SPECIFIC SUPPRESSION OF TRANSPLANTATION IMMUNITY IN RABBITS BY ANTISERUM OF DONORS IMMUNIZED WITH RECIPIENTS' LEUKOCYTES

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UDC 612.6.02.017.1.014.46:615.365.018.53

The antiserum of donor rabbits obtained against leukocytes of future recipients prolonged the life span of the donor's skin graft compared with a graft from an intact rabbit. This serum contained cytotoxins and induced blast-transformation of the recipients' leukocytes in vitro. The complement was cytotoxic for lymph gland cells of recipients which had received these sera. The fluorescent antibody method revealed globulin on these cells.

Preliminary findings [4] showed that allogeneic antileukocytic serum can inhibit transplantation immunity in rabbits and rats.

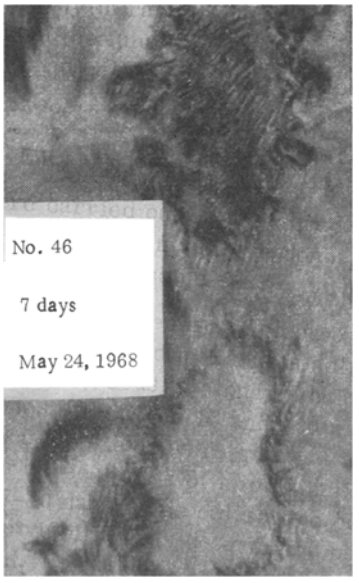

Fig. 1. Skin grafts on a recipient rabbit. Top graft from intact rabbit (extensive hemorrhages visible); bottom graft from AALS donor (normal).

The object of the investigation described below was to study the effect and mechanism of action of an allogeneic antileukocytic serum (AALS) on immunity in rabbits receiving skin grafts. The AALS was obtained from the future donor after preliminary immunization with living and dead cells from the recipient's spleen and lymph glands.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred rabbits, mostly females, weighing 2.5-3.5 kg. As a rule the donor and recipient were different in color but of the same sex.

Suspensions of cells from pieces of spleen or lymph glands of the future recipients were prepared in Hanks's solution and injected into the donor rabbits subcutaneously, along with Freund's adjuvant, and intravenously in doses of between 10 and 100 million cells. Between three and five such injections were given at intervals of 3-5 days. Altogether two or three cycles of immunization were carried out, with intervals of 17-30 days. The cells were counted in a Goryaev's chamber and their viability was determined by staining with trypan blue and eosin [1].

The AALS was obtained on the 7th-10th day after the last immunization of the donor rabbits. The titer of cytotoxins [1] and of leukoagglutinins [2] against cells of the recipients' lymph glands was determined. The same serum was injected into intact rabbits,

Central Research Laboratory, Vitebsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 70, No. 11, pp. 82-85, November, 1970. Original article submitted December 29, 1969.

TABLE 1. Results of Skin Grafting Procedures on Rabbits Receiving AALS

Group	Treatment of recipients	Number of rabbits	Beginning of rejection of grafts (days)		Titer of antibodies in AALS
			experimental	control	
1	AALS during grafting and 1-3 days thereafter in doses of 6-18 ml	10	14 ± 0.67 $P_{exp-c} < 0.05$ $P_{exp-4} < 0.001$	11.7 ± 0.85 $P_{c-4} < 0.001$	1:10-1:160
2	AALS daily, starting 5-9 days before grafting, in doses of 2-8 ml	7	10 ± 1.0 $P_{exp-c} < 0.05$ $P_{exp-4} < 0.02$	7 ± 1.0 $P_{c-4} > 0.1$	whole-1:16
3	AALS as in group 1, but from two donors	4	9.5 ± 1.32 10 ± 1.22 $P_{exp-4} < 0.05$	9.7 ± 1.1 $P_{c-4} < 0.02$	1:4-1:8
4	SND by the same scheme as AALS	9	6.7 ± 0.4	6.6 ± 0.53	——
	Nothing injected	8	6.5 ± 0.08	——	——

and the serum of these animals was injected into the recipients together with AALS. The intensity of the reaction (denoted by + signs) was read after 6-24 h.

