EFFECT OF CELLS AND SERUM OF DONORS IMMUNIZED WITH RECIPIENTS' LEUKOCYTES ON TRANSPLANTATION IMMUNITY IN RABBITS

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Cells from the lymph glands and spleen of donor rabbits, immunized with recipients' leukocytes, prolonged the life span of a donor's skin graft by comparison with a graft from an intact rabbit. Both grafts of these rabbits survived longer than grafts on the control groups of recipients. Allogeneic antileukocytic serum weakened the effect of the immune cells. Changes progressing to necrosis were observed in the lymph glands of rabbits receiving the immune cells, and the cells of these lymph glands were sensitive to the cytotoxic action of complement.

The graft versus host reaction (GVHR) can be used as a procedure to inhibit the primary immune response [6-9]. Foreign nonimmune and immune cells injected while the recipient is in a state of areactivity to them can depress both the synthesis of antibodies against different antigens and transplantation immunity [3, 4, 6-9]. Both a general and a local GVHR may occur, not withstanding a simultaneous response of the recipient to the graft [4, 6, 12]. This phenomenon evidently explains the increased survival of grafts in rabbits after preliminary regional transplantation of the lymph glands of donors immunized with antigens of the future recipients [1, 14, 15].

The object of the present investigation was to study the effect of cells and antiserum of specifically sensitized donors on the transplantation immunity of normal adult recipients.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred female rabbits weighing 2.5-3.5 kg. The donor and recipient had hair of different colors. The donor rabbits were immunized with suspensions of the spleen and lymph gland cells of the future recipients [2]. On the 5th-6th day after immunization the inguinal or axillary lymph glands were taken from the donors and suspensions containing up to 95% of viable cells were prepared from them [2]. The suspensions of lymph gland cells (up to $2 \cdot 10^7$-$18 \cdot 10^7$ cells) were injected intravenously into the recipients at the time of the skin grafting, and the donors were again immunized with the recipients' cells. From 5 to 6 days later viable donors' spleen cells were injected into the recipients (in a dose of $2 \cdot 10^8$-$2.8 \cdot 10^9$ cells per animal). The animals of one of the groups were injected with the cells of the immunized donors 30 min-2 h after intravenous injections of 10-20 ml allogeneic antileukocytic serum (AALS) of the same donors, the characteristics of which were described previously [2].

Two full-thickness skin grafts (each measuring 6-9 cm^2) were transplanted on the dorsal region of the recipient rabbits. One graft (experimental) was taken from the donor of the immune or normal cells, while the second (control) was taken from an intact rabbit.

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TABLE 1. Results of Skin Grafting in Rabbits Injected with Donors' Immune Cells (M ± m)

Group of animals	Materials for immunization of recipients	Number of rabbits	Beginning of rejection of grafts (in days)	
			experimental	control
1-я	Immune cells	11	14±1,3 (10—23) P_{e-c} <0,05 P_{e-2-5} <0,05 —0,001	10±1,4 (6—20) $P_{c-2,5}$ <0,05 $P_{c-3,4}$ >0,1
2-я	Immune cells disintegrated by freezing	4	6,5±0,58 (6—7)	6,5±0,6 (6—7)
3-я	Normal cells	7	7±0,6 (6—10) P_{e-c} >0,1	8±1,0 (6—14) P_{c-5} >0,1
4-я	AALS and immune cells	7	10±1,2 (7—16) P_{e-c} >0,1 $P_{e-2,3,5}$ <0,05	9±0,9 (7—14) $P_{c-2,5}$ <0,05 P_{c-3} >0,1
5-я	Control	8	6,5±0,1 (6—7)	6,6±0,3 (6—7)

Legend: e) experimental graft; c) control; numbers denote group numbers.

The action of complement [2] on the lymph gland cells of the recipient rabbits was studied. The significance of differences between the survival period of the experimental and control grafts within each group was determined by the criterion of signs [5] and between groups by Student's criterion.

EXPERIMENTAL RESULTS

The life span of the grafts was determined from the time until they began to be rejected, as shown by the appearance of the first signs of a disturbance of the circulation (arterial or venous hyperemia, petechial hemorrhages). This time corresponded to the period in which the state of the grafts was good [1]. This method of assessment eliminated the possibility of errors which arise when the life span of grafts is determined from their total necrosis.

