CYTOPATHOGENIC ACTION OF RAT LYMPHOID CELLS TRANSPLANTED BENEATH THE RENAL CAPSULE INTO MICE WITH DEPRESSED IMMUNOLOGICAL REACTIVITY

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UDC 616-056.3-092.9-02:
612.42-018.1-089.843

A local "graft versus host" reaction was induced in CBA mice by inoculation of normal and specifically sensitized spleen and lymph gland cells of rats beneath the capsule of the left kidney. The most marked invasive and destructive changes in the kidney parenchyma (especially the tubules) were observed in animals irradiated in a sublethal dose of 600 R and mice with artifically induced tolerance to rat antigens. Tolerance was induced by transplantation in the adaptive period of about 20 million rat spleen cells. Donors' cells in the zones of infiltration were identified by the indirect Coons' method. The highest cytopathogenic activity was shown by lymph gland cells of a rat sensitized with mouse kidney antigens.

Investigations have shown that sensitized lymphocytes in tissue cultures can establish contact with and destroy the corresponding allogeneic and xenogeneic target cells [1, 8, 10, 13]. A similar process of local "graft versus host" type can also develop after intradermal injection of normal allogeneic lymphocytes [3, 12] and also after injection of parental lymphoid cells into the kidney [5, 6] or skin [7] of F_1 hybrids.

Changes in the kidney tissue after inoculation of normal and specifically sensitized rat lymphoid cells beneath the renal capsule of mice with partially or totally suppressed immunological reactivity were studied in the investigation described below.

EXPERIMENTAL METHOD

Male Wistar rats aged 4-6 months were used as the cell donors and CBA mice as recipients. In the experiments of series I 58 mice were irradiated in a lethal dose of 850 R with the RUM-11 apparatus (skin-focus distance 60 cm, filter: 0.5 mm Cu + 1 mm Al, current 10 mA, voltage 180 kV, dose rate 7 R/min). In series II 46 mice were irradiated in a sublethal dose of 600 R. The donor's cells were injected by Elkins's method [5] beneath the capsule of the upper pole of the left kidney 24 h after irradiation. In series III tolerance to rat antigens was induced in 59 mice by intraperitoneal injection of 2.3×10^7 to 2.6×10^7 rat spleen cells during the first 24 h of the postnatal period. The degree of tolerance was assessed from delay in rejection of full thickness rat skin grafts measuring 1.5×1.5 cm, grafted on to the lateral surface of the trunk of recipient mice aged 6-8 weeks [4]. Survival of the grafts was assessed by the criteria of Levey and Medawar [9] (Table 1). In the experiments of series IV, intact mice of the same age were used. The mice of each series were divided into six groups: in group 1 spleen cells of an intact rat were transplanted in doses of 4.12×10^7 to 4.72×10^7 in 0.05 ml medium no. 199, in group 2 from 3.6×10^4 to 4.2×10^7 normal rat lymph gland cells were injected, in group 3 from 3.9×10^7 to 4.4×10^7 sensitized spleen cells, and in group 4, from 2.3×10^7 to 2.4×10^7 lymph gland cells from a rat previously stimulated with mouse kidney antigens (20 mg homogenate injected subcutaneously into all four limbs plus 30 mg intraperitoneally) were injected. Cells were obtained from the spleen and lymph glands (inguinal, axillary, retroperitoneal,

Department of Pathological Physiology, Kazan Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR, A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 73, No. 6, pp. 65-68, June, 1972. Original article submitted January 19, 1971.

