perfusion fluid into them. For instance, in mice aged 10 months, after eight injections of perfusion fluid the mean score in points, based on estimation of the area occupied by IC in the renal glomeruli, was 2.26 ± 0.06, compared with 3.67 ± 0.08 in the control ($p < 0.001$). The number of glomeruli whose area was completely filled with IC deposits, with a score of 4 points, was considerably reduced (Fig. 1a). In the control group, glomeruli of this kind were found in 86% of observations. In the experimental group their number was reduced to 9.1% (Fig. 2b). In the experimental group glomeruli with single large and (or) small granules, scoring 2 points, began to predominant (38.1%; Fig. 1c). In the control group the number of glomeruli scoring 2 points was 2.9%. The number of glomeruli with residual signs of IC deposition, scoring 1 point, was considerably reduced (16.7%; Fig. 1f), whereas in the control they were found in only 1.2% of observations.

Similar results were obtained after injection of perfusion fluid into 6-month-old mice. Toward the end of the course of injection of the perfusion fluid the mean score relating to the area of IC deposits in the renal glomeruli was 1.08 ± 0.08 in the experimental group and 2.89 ± 0.04 in the control ($p < 0.001$). In the control group glomeruli scoring 3 points were found most frequently (65.3%; Fig. 1b), whereas in the experimental group their number fell to 11.3% (Fig. 2a). Glomeruli scoring 2 points (32.1%) and glomeruli not containing IC (29.1%; Fig. 2a) began to predominate in the experimental group, whereas in the control the number of glomeruli free from IC was only 0.9%. The number of IC in the kidneys of the experimental mice aged 6 and 10 months was not increased 1 month after the end of the course of injection of perfusion fluid, and differed from that in the control animals as before. No marked side effects of administration of the perfusion fluid were observed.

Consequently, during administration of splenic perfusion fluid to autoimmune NZB/N mice, elimination of IC granules from the vascular glomeruli of the kidneys was observed. Elimination took place in animals in different stages of development of the autoimmune process, and even in mice with very prominent kidney lesions. The mechanism of action of the perfusion

fluid on the process is not yet clear. Mononuclear phagocytes and tissue macrophages are known to remove IC from the circulation and tissues. Activation of the mononuclear phagocytic system by the action of splenic perfusion fluid may probably lead to the more rapid elimination of IC both from the kidneys and from the blood stream.

The method of elimination of IC developed in New Zealand mice is capable of being utilized, as the results show, to act on an already developed auto-immune process, it does not cause any marked side effects, and it is simple in use, for the course of injections of perfusion fluid lasts only 1 month, a great advantage when compared with known existing methods, requiring injections of preparations for many months.

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EFFECT OF T-ACTIVIN ON EXPERIMENTAL VIRAL MYOCARDITIS

N. N. Kipshidze, M. M. Dzhaparidze, R. V. Bulusashvili, A. V. Khuchua, and N. A. Zviadadze

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Among infectious factors facilitating the development of cardiomyopathies (CMP) in man, Coxsackie virus continues to attract increasing attention of research workers. In recent years much information has been obtained on its role in the development of viral myocarditis (VM) [2, 3, 8, 12, 15]. The hypothesis of a progressive, infectious, predominantly viral myocarditis and its possible transformation into CMP is very tempting [14]. The role of autoimmune disturbances in the pathogenesis of the disease and of the pathogenetic role of T suppressor deficiency in myocarditis and CMP has been widely discussed [6, 13].

The aim of this investigation was to study the effectiveness of the immunomodulating agent T-activin in myocarditis caused by Coxsackie virus in BALB/c mice. To produce experimental VM, the procedure followed in previous studies was adopted [12, 14, 15].

EXPERIMENTAL METHODS

BALB/c mice, male and female, aged 2 months and weighing 16-20 g were infected intraperitoneally with 0.2 ml of Coxsackie B_1 virus in a titer of 10^{-3}-10^{-7} TCD/ml, obtained from the Virus Museum of the Institute of Poliomyelitis and Virus Encephalitis, Academy of Medical Sciences of the USSR. The animals were killed (under general anesthesia) by decapitation

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Institute of Experimental and Clinical Therapy and Institute of Experimental and Clinical Surgery, Ministry of Health of the Georgian SSR, Tbilisi.

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