INTERLEUKIN-1 PRODUCTION BY MONONUCLEAR LEUKOCYTES AND MESANGIAL CELLS IN EXPERIMENTAL NEPHROTOXIC NEPHRITIS

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The study of the mechanisms and factors involved in intercellular interaction in the renal tubules under normal and pathological conditions is a promising approach to the study of the pathogenesis of glomerular nephritis (GN). Proliferation of mesangial cells (MC), one of the principal manifestations of GN, is often due to infiltration of the glomeruli by circulating mononuclear (MN) leukocytes, which secrete various cytokines and growth factors [4, 5, 8, 11]. A central position among them is occupied by interleukin-1 (IL-1), which is involved in the development of immune information and induces proliferation of cells of many types [7].

In the investigation described below a culture of MC was used to study the mechanisms of their proliferation, on a model of nephrotoxic serum nephritis (NTS-nephritis) in rats.

EXPERIMENTAL METHOD

NTS-nephritis was induced in male Wistar rats weighing 150-200 g by intravenous injection of nephrotoxic serum, obtained by immunizing rabbits with rat renal glomeruli [3], on the 1st and 2nd days of the experiment in a dose of 0.8 ml/100 g body weight. Control rats received the same volume of physiological saline. On the 5th day of the experiment the animals were killed under phenobarbital anesthesia, blood was taken for biochemical testing, and the kidneys were removed with aseptic precautions for morphological investigation and to obtain cultures of glomerular cells. During the 18 h before injection of NTS and 18 h before sacrifice, the animals' urine was collected in order to determine the presence of proteinuria (by the sulfosalicylic acid method). Total protein, cholesterol, and nitrogen in the urine and blood serum were determined on a "Technicon" biochemical analyzer (England). A primary culture of MC from the renal glomeruli of the control rats and rats with NTS-nephritis was obtained by the method described by the writers previously [1]. The renal glomeruli were pressed through a sieve (pores 110 and 40 μ in diameter), and treated with collagenase solution in a concentration of 750 U/ml (type IV, from "Sigma," USA) for 1 h at 37°C. The glomeruli were then placed in wells of 24-well plates ("Flow Laboratories," England), whose surface had been treated beforehand with collagen solution, and cultured at 37°C in 95% humidity and with 5% CO_2 in the air, in DMEM culture medium ("Flow Laboratories") containing 20% fetal calf serum ("Serva," West Germany), glutamine (2 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml). Culture continued for 20 days under control of the phase-contrast microscope. On the 20th day of culture the number of cells were counted in five fields of vision, chosen arbitrarily in each well (magnification 320) and expressed as the average number of cells in a field of vision of the microscope. The intensity of MC proliferation was estimated as incorporation of ^{3}H-thymidine into nuclear DNA. The results were expressed as the number of counts per minute (average from three wells). The IL-1 concentration in the culture medium, collected on the 20th day of culture, was estimated in a biological test by measuring the increase in proliferative activity of PHA-stimulated thymocytes of 4-6-week-old BALB/c mice [9]. Samples of culture fluid were tested in dilutions of 1:2, 1:4, and 1:8. The results were expressed as the number of counts per