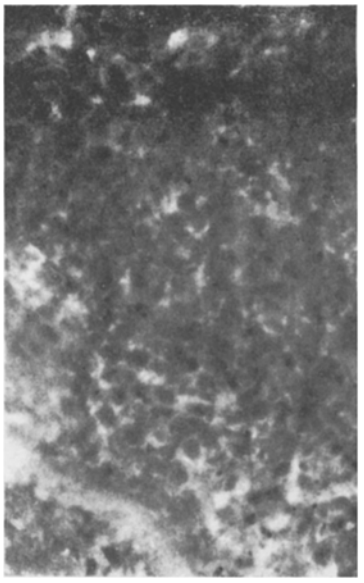

Fig. 1

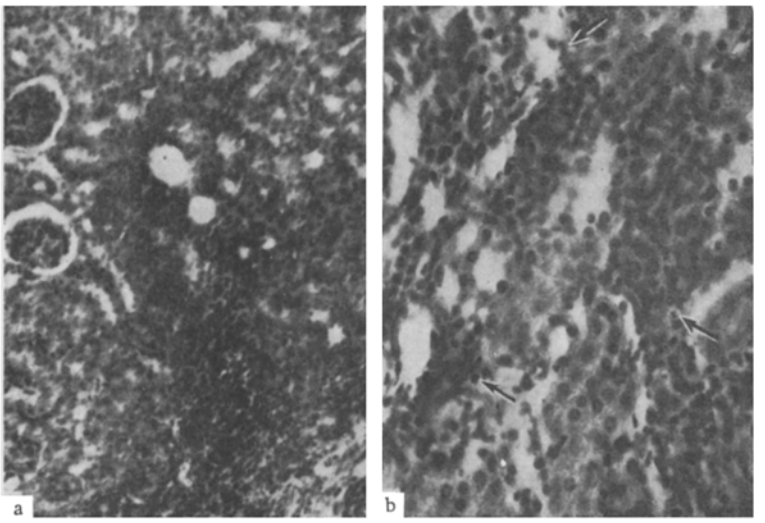

Fig. 2

Fig. 1. Cryostat sections of the kidney of a mouse irradiated in a dose of 600 R 7 days after transplantation of 4.4×10^7 sensitized rat spleen cells. Fluorescence of donor's cells in subcapsular zone of infiltration (on the right). Coons' indirect method, 400 ×.

Fig. 2. Section through the kidney 7 days after transplantation of 2.4×10^7 sensitized rat lymph gland cells (hematoxylin-eosin); a) massive infiltration of monocytes in subcapsular zone with high invasive activity (top right). Complete obliteration and disintegration of tubules in center of zone of infiltration; 140 ×; b) invasion of individual tubules by infiltrating monocytes (arrows). Structure of glomeruli intact (below); 280 ×.

TABLE 1. Survival of Skin Heterografts on Intact Mice with Artificially Induced Tolerance (M ± m)

Mice	Number of observations	Mean period of survival of skin graft (in days)	
		rat	guinea pig
Intact	26	5,3±0,8	4,3±0,6
Tolerant	34	12,6±1,9	4,8±1,2

Note: The test with both grafts was carried out simultaneously.

and mesenteric) 7-11 days after immunization. The method of preparation of the cell suspension was described previously [2]. Films were made from the suspension and the cell formula determined. The mice of group 5 received injections of completely disintegrated sensitized lymph gland cells (homogenization at 2000 rpm for 3 min followed by freezing and thawing four or five times). The completeness of disintegration was verified by the trypan blue test. The mice of group 6 were transplated with syngeneic spleen cells. The animals were sacrified on the 3rd and the 7th-10th days. Both kidneys were fixed and embedded in paraffin wax by Sainte-Marie's method [14]. Sections were stained with hematoxylin-eosin and methyl green-pyronine by Brachet's method. The donors' lymphoid cells in cryostat sections of the kidney were identified by the indirect Coons' method. Hyperimmune rabbit serum against rat globulins, exhausted with a mixture of mouse serum proteins, as verified by immunoelectrophoresis and immunofluorescence, and monospecific luminescent ass serum against rabbit globulins (prepared at the N. F. Gamaleya Institute of Epidemiology and Microbiology) were used as the reagents.

