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Immunosuppressive Effect of Syngeneic Thymus Cells on Allograft Rejection

(suppressor cells/immunosuppression/skin graft rejection)

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ABSTRACT Transfer of thymus cells from young chickens to syngeneic recipients suppresses the allograft rejection between strains differing at the major histocompatibility (B) locus. Thymus cell transfer in combination with a light whole body irradiation (360 R) prolongs significantly the mean rejection time of skin allografts and leads in a proportion of recipients to long-lasting graft survival (>200 days). Three weeks after the cell transfer, the suppression appears to be antigen specific, as judged by the normal reactivity against third-party skin grafts. From the types of thymus cell preparations that are effective in these experiments, it is inferred that the suppressor cell is a bursa-dependent lymphocyte, which is predominantly found in the young chicken thymus and which is different from B-lymphocytes, B-precursor cells, or graft-versus-host-reactive T-cells.

Suppressive effects of thymus cells or thymus-derived cells on various types of immune responses have been observed in different species (for review, see refs. 1–3). In the chicken, thymus cells given together with antigen were found to suppress the serum antibody titers against this antigen (4), and also the number of plaque-forming cells in the spleen (W. Droege, unpublished). Experiments suggest that this suppression is mediated by a cell type (suppressor cell) which is different from typical B-cells as well as T-cells, and which in chickens is found predominantly in the young thymus (5, 6). This suppression is antigen-specific and by several criteria similar to the tolerance observed after neonatal application of antigen ("neonatal tolerance") (5).

Neonatal or prenatal contact with allogeneic cells can lead to specific tolerance and long-lasting skin graft survival in mice (7, 8) as well as in chickens (9). The analogy between the suppressive effect of young chicken thymus cells and the phenomenon of "neonatal tolerance" suggested that such thymus cell preparations might be able to mediate tolerance to allografts if allogeneic cells or tissue were used as antigen. The present experiments confirmed this partially. Transfer of syngeneic thymus cells in combination with sublethal whole body irradiation was found to mediate long-lasting skin graft survival in a significant proportion of experimental animals.

MATERIALS AND METHODS

White Leghorn chickens of the WC-line from Hy-line International, Johnston, Iowa, U.S.A. were used in all experiments as recipients and thymus cell donors. These birds were F_1 hybrids between two inbred strains identical with respect to the major histocompatibility locus (B^2/B^2). Thymus cells were always injected intraperitoneally (2×10^8 cells). Skin grafts (1×1 cm) were taken from 3-week-old White Leghorn chickens of the FS-line (Hy-line International) which were F_1 hybrids between two other inbred lines (B^{15}/B^{21}). Some animals received also skin grafts from 3-week-old random bred donors (Babcock White Leghorn from Hallauer Zuchtfarm, Hallau, Switzerland). The skin was grafted to WC recipients underneath the wing as shown in Figs. 2–4. The grafts were sealed with Histoacryl-Blau (Braun, Melsungen, Germany), covered with a thin film of nobecutan (Bofors, Nobel-Pharma, Sweden) and protected for the first 5 days with 12×2 cm bandage (Beiersdorf, Hamburg, Germany). The grafts were usually turned so that the follicles would grow in the opposite direction and could be distinguished from the animal's own feathers (see Fig. 2). The grafts were checked every second day for the first 4 weeks, and once a week for grafts surviving more than 30 days. Three types of results were observed: (*1*) acute necrosis (identified by the black color of the graft) starting on average 7 days after grafting and leading to complete rejection around day 15; (*2*) the chronic rejection process which is indicated by a dry, yellow surface of the graft; and (*3*) the long-lasting graft survival. The skin grafts in the latter case were soft like the animal's own skin and started to grow feathers about 30–45 days after grafting. FS thymus cell extracts were prepared essentially according to the procedure of Brent and Medawar (10, 11).

RESULTS

In the groups of experiments summarized in Table 1 (experimental schedule of Fig. 1), thymus cells were transferred 1 day prior to skin grafting. Additionally, whole body irradiation of 400 R or 360 R was given 2 days before thymus cell transfer. The thymus cell preparations were in some cases fractionated on bovine serum albumin step gradients with 9 ml steps from 230 to 300 mg of serum albumin per ml (Table 1, groups VI and VII).