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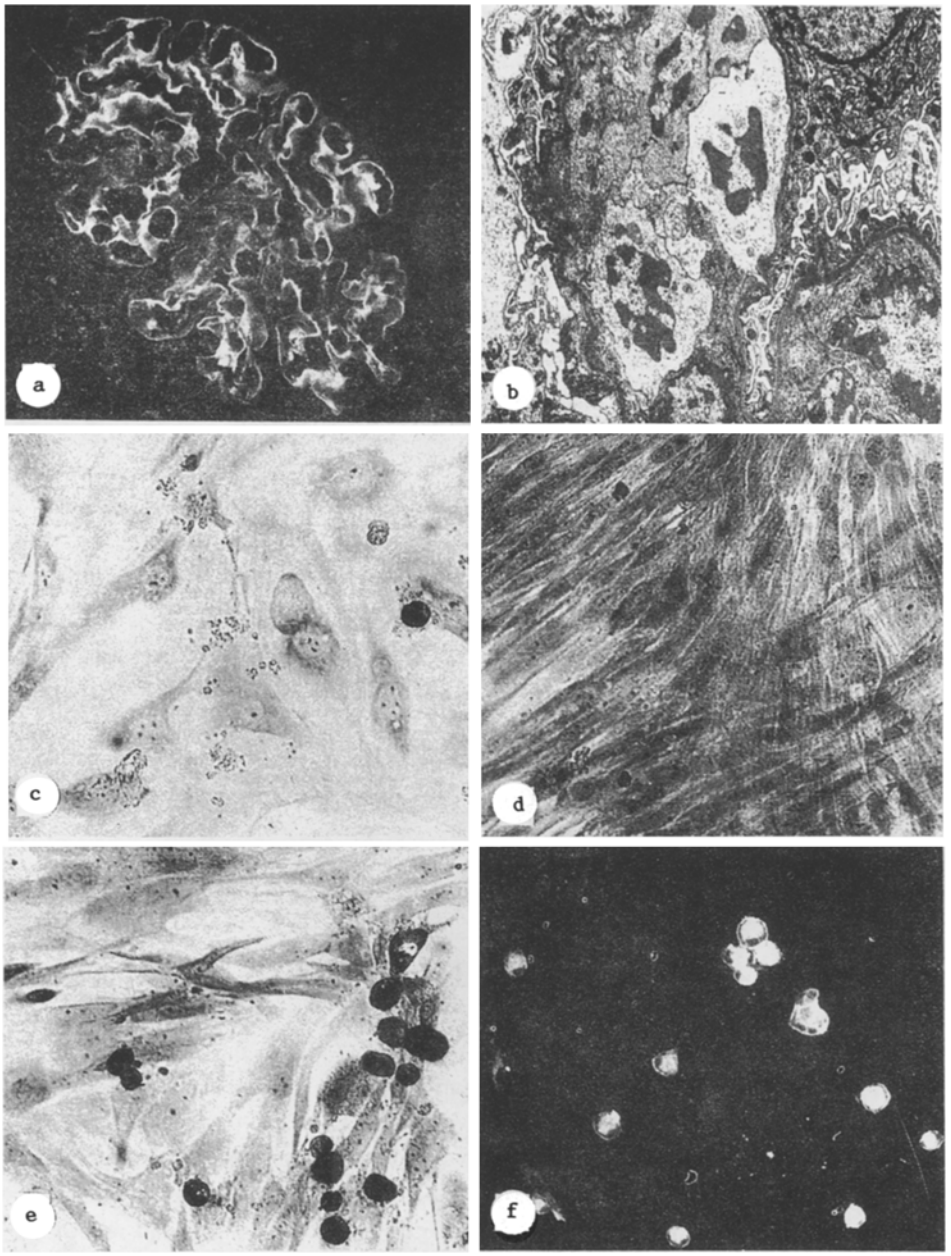
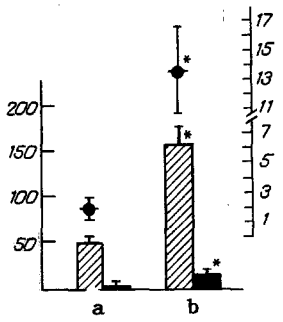


Fig. 1. Immunomorphological characteristics of NTS-nephritis in vivo and in vitro. a) Linear deposition of rabbit immunoglobulins along glomerular basement membrane. 400×. Direct immunofluorescence method. b) Fragment of renal glomerulus with proliferating MC and endothelial cells. 7100×. c) Monolayer of fibroblastlike spreading MC and solitary MN leukocytes. Azure II-methylene blue — fuchsine. 320×. d) Formation of monolayer culture of fusiform MC. 320×. e) Concentration of MN leukocytes around proliferating fusiform MC. 320×. f) Specific reaction of MN-leukocytes with antibone marrow serum. Indirect immunofluorescence method. 320×.

minute (average of three wells), and the level of IL-1-like activity in the culture fluid was judged by the magnitude of the stimulation index (SI).

The light-optical Investigation was conducted on semithin sections and in a monolayer of cells stained with methylene blue and azure II-fuchsine. Fragments of the kidneys for ultrastructural investigation were fixed with a mixture of glutaraldehyde and paraformaldehyde by Karnowsky's method, dehydrated, and embeddded in Araldite by the standard technique. Stained ultrathin sections were examined under the EM 410 electron microscope ("Philips," The Netherlands). The immunofluorescence investigation was conducted on unfixed frozen sections through the kidneys with sera against rabbit immunoglobulins, labeled with FITC ("Dakopatts," Denmark) and with sera against rat IgG ("Sigma," USA), and also in wells of culture plates with



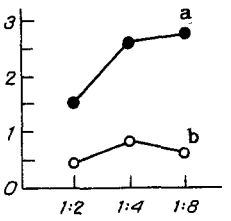


Fig. 2 Fig. 3

Fig. 2. Incorporation of ^{3}H-thymidine and number of MC per field of vision in control (a) and in NTS-nephritis (b). Dark columns — MN-leukocytes, shaded columns — MC. Ordinate, left — number of cells in field of vision, right — incorporation of ^{3}H-thymidine (in cpm · 10^{-2}).