The general state of the recipient rabbits injected with lymph gland and spleen cells of the unimmunized donors was satisfactory. A temporary lymphocytopenia was observed in the blood, accompanied by a marked leukocytosis. In some rabbits after injection of spleen cells (5th–6th day), which was preceded by injection of lymph gland cells on the day of transplantation, dyspnea was observed and 3 of the animals died with signs of shock soon after this injection.

In all rabbits of group 1 receiving lymph gland and spleen cells of the immunized donors, as a rule the donor's graft survived 2–10 days longer than the graft from the intact rabbit. Besides this specific effect, a nonspecific effect was observed: increased survival of the experimental and control grafts on the recipients of group 1 compared with the grafts on animals of all other groups. In some rabbits signs of rejection of the graft appearing on the 6th or 14th day (patches of venous hyperemia and petechial hemorrhages) disappeared later, moderate desquamation of the epidermis was observed with loss of hair, and the grafts remained in this satisfactory state until their eventual death, which was preceded by a more abrupt disturbance of the circulation (edema, large hemorrhages).

The results of the histological study of the grafts also confirmed that whereas grafts from the cell donors were viable, those from the intact rabbits had undergone necrosis. The epithelium of the former proliferated, the dermis was moderately collagenized, and evidence of hyperkeratosis was present in the epidermis, although no infiltration by the recipient's cells was observed (Fig. 1a). The grafts from intact donors were considerably infiltrated by the recipient's monocytes and lymphocytes, no epithelial proliferation was present, but instead its destruction could be observed (Fig. 1b). The prolongation of the life of the grafts did not depend significantly on the number of donor's cells injected. In four rabbits into which spleen and lymph gland cells were injected only on the day of grafting, no delay in rejection of the grafts occurred.

It must be emphasized that in the rabbits of group 2, which also received cells which were of immunized donors, but disintegrated by freezing and thawing, no prolongation of survival of the grafts was obtained.

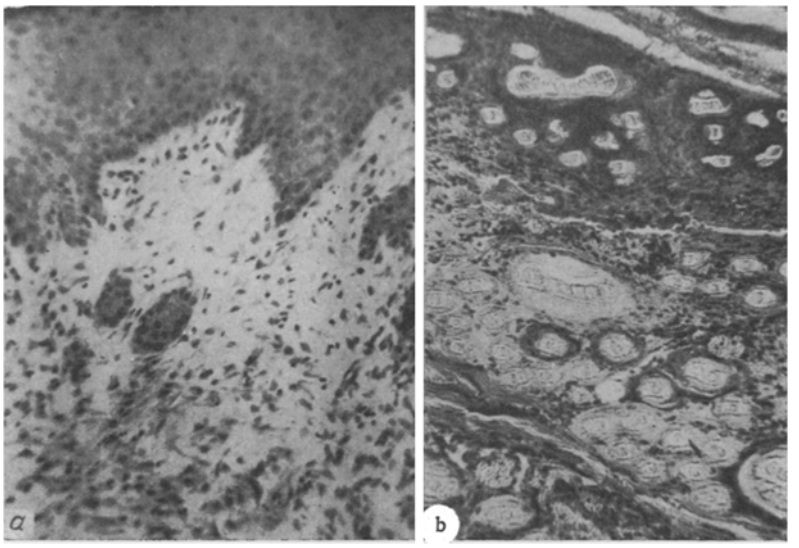

Fig. 1. Grafts on rabbit No. 43, 16 days after transplantation: a) graft from donor of immune cells; b) graft from intact rabbit. Explanation in text. Hematoxylin-eosin. a) 140 ×, b) 100 ×.

Injection of viable cells of unimmunized donors into the recipients under these experimental conditions did not significantly affect the result of transplantation (Table 1, group 3). Longer survival of the graft from the donor of the cells (by 3 days) or, conversely, the grafts from the intact rabbits (by 8 days) was observed in only 2 rabbits of this group.

Cells of the lymph glands or spleen of the immunized donors, if injected intradermally in a dose of 0.05 ml (from 300,000 to 1,000,000 cells) simultaneously with their intravenous injection evoked, after 24 h, a reaction (from ++ to +++) in only 4 rabbits of group 1 (in 2 of them the longest increase in survival of the experimental grafts was observed). Cells of normal donors usually did not give rise to reactions of this type from − to +).

