Brief Definitive Report

INFLUENCE OF THE THYMUS ON THE CAPACITY OF FEMALE MICE TO REJECT MALE SKIN GRAFTS

By ELIZABETH S. De PIRRO and ELLEN H. GOLDBERG

From the Department of Microbiology, University of New Mexico School of Medicine, Albuquerque, New Mexico 87131

Rejection of male skin grafts by otherwise histocompatible female mice varies greatly in strength from one inbred strain to another and is largely controlled by genes of the MHC (H-2) (1, 2). Females rejecting male skin grafts commonly produce CTL that destroy male but not female cells, as indicated in the cell-mediated cytolysis (CMC) assay in vitro, defining H-Y antigen (3, 4).

B6 females ($H\text{-}2^b$) are called H-Y rejectors because they invariably reject male skin and produce H-Y-specific CTL, as also do (B6 × C3H)F_1 hybrids.

C3H females ($H\text{-}2^k$) are called H-Y nonrejectors because usually they neither reject male skin nor produce H-Y-specific CTL. Nevertheless, C3H females retaining male skin grafts produce antibody specific for male cells, and a second male skin graft may undergo an abortive rejection response (5). Also, some aged C3H females, and some C3H females that have been thymectomized or splenectomized, will reject male skin, and CMC assay has revealed H-Y-specific CTL in thymectomized C3H females rejecting male skin (6–8). Therapy with TP-5, a pentapeptide analogue of the thymic factor thymopoietin, substantially counteracts the rejection-facilitating effects of aging and thymic deprivation in C3H females (7, 9).

There are further instances of the complexity of the H-Y immune response. Thus, H-Y-specific CTL may be generated in females of "low-responder" strains by altering the route of immunization or the source of immunizing male cells (10, 11), or by transferring immunopoietic cells of low-responder donors to the environment of high-responder recipients (12). Finally, in females of some strains, rejection of male skin grafts may not invariably accord with demonstrable generation of H-Y-specific CTL (13).

The aim of the following studies was to disentangle the influence of the thymus from all other circumstances affecting male skin rejection by observing the fate of identical male skin grafts in females that bore a substituted and histocompatible thymus of either rejector or nonrejector origin but were otherwise identical.

Materials and Methods

Mice. C3H/HeJ (C3H), C57BL/6 (B6), and (B6 × C3H)F_1 females (in designating hybrids the mother's strain is named first) were obtained from the Jackson Laboratory, Bar Harbor, ME. C3H females of the University of New Mexico (UNM) Animal Resource Facility were crossed with B6-congenic B6-Ly-5^a males, provided by Dr. E. A. Boyse from colo-

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nies at Memorial Sloan-Kettering Cancer Center (New York), to obtain (C3H × B6-Ly-5^a)F_1 fetuses, identified as female by the presence of ovaries, yielding liver cells, harvested at 14 d of gestation for hematopoietic reconstitution. Newborn donors of female thymic grafts and (B6 × C3H)F_1 donors of spleen or bone marrow cells for hematopoietic reconstitution were bred in the UNM facility.

Thymectomy and Thymic Grafting. Females were thymectomized at 4–5 wk of age. At no less than 10 wk of age, each received a renal sub-capsular implant of two or three lobes of thymus, taken from newborn females on the day of birth, and was irradiated within 24 h (see below). At the conclusion of experiments, all these mice were examined post mortem and found to possess a healthy thymic graft and to lack remaining host thymus.

Radiation Chimeras. On the basis of prior separate conventional estimations of the lethal dose 100/30 d for females of appropriate age, B6 females received a dose of 1,000 rad, and C3H and (B6 × C3H)F_1 females received 1,100 rad (250 X ray source; Phillips Electronic Instruments, Inc., Mahwah, NJ). Reconstituting female spleen cells (4×10^7), bone marrow cells (10^7), or fetal liver cells (10^7) were injected by tail vein within 24 h.