The four experiments in Table 1 differed slightly in respect to dose and time of irradiation but gave essentially similar results. All control groups (I, III, V, VIII, XI) showed mean rejection times of about 15 days, and only a small proportion (0–7%) of long-surviving healthy grafts. Thymus cells from syngeneic young donors that were either untreated, sham-bursectomized or previously immunized with extracts from FS-thymus cells produced a significant prolongation of the graft rejection time and led in a proportion of recipients (17–42%) to long-surviving healthy allografts (Table 1, experi-

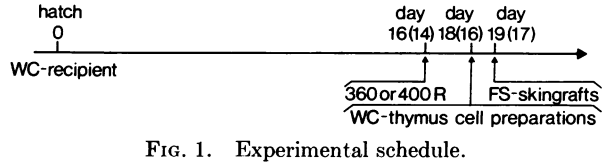

FIG. 1. Experimental schedule.

TABLE 1. *Effect of thymus cells plus irradiation on the allograft rejection*

Exp. group	Cell preparation	No. of animals	Rejection completed (days)*	% Graft survival†	
				Beyond 20 days	Beyond 100 days
I	—	40	14.6	13	5
II	18 days TN	11	24.6 (<0.005)	54	27
III	—	32	15.4	13	3
IV	9 days TN	6	23.3 (<0.05)	50	33
V	—	14	14.6	7	7
VI	5 weeks TSBx	12	25.1 (<0.025)	42	42
VII	5 weeks TBx	12	20.0 (N.S.)	25	25
VIII	—	22	16.6	14	4
IX	8 weeks TSBx	13	21.7 (<0.05)‡	46	23
X	8 weeks TBx	12	16.5 (N.S.)	17	0
XI	—	10	14.9	0	0
XII	7 weeks TSBx	18	21.1 (<0.01)§	33	17
XIII	7 weeks TBx	18	16.0 (N.S.)	17	0

WC chickens received 400 R on day 14 (groups V–VII), 400 R on day 16 (groups I and II), or 360 R on day 16 (all other groups). Two days after irradiation, the animals received thymus cells from normal syngeneic donors (TN), thymus cells from neonatally bursectomized (TBx) or sham-bursectomized WC-chickens (TSBx), or no cells (—). The age of the donors ranged from 9 days to 8 weeks. All animals received FS-skin grafts 3 days after irradiation. The thymus cells used in groups VI and VII had been fractionated in a bovine serum albumin density gradient and were recovered from the dense fractions (>280 mg/ml of BSA). The thymus cell donors in group II had been immunized 7 days before sacrifice with FS thymus cell extracts. This material was prepared essentially according to the procedure of Brent and Medawar (10, 11). Material from 0.5 ml of packed thymus cells was injected into recipients of 80 g. The recipients in groups VIII–X received FS-thymus cell extracts (material from 0.2 ml of packed cells per recipient) on day 18, 1–3 hr before the thymus cell transfer.

* Harmonic mean (P values for difference to controls, computed by Student's t-test; N.S. = not significant).

† Proportion of birds with healthy grafts.

‡ $P < 0.06$ for group IX different from group X.

§ $P < 0.05$ for group XII different from group XIII.

mental groups II, IV, VI, IX, XII). Thymus cells from neonatally bursectomized donors (groups VII, X, XIII) did not significantly prolong the graft rejection time. Thymus cells from 5-week-old bursectomized donors (group VII) were less effective than cells from the normal donors of the same age, but still produced some suppression. Presumably, the effect of bursectomy was not complete at this age. In the experimental group II, the thymus cell donors had been immunized with antigen extracts from FS-thymus cells, and in the experimental groups VIII–X the recipients had been immunized with similar antigen extracts several hours before the thymus cell transfer. So far, such additional immunizations did not seem to have much effect on the suppression of the graft rejection by thymus cells.

The good condition of the long-surviving grafts was evident from the feathers, which appeared between 30 and 45 days after grafting (see Fig. 2). Additional evidence for the persistence of donor tissue in the graft area was obtained by regrafting 5-month-old healthy grafts together with a rim of host skin to normal untreated recipients (Figs. 3 and 4). Six out of six secondary WC-recipients produced an acute necrotic reaction in the area of the original FS-grafts.

The specificity of the suppressive effect was tested in another experiment by setting two additional skin grafts 3 weeks after the first one. One of the secondary grafts was syngeneic, the other allogeneic to the first graft. Animals that received thymus cells and carried intact primary FS-skin grafts rejected the second FS-grafts usually not in an acute necrotic reaction and on average significantly slower than the third party grafts (Table 2). Control animals that did not receive thymus cells and which had already rejected the first FS-grafts rejected the second FS-grafts slightly faster than the third-party grafts and also slightly faster than untreated (unprimed) control animals did. The second FS-grafts in suppressed animals did, however, not survive as long as the first grafts, suggesting that the specific suppression is not the only mechanism operating in this system.