The most marked changes were observed in the lymph glands of recipient rabbits which received lymph gland cells on the day of grafting and which died on the 5th-6th day after injection of spleen cells of the immunized donors. Complete and, evidently, allergic necrosis of the central part of the lymph gland was observed in these recipients so that all that remained of the gland was cellular debris and a rim of cells at the periphery, most of which were immature eosinophils. Individual lymphocytes and lymphoblasts also were seen (Fig. 2a). In the rabbits which died 6 days after injection of the spleen cells of immunized donors (12th day after grafting) devastation of the medullary layer was observed. Only small pericapillary collections of lymphocytes were present in it. The stroma was structureless or only cell ghosts could be seen. Subcapsular foci of proliferation were present, consisting of large blast-like reticulum cells and lymphocytes with a Rieder type of nucleus. As a rule follicles were absent (Fig. 2b). The changes were less marked in the lymph glands of the surviving recipient rabbits. However, zones of devastation, lysis of the nuclei, and dedifferentiation of the cells were observed. These changes in the lymph glands of the recipients resembled those found in the GVHR [13].

Since immune cells and humoral antibodies may act synergically, potentiating each other's effect [10, 11], it was decided to study the combined action of the cells and serum of immunized donors on the immunity of the recipients (Table 1). In group 4, moderate delay (by 3-5 days) of rejection of the experimental graft compared with the control was observed in only 3 rabbits. However, the life of the grafts on the animals of this group was prolonged by comparison with that of grafts on the control group of rabbits (Table 1). Weakening of the immunodepressive action of the cells by antiserum is evidently explained by a phenomenon of the "potentiation effect" type [11]. The injected immune cells possibly formed antibodies which were adsorbed on the lymph gland cells of the recipient rabbits. This is confirmed by the fact that cells of 4 of 10 such rabbits underwent lysis under the influence of active (but not inactivated) guinea pig complement. Lymph gland cells of the other groups of rabbits, not receiving immune cells, were not lysed by complement.

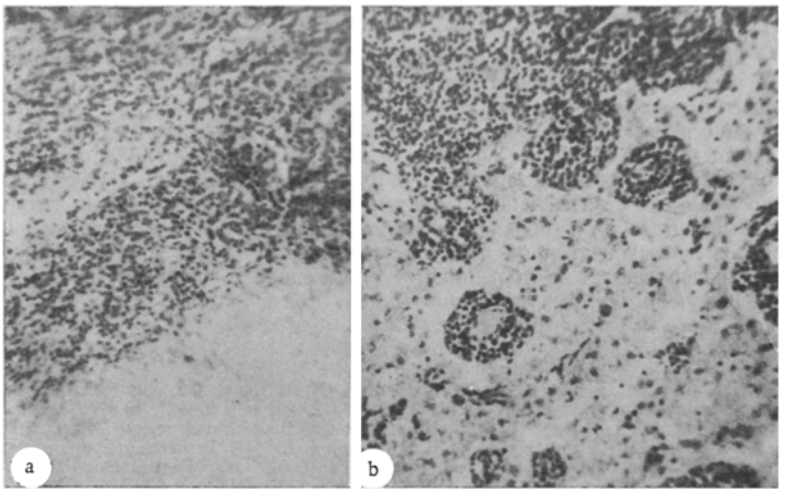

Fig. 2. Lymph glands of rabbits after injection of cells from immunized donors: a) lymph gland of rabbit No. 28; pale area below is a zone of necrosis; b) lymph gland of rabbit No. 51. Explanation in text. Hematoxylin-eosin, 140 ×.

The cells of immunized donor rabbits, injected into recipients, thus induced both a specific increase in the survival of the graft from the cell donor compared with the graft from the intact rabbit, and also a nonspecific depression of transplantation immunity in these animals compared with the control groups of recipients. Specific inhibition of transplantation immunity was evidently due to interaction between the GVHR and the newly developed reaction of the recipient to 2 grafts with a different isoantigenic spectrum, as a result of which clones of immunocompetent cells responding to the graft, and having common isoantigens with the immune cells injected, were selectively inhibited.

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