Fig. 3. IL-1 activity in culture fluid of intact rats (a) and rats with NTS-nephritis (b). Abscissa, dilution of culture fluid; ordinate, SI.

monoclonal antibodies to myosin, vimentin, and desmin ("Dakopatts," Denmark) and with serum against rat bone marrow cells [2].

EXPERIMENTAL RESULTS

The protein concentration in the urine of rats with NTS-nephritis rose from 3.1 ± 0.46 to 109.5 ± 18 mg ($p < 0.01$) by the 5th day, whereas levels of cholesterol, creatinine, and nonprotein nitrogen in the blood serum showed no significant change. These parameters in the control group remained within normal limits.

During immunofluorescence investigation of the kidney tissues of rats with NTS-nephritis, linear deposits only of rabbit immunoglobulins were observed along the glomerular basement membrane (GBM) (Fig. 1a). On ultrastructural investigation the glomeruli were enlarged, and swelling and proliferation of the endothelial cells and areas of detachment of the endothelium from GBM were detected in the dilated capillary loops. In the lumen of the capillary loops and beneath the detached endothelium deposits of fibrin were observed. The mesangial zone was widened due to segmental proliferation of MC (Fig. 1b).

The culture of renal glomerular cells of intact rats on the 20th day of cultivation consisted of a monolayer of spreading fibroblastlike MC of smooth-muscle type and single round cells with a large nucleus and a narrow rim of cytoplasm (Fig. 1c). Concentrations of round cells were observed in the culture of renal glomerular cells of rats with NTS-nephritis on the 20th day, and fusiform MC of smooth-muscle type formed several cellular layers (Fig. 1d, e). On immunofluorescence study with antiserum to rat bone marrow cells, specific fluorescence was observed only on the round cells, indicating that they were MN-leukocytes (Fig. 1f). The rate of proliferation of MC of rats with NTS-nephritis was significantly higher than in the case of intact rats: incorporation of ^{3}H-thymidine into cellular DNA amounted to 1383 ± 288 and 218 ± 58 cpm respectively ($p < 0.05$; Fig. 2). This coincided with the greater content of MC in the cultures of rats with NTS-nephritis than in the control (155.0 ± 16.0 and 50.3 ± 2.6 cells per field of vision, $p < 0.05$) (Fig. 2). Meanwhile, there were five times as many MN-leukocytes in cultures of renal glomeruli of rats with GN (15.7 ± 4.5 and 3.3 ± 1.5 cells per field of vision respectively, $p < 0.05$) (Fig. 2). On testing of the supernatant of the culture of experimental and intact rats, IL-1-like activity was found in rats with NTS-nephritis (Fig. 3). IL-1 activity was intensified on dilution of the medium. In a maximal dilution of 1:8, SI amounted to 2.7.

Thus, judging by incorporation of ^{3}H-thymidine, active proliferation continued in the primary culture of MC of rats with NTS-nephritis, even on the 20th day, whereas in the control, cell division was already virtually absent. As a result, from the culture of MC, consisting of only one layer under ordinary conditions of culture, became stratified, forming "hillocks" in places — regions of accumulation of mesangial matrix, which are regarded as analogs of the zones of mesangial sclerosis in patients with GN [13]. The number of MN-leukocytes also was considerably higher in the culture of MC from rats with NTS-nephritis. By contrast with the control, growth factor with biological activity of IL-1 was present in the supernatant of this culture. With increas-

ing dilution of the supernatant its ability to stimulate thymocyte proliferation increased, evidently reflecting the presence of factors inhibiting proliferation in it also. On dilution of the supernatant, the effective concentration evidently fell faster than the IL-1 concentration.

When active prolonged proliferation of MC is compared with the presence of a large number of MN-leukocytes, it must be recalled that the IL-1 secreted by them potentiates MC proliferation, and at the same time, it is produced by them also, as an autocrine growth factor of MC [12]. Thus IL-1 in our model may have two sources: MC and MN-leukocytes.

MN-leukocytes play the leading role in the development of immune inflammation in NTS-nephritis. Signs of glomerular damage (hypercellularity, proteinuria, etc.) can be prevented by measures aimed at damaging MN-leukocytes (irradiation, injection of "antilymphocytic" and "antimacrophagal" sera) [8, 10]. The fact that prolonged active proliferation of MC and IL-1 production in a culture of renal glomerular cells of rats with NTS-nephritis were combined with the presence of a large number of MN-leukocytes suggests that it is these cells (through IL-1 production) that trigger and maintain proliferation of renal glomerular cells, and especially of MC, and that they are perhaps responsible for the analogous processes in GN in man.

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