Donor Cell Typing of Radiation Chimeras. Essentially complete hematopoietic restoration of lethally irradiated (B6 × C3H)F_1 females (Ly-5.2) by (B6-Ly-5^a × C3H)F_1 donor cells (Ly-5.1) was verified by positive serotyping with Ly-5.1 mAb provided by Dr. E. A. Boyse (Sloan-Kettering Cancer Center). Hematopoietic restoration of lethally irradiated B6 or C3H females by (B6 × C3H)F_1 donor cells was verified with a relevant H-2 antibody.

Skin Grafting. Male tail skin grafts (~1 cm^2) were placed on a bed prepared on the dorsal thorax and were secured by sutures. Grafts were inspected at least twice a week and scored for unscathed acceptance, rejection with no more than a scab or scar remaining at the graft site, or transient crisis signifying an abortive homograft response, throughout the observation period of 90–100 d. Controls, identically prepared, received similar female skin grafts, which were invariably accepted, signifying H-Y rejection specificity.

Results and Discussion

Comparative Survival of (B6 × C3H)F_1 Male Skin Grafts in B6 vs. C3H Female Radiation Chimeras Restored with (B6 × C3H)F_1 Female Cells. The purpose of this study with radiation chimeras was to ascertain the H-Y rejector vs. nonrejector status of immune systems generated from identical immunopoietic cells within hosts of rejector (B6) vs. nonrejector (C3H) type.

F_1 hybrid hemi-syngeneic female donor cells, (B6 × C3H)F_1, were used in restoring lethally irradiated B6 and C3H females, thus precluding the occurrence and possible complications of graft vs.-host (GVH) disease in both B6 and C3H recipients. The results of male skin grafting, summarized in Table I, were decisive.

The B6 female chimeras displayed essentially the usual rejector reponses of normal B6 females, with all but one of 57 recipients rejecting their male skin grafts within the observation period of 90–100 d.

In contrast, the C3H female chimeras, restored with identical cells and receiving identical skin grafts, displayed the usual nonrejector characteristics of C3H mice, with all 54 recipients retaining their grafts throughout the entire observation period of 90–100 d.

As regards controls, essentially complete reconstitution by donor F_1 cells was verified by H-2 typing of lymphocytes, and H-Y rejection specificity was confirmed by identical control chimeras, all of which retained unscathed female skin grafts of the same F_1 origin as the rejected male grafts.

As Table I shows, there was no significant difference between spleen, bone marrow, and 14-d fetal liver as alternative sources of reconstituting donor cells.

Thus, the H-Y rejector vs. nonrejector status of an initially indifferent immunopoietic

TABLE I

Comparative Survival of (B6 × C3H)F1 Male Skin Grafts on Lethally-irradiated B6 or C3H Females Restored with (B6 × C3H)F1 Female Cells

Irradiated recipients	Source of (B6 × C3H)F_1 cells	No. of mice	Survival of (B6 × C3H)F_1 male skin grafts		
			Accepted	Rejected	Mean survival time*
					d
B6 ♀♀	Spleen	23	0	23	33.0 (19-42)‡
	Bone marrow	22	0	22	29.5 (21-46)
	Fetal liver	12	1	11	28.7 (18-34)
	Total	57	1	56	
C3H ♀♀	Spleen	22	22	0	-
	Bone marrow	15	15	0	-
	Fetal liver	17	17	0	-
	Total	54	54	0	

* Observation period, 90-100 d.
‡ Range.

cell population was seen to be determined during subsequent differentiation, presumably (at least primarily) within the thymus, although the design of this first study does not disclose whether the thymus was the sole or principal determining agent; but that becomes clear from the following study.

Comparative Survival of Identical Male Skin Grafts in Female Radiation Chimeras that have a Substituted Thymus of Rejector vs. Nonrejector Origin but are Otherwise Identical. The essential role of the thymus in determining rejector vs. nonrejector status was demonstrated in chimeras procured and skin grafted as follows. (*a*) (B6 × C3H)F_1 females were thymectomized at 4-5 wk of age. (*b*) Soon after 10 wk of age, they received a histocompatible graft of either a B6 or a C3H neonatal thymus. (*c*) About 24 h later they were lethally irradiated and restored with (C3H × B6-Ly-5^a)F_1 14-d female fetal liver cells. (*d*) 8-10 wk later they were grafted with (B6 × C3H)F_1 male skin.