EXPERIMENTAL RESULTS

The general changes in the kidneys observed in the mice of all groups regardless of the type of cells injected included a hematoma at the site of injury to the parenchyma, fibrosis and thickening of the capsule, and focal interstitial hemorrhages. On the third day of the experiment the transplanted lymphoid cells were arranged as a thin layer on the surface of the kidneys, and covered by the partially detached capsule. The

TABLE 2. Character of Local "Graft Versus Host" Reactions 7-10 Days after Transplantation of Different Types of Cells

Type of cells injected	Series I (irrad. in dose of 50 R)			Series II (irrad. in dose of 600 R)			Series III (induct. of tolerance)			Series IV (intact mice)		
	I	II	III	I	II	III	I	II	III	I	II	III
Normal spleen cells	3/6	0/6	1/4	0/4	0/4	2/8	0/8	0/8	0/7	0/7	0/7	0/7
Normal lymph gland cells	3/8	1/8	0/8	2/6	1/6	0/6	3/7	1/7	0/7	1/6	0/6	0/6
Sensitized spleen cells	3/10	2/10	0/10	1/8	4/8	3/8	4/12	5/12	3/12	2/8	0/8	0/8
Sensitized lymph gland cells	3/9	4/9	2/9	1/8	2/8	5/8	2/11	2/11	7/11	2/9	2/9	0/9
Disintegrated lymph gland cells	0/6	0/6	0/6	0/3	0/3	0/3	0/5	0/5	0/5	0/6	0/6	0/6
Syngeneic spleen cells	2/7	0/7	0/7	2/5	0/5	0/5	1/4	0/4	0/4	2/5	0/5	0/5

Note: Numerator gives number of kidneys with a marked reaction; denominator, total number of kidneys investigated.

zones of infiltration consisted of a few small cells with virtually no penetration into the interstitial tissue of the kidneys. On the 7th-10th day, with a well-marked reaction present at the upper pole of the kidney, a white, elastic nodule was formed and occupied between one-third and one-quarter of the total surface of the kidney. Depending on the character of the infiltration and the degree of injury to the renal parenchyma, the reactions could be divided into three types. Type I) a subcapsular nodule composed of monocytes covering the surface of the kidney and with no tendency toward invasion. Type II) subcapsular infiltration with limited power of invasion. Most of the cells were on the surface of the organ, but bands of cells were beginning to diverge from it and to penetrate into the cortex of the kidneys. The renal tubules, surrounded by monocytes, showed degenerative changes. Type III) extensive invasive and destructive processes [15]. The extensive foci of cellular infiltrations contained lymphocytes of different sizes and different degrees of maturity, histiocytes, and large pyroninophilic blast cells.

Some cells of donor type gave a positive indirect Coons' reaction (Fig. 1).

The renal tubules in the boundary zone between the kidney and the graft were "excised" by the infiltrating cells and destroyed. The glomeruli and adjacent tubules were less severely affected. Sometimes the kidney tissue was almost completely replaced by massive zones of cellular infiltration, among which only scattered and necrotic renal structures remained (Fig. 2). In many cases fragmentation of the tubules with desquamation of the epithelial cells, which showed vacuolation and necrobiosis and were found in the interstitial zones of infiltration and the lumen of the tubules, was observed. Many tubules were converted into shrunken bands of cells which had lost their characteristic structure. The glomeruli in the affected zones were sclerosed, and at the boundary with the graft they were in an ischemic state which contrasted with the hyperemia outside the reaction zone.

As Table 2 shows, the normal lymphoid cells had a weak cytopathogenic action. Meanwhile, after inoculation of the spleen cells and, in particular, the lymph gland cells from rats sensitized with mouse kidney antigens the frequency of the invasive and destructive reactions was appreciably increased. The results showed that a type III reaction developed most frequently in mice with artificially induced tolerance to donor's antigens. The lesions in the irradiated animals were more widespread and severe in character if a sublethal dose of 600 R was used, whereas after a lethal dose of 850 R, the reaction was most frequently abortive (types I and II).

In the intact mice (series IV) the lesions were localized in a narrow area, and they extended beyond the surface layer of the kidney cortex in only two of nine cases after transplantation of sensitized lymph gland cells.

Optimal conditions for manifestation of the cytopathogenic action of xenogeneic lymphoid cells thus occurred in these experiments after partial general suppression of the functions of the recipients' immunogenic system by irradiation and also after selective suppression of sensitivity to the donor's antigens by the induction of tolerance in the adaptive period. These results confirm the view that not only the harmful action of the donor's lymphocytes themselves on the host's target cells, but also the recipient's reactions in response to injection of the donor's cells [2, 6, 11], play an important role in the development of the "graft versus host" reaction.

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