Suspensions of cells of the rabbits' lymph glands, made up in medium No. 199 (0.5 million/ml), containing 20% AALS or inactivated intact serum, were incubated for 3 days at 37°C in Carrel's flasks. Cells from lymph glands of intact rabbits were incubated in a similar manner. Cytohemagglutinin (0.025 ml/ml) was added to some of the flasks with intact serum. Blasts were counted in 3-5 flasks in each variant of the experiment.

On each of the 38 recipient rabbits, two full-thickness skin grafts were applied to the dorsal region (6-9 cm^2 in each case). One graft (experimental) was taken from the donor of the antiserum, while the second (control) was taken from an intact rabbit. Rabbits injected with the serum of normal donors (SND) likewise each received two grafts of skin from different donors. The inguinal or axillary lymph glands were removed from some of these control recipients and splenectomy performed as a preliminary measure. These operations had no significant effect on the rate of survival of the grafts.

The cytotoxicity of complement for lymph gland cells of the recipient rabbits was tested at various times after the animals received injection of antiserum from the donors or of normal serum. For this purpose, 0.1 ml of whole or diluted active guinea pig complement was added to 0.1 ml of the suspension of lymph gland cells (2 million/ml, 90-95% of living cells), and in the control the same volume of inactivated complement (56°C for 30 min) was added. A second control test was set up with the same complement, but with lymph gland cells from an intact rabbit. Incubation continued for 1 h at 37°. The cytotoxic index was calculated.

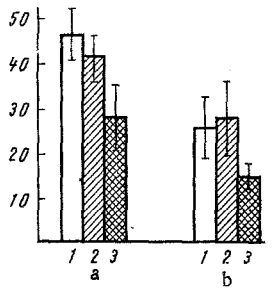

Fig. 2. Blast-transforming action of AALS: a) lymph gland cells of recipients of graft (donors of cells for preparing AALS); b) lymph gland cells of intact rabbits; 1) AALS; 2) normal serum + cytohemagglutinin; 3) normal serum. Ordinate: percentage of blast cells.

The direct and indirect fluorescent antibody methods [3] were used to study adsorption of globulin, injected with the AALS, on cells of the recipients' lymph glands. Suspensions of living cells obtained after injection of AALS were treated with sera labeled with fluorescein isothiocyanate (from the Gamaleya Institute of Epidemiology and Microbiology, Batch 400, working dilution 1:64, and batch 524-1, working dilution 1:32), and with preliminarily absorbed recipient's lymph gland cells obtained before injection of AALS, or cells of a control rabbit.

Morphological and histochemical studies of the rabbits' lymph glands were carried out before and after injection of AALS or SND. Sections through the lymph glands were stained with hematoxylin-eosin and Schiff's reagent, DNA was demonstrated by Feulgen's method, RNA by Brachet's method, and reticulin by the method of Gordon and Sweet [6].

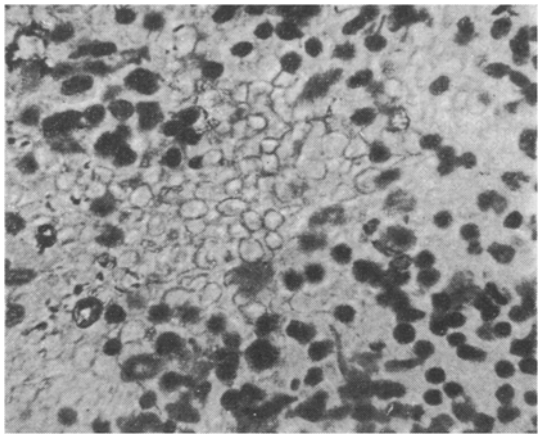

Fig. 3. Lymph gland of rabbit receiving AALS (10 days before allotransplantation). Stained for DNA by Feulgen's method, 280×. Explanation in text.