DISCUSSION

All experiments taken together show that thymus cells from young chickens can mediate a specific suppression of the allo-

TABLE 2. *Specificity of the suppressive effect of thymus cells*

Cell transfer	No. of animals	Rejection completed (days)*		P (Student's t-test)
		2nd graft from FS-donor	Graft from random-bred donor	
Thymus cells	6	19.8†	13.9	<0.005
None	3	11.8‡	13.3	N.S.

A group of chickens that received thymus cells and carried intact FS-skin grafts, and a group of control animals that already rejected the first FS-skin grafts, were grafted with random-bred skin and contralaterally with a second FS-skin graft 3 weeks after the first grafting.

* Harmonic mean.

† The first FS-grafts remained intact in all animals throughout the observation period.

‡ The mean rejection time for primary FS-grafts was 14.8. days.

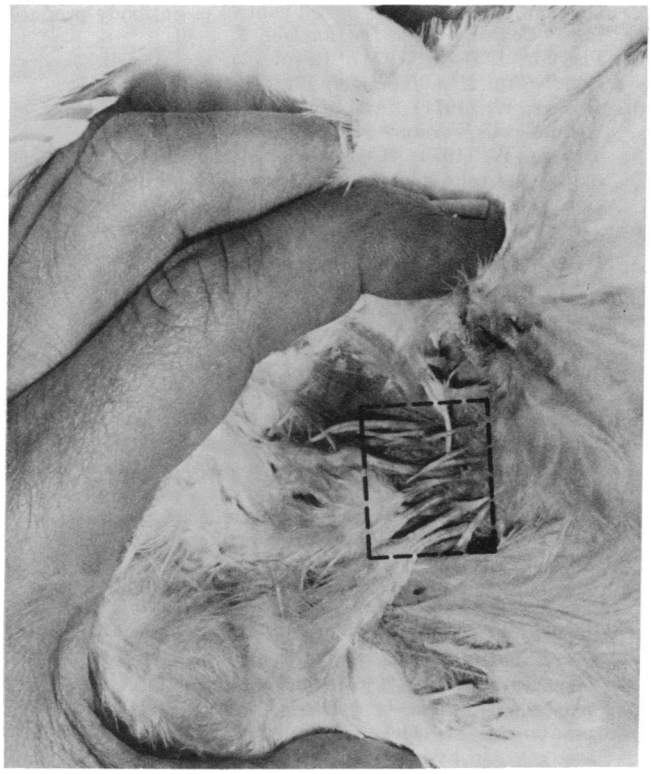

FIG. 2. Healthy skin graft with growth of feathers oriented in opposite direction to the animal's own feathers. The animal's front is on the left-hand side.

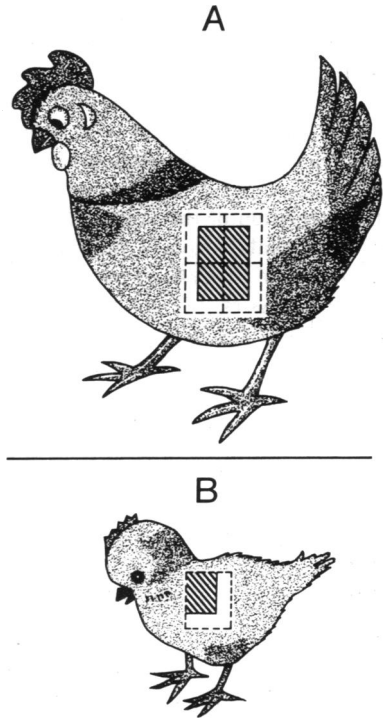

FIG. 3. Experiment to demonstrate the presence of donor tissue in the grafted area. Five-month-old healthy intact grafts were freed from feathers, excised together with a rim of surrounding host skin, and cut into four pieces of even size (A). Two of the pieces were grafted to WC-recipients (syngeneic to the former host), the other two to FS-chickens (syngeneic to the original graft donor). The area of the original graft was always placed into the upper left corner (B).

graft rejection. Syngeneic thymus cell transfer was found to prolong the rejection time and to mediate long-lasting healthy skin graft survival across a strong histocompatibility barrier in a proportion of experimental animals. However, not all types of thymus cell preparations were effective. Thymus cells from neonatally bursectomized donors never did produce a significant prolongation of the rejection time (Table 1). The activity of thymus cells from more than 8-week-old donors remains to be tested.