The Ly-5 congenic strain B6-Ly-5^a, rather than B6, was used as a parent of the F_1 hybrid cell donors to provide a serological marker distinguishing donor cells from host cells and thus verify reconstitution by donor cells in both B6 and C3H (both strains being Ly-5^b). Although this might create a minor histoincompatibility, control data showing uniform acceptance of female skin indicate that this minor deviation from complete syngeneity was not a significant factor.

The results of male skin grafting, summarized in Table II, show a decisive difference between the recipients of B6 vs. C3H thymus grafts indicative of corresponding rejector vs. nonrejector status, respectively.

The thymectomized chimeras bearing a C3H thymic graft displayed the nonrejector status of C3H mice, with all 15 recipients retaining their male skin grafts throughout the entire observation period of 90-100 d.

In contrast, the otherwise identical chimeras bearing a B6 thymic graft displayed, in general, the rejector status of B6 mice; 19 of the 24 recipients rejected identical male skin grafts, and the male skin grafts in three of the five remaining recipients

TABLE II

Comparative Survival of (B6 × C3H)F1 Male Skin Grafts on Thymectomized Lethally-irradiated (B6 × C3H)F1 Females Bearing a B6 or C3H Thymic Transplant and Restored with (C3H × B6-Ly-5a)F1 Female Fetal Liver Cells

Recipient	Thymic transplant donor	No. of mice	Survival of (B6 × C3H)F1 male skin grafts		
			Accepted*	Rejected	Mean survival time
					d
Thymectomized (B6 × C3H)F1 ♀♀	B6	24	5	19	69 (35-90)§
	C3H	15	15	0	-
Thymus-intact B6 ♀♀	-	4	0	4	44 (39-47)
Thymus-intact C3H ♀♀	-	7	7	0	-

* Observation period 90-100 d.

‡ Three of which underwent a severe but abortive rejection crisis.

§ Range.

underwent a severe but abortive rejection reaction. In two respects the rejection response in this group was less intense than expected for intact B6 mice, since not all recipients completely rejected their male skin grafts and the mean survival time of rejected grafts was appreciably longer; these observations are the subject of further study, particularly in regard to possible secondary influence of an organ other than the thymus and of genes other than those of the MHC.

As regards controls, essentially complete reconstitution by donor cells was verified by Ly-5 typing of lymphocytes, and H-Y rejection specificity was confirmed by identical control chimeras, all of which retained female skin grafts of the same origin as the rejected male skin grafts.

Clearly, the genetic constitution of the thymus is the main agent determining H-Y rejection capability.

Summary

The ability of female mice to reject H-Y-incompatible, but otherwise histocompatible, male skin grafts differs greatly from strain to strain, as is illustrated particularly by the C57BL strain (B6 and other sublines), termed "H-Y rejector," because females invariably and promptly reject C57BL male skin, in comparison with the C3H strain, termed "H-Y nonrejector," because females characteristically accept male C3H skin. To assess the extent to which the thymus governs this rejector vs. nonrejector status, two studies were made. In the first, lethally irradiated B6 (C57BL) and C3H females were restored with (B6 × C3H)F1 female cells, providing a graft-vs.-host-free milieu for differentiation of the same immunopoietic cell population in B6 vs. C3H hosts. With respect to (B6 × C3H)F1 male skin grafts, B6 hosts responded as rejectors and C3H hosts as nonrejectors, signifying that rejector vs. nonrejector status was determined by the host during immunopoiesis. That the main organ responsible for rejector vs. nonrejector determination is the thymus was shown in a second study. Previously thymectomized (B6 × C3H)F1 females received a histocompatible graft

of thymus from either B6 or C3H neonatal females and were restored with donor-marked (B6-Ly-5^a × C3H)F_1 female cells after lethal irradiation. Wtih respect to (B6 × C3H)F_1 male skin grafts, the recipients of B6 thymus grafts responded generally as rejectors and the recipients of C3H thymus grafts responded uniformly as nonrejectors.

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