EXPERIMENTAL RESULTS

The criterion used to determine the beginning of rejection of the grafts and, consequently, to determine their life span was the time of appearance of the first signs of disturbance of the circulation on their surface (arterial or venous hyperemia, petechial hemorrhages). In some cases (some recipients after receiving AALS), when these signs were not present, the life span of the grafts was estimated from the beginning of their drying and compaction.

The general condition of the rabbits receiving AALS was not significantly affected. Depending on the doses and scheme of administration of AALS, the survival period of the grafts applied to the rabbits differed slightly. However, as a rule, a graft from the AALS donor (experimental) survived longer than a graft from an intact rabbit by 1-6 days (Table 1, groups 1 and 2; Fig. 1). At the same time, the life span of both grafts was increased on recipients receiving AALS compared with the grafts on rabbits injected with SND (Table 1, group 4). In the group of rabbits (Table 1, group 3) receiving AALS from two donors of the same litter (the two experimental grafts were taken from the same animals), no difference was observed in the survival period of the experimental and control grafts. However, in this group also the life span of all the grafts was longer than that of grafts on rabbits of group 4.

The titers of lymphocytotoxins in the AALS usually were low (Table 1). The lowest titers were observed in the sera of the rabbits of group 2, for which the last cycle of immunization was carried out with living lymph gland cells only. Cytotoxins were absent from the sera of some donors, and in such cases no lymphagglutinins likewise were found.

When intradermal injections of AALS from the donors were given to the recipients, the reaction in three of the seven rabbits was positive (from + to +++). The same antisera evoked no reaction or a weak reaction in the intact rabbits, and the sera of intact rabbits evoked no reaction in the experimental recipients.

AALS, when added in vitro to lymphocytes of rabbits whose cells were used for immunization, led to blast-transformation of the lymphocytes to a greater degree than the serum of intact rabbits. It also induced blast-transformation of the lymph gland cells of intact rabbits, but evidently to a lesser degree (Fig. 2). Blast-transformation of the cells was even induced by AALS in which no cytotoxins were detected.

Treatment with absorbed labeled sera of the lymph gland cells taken from rabbits after injection of AALS revealed the presence of globulin on their surface. This was shown by fluorescence of their surface in the form of half-rings or dots. The luminescence index was low (0.15-0.22). Either diffusely luminescent cells or single dots on their surface were visible in the controls.

Active (but not inactivated) guinea pig complement had a cytotoxic action on lymph gland cells taken at different times from rabbits injected with AALS. At the same time, it was not toxic for cells of control rabbits. Evidently antibodies injected with AALS were adsorbed on the lymph gland cells of the experimental rabbits, but in vivo, for some reason or other, rabbit complement had no cytotoxic action on them.

After injection of AALS into recipient rabbits, marked dedifferentiation of cells, an increase in the number of blast cells, and proliferation of the reticular cells and vascular endothelium were observed in their lymph glands taken before grafting. In some cases, karyopyknosis and karyolysis were observed in parts of the lymph glands in the paracortical zone, so that only cell ghosts and their reticular stroma could be seen (Fig. 3). No such changes were found in the lymph glands of rabbits receiving SND. Moderate hyperplasia was usually observed in such glands.

Either a temporary, moderate lymphocytopenia or, on the contrary, lymphocytosis was observed in the blood of the rabbits receiving AALS.

AALS obtained from donor rabbits against isoantigens of the recipients' leukocytes thus selectively inhibited the immune response to a graft from the donor to a greater degree than from an intact rabbit.

With its evidently largely specific cytotoxic or blast-transforming action on the recipients' lymphoid cells, it temporarily eliminated the clones of immunocompetent cells most incompatible with the donor.

The immunodepressive action of AALS during heart transplants in rats has also been demonstrated recently [5].

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