Previous experiments on the suppression of antibody production (4, 5) have shown that thymus cells from bursectomized donors were less suppressive than thymus cells from normal birds. Bursectomy, on the other hand, has no effect on the graft-versus-host reactivity of thymus cells (W. Droege, unpublished observation). Suppressive activity has also been found in the very young chicken thymus 10 days after hatching (5), when thymus cells have practically no graft-versus-host reactivity (16) and no detectable B-cell activity (13), or B-cell precursors (14). These and other

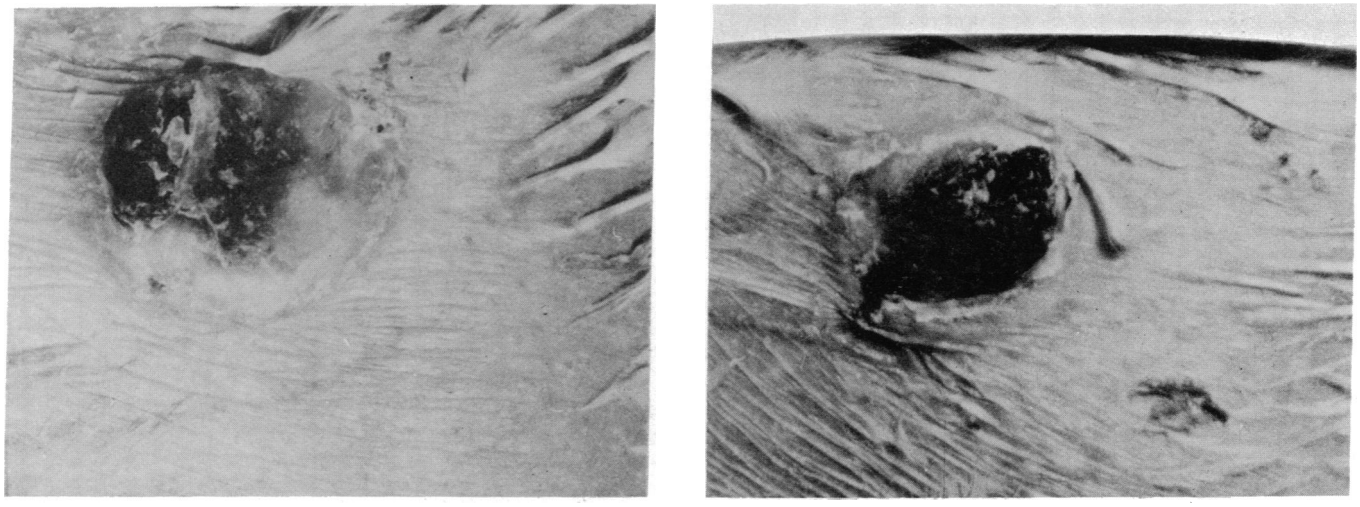

FIG. 4. Rejection pattern of regrafted skin (according to Fig. 3). The WC-recipient (left, no. 9671) shows acute rejection in the original graft area but not in the rim of WC-skin. The FS-recipient (right, no. 9562) shows acute necrosis in the rim of WC-skin, and a less acute rejection in the original graft area.

observations (see refs. 5, 6) suggest that the suppressor cells are different from graft-versus-host reactive T-cells, B-cells, and B-cell precursors. The present results with thymus cells from 8-day-old donors and with thymus cells from neonatally bursectomized donors indicate that the suppressive effect on the allograft rejection is mediated by the same type of suppressor cell.

The cellular composition of the young chicken thymus has been analyzed by a combination of physical cell separation techniques (12, 17). The analysis reveals the presence of at least three distinct classes of small lymphocytes in the chicken thymus of 8–16 weeks of age (12). The early postnatal chicken thymus (0–3 weeks) contains only one of these cell types (17), and the adult chicken thymus contains again only one type, but a different one (17). A dense subset of the early cell type is substantially decreased in neonatally bursectomized birds (12). These and other experimental observations indicate that the suppressive activity is associated with a bursa-dependent cell type of high buoyant density, which is found predominantly in the young chicken thymus and which is virtually absent in the adult chicken thymus (6).

The data allow no further conclusions towards the mechanism of suppression. Various authors have proposed mechanisms for suppression that are based on the assumption that suppressor cells and helper T-cells are identical or belong at least to the same cell lineage (13, 15). The bursa dependence of the suppression of graft rejection and humoral antibody responses suggest strongly that this is not the case in these experiments (see ref. 6). A possible mechanism for this type of suppression is discussed elsewhere (W. Droege, manuscript submitted for publication).

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