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Antibody Responses in Athymic ('Nude') Mice Implanted with Neonatal or Adult Allogeneic Thymus[1] (37808)

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Great interest has been generated recently by the nude (*nu/nu*) mouse because of its congenital thymic dysgenesis. Early work with this mouse has repeatedly shown lack of cell-mediated immune function (1, 2). Nude mice accept indefinitely skin allografts and xenografts (3, 4), lack delayed hypersensitivity (5), and are subject to wasting disease (6).

As in neonatally thymectomized mice, the antibody response to sheep red blood cells (SRBC) is also depressed in nude mice (7, 8). Humoral responsiveness in the thymectomized mouse can be restored by implantation of thymus cells (9, 10), indeed even with xenogeneic thymus (10, 11). In contrast, it was recently reported (12, 13) that restoration of the antibody response to SRBC in nude mice required grafting of thymocytes from histocompatible donors. Thus, Kindred (12) injected suspensions of thymocytes from Balb/c or C57 BL adult donors into recipients derived from the original outbred nude stock and backcrossed to the Balb/c strain. One group of nude recipients carried 50% or more Balb/c genes due to backcrossing. Another group carried less than 50% Balb/c genes. SRBC injections and titrations for serum hemagglutinins were done weekly on surviving mice. Kindred concluded that the restoration of antibody-forming potential by thymus grafts in nude mice requires a high degree of histocompatibility (50% or more backcrossing) between recipient and donor. She suggested that since nude mice lack graft rejection capability, the genetically dissimilar and presumably histoincompatible donor thymus cells survived in the nude host but could not cooperate with recipient cells.

We wish to report here the restoration of antibody-forming capacity in nude mice by thymus transplants from genetically dissimilar neonatal donor mice but not by thymus from 3-week-old donors.

Materials and Methods. Animals. The recessive nude mutant, *nu/nu,* arose in a closed but not deliberately inbred stock at the Ruchill Hospital, Glasgow, England. We received heterozygous breeders, derived from this original stock, as the generous gifts of Drs. S. P. Flanagan and K. Artzt. These mice were either +/*nu* or Balb/c/*nu* (single backcross). They were mated at random in our laboratory to produce homozygous nude offspring and phenotypically normal littermates. Mice heterozygous for the nude gene (+/*nu*) are not distinguishable from homozygous normal mice (+/+) except by mating tests. Confirming the observations of Pantelouris (1), we demonstrated normal first-set (11–13 day) rejection by phenotypically normal recipients of skin grafts from nonlittermate donors, indicating that the relationship among nonlittermate members of our colony was allogeneic.

Grafting. Thymus grafts were obtained from neonatal (less than 24-hour-old) or three-week-old donors which were phenotypically normal and had no nude littermates. Three-week-old nude animals, weighing 8–10 g, were the recipients. A single three-week-old thymus (approximately 70 mg), minced into 3-mm fragments, or a single whole neonatal thymus (approxi-

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mately 20 mg) was placed subcutaneously over the right flank. Graft sites were examined macro- and microscopically 4 weeks after implantation.

Immunization and serum titration. At seven weeks of age, all grafted mice, as well as normal and nude control animals, were injected intraperitoneally with 0.2 ml of a 20% saline suspension of SRBC. Sera were obtained 7 days after injection and stored at −20° until analyzed. Fifty-percent anti-SRBC hemolysin units/ml serum were determined as described earlier (14).

Results and Discussion. Table I lists mean arithmetic and logarithmic serum hemolysin titers in nude mice reconstituted with neonatal or three-week-old thymus, as well as in nude and phenotypically normal control animals.

As observed previously (8; McCann and Sussdorf, unpublished data), the mean anti-SRBC titer in nude animals was approximately 1 log unit lower than in phenotypically normal mice. Our failure to restore this depressed response with thymus grafts from histoincompatible young adult donors is consistent with the results of Kindred (12, 13). Successful restoration of antibody-forming capacity by thymus from histoincompatible neonatal donors is therefore a surprising finding.

Histologically verifiable thymus tissue was found at the graft site of all mice implanted with neonatal thymus but not in mice that received 3-week-old thymus. This absence of viable tissue is perplexing since it has been shown repeatedly that nude mice accept skin allografts indefinitely (2, 3). In our own laboratory, we have successfully grafted CBA mouse skin and even rabbit skin onto nude mice. However, because of the presence of mobile cells in the thymus, its subcutaneous implantation may constitute a different antigenic stimulus than does a skin graft and may produce a thymus-independent humoral response resulting in cytotoxic rejection. Thus, a factor cytotoxic for mouse thymocytes has been found in the serum of nude mice (16). The neonatal thymus, on the other hand, may survive because immunogens present on the older graft are not yet expressed.

An alternate explanation for the inability of the 3-week-old thymus to reconstitute the nude mouse hinges on the immunocompetence of the graft. Thymocytes have been shown to contain a minor population of cells capable of mounting a graft-versus-host (GVH) reaction (17). Also, interference with the antibody response to SRBC by a GVH reaction has been demonstrated in the rat (18). It is possible that in the nude mouse, thymocytes from an immunocompetent, allogeneic donor produce a low-grade GVH reaction which interferes with the reconstitution of antibody-forming capacity. Such an event is suggested in nude mice grafted with 3-week-old thymus (mean relative spleen weights of 5.5 and 8.5 mg/g in Groups A and D, respectively). The possibility of a GVH response is consistent with the data of Kindred's experiments (12) in which adult thymus from less than 50% inbred donor would be expected to produce a greater GVH reaction than would thymus from more inbred animals. Differentiation

TABLE I. Serum Hemolysin Titers 1 Week after Injection of SRBC into Athymic (Nude) Mice Previously Implanted with Thymus from Neonatal or 3-week-old Allogeneic Donors.

Group	Genotype	Thymus implant	No. of animals	50% Hemolysin units per ml serum (group mean)		P[a]	Viable thymus tissue found
				Arithmetic	Log		
A	*nu/nu*	none	7	51	1.63 ± .30[b]	—	—
B	+/?	none	9	720	2.84 ± .09	<.01	—
C	*nu/nu*	neonatal	8	770	2.70 ± .44	<.01	8/8
D	*nu/nu*	3-week-old	6	73	1.83 ± .16	n.s.	0/6

[a] *P* values for multiple comparisons against Group A (according to Dunnett (15)) after analysis of variance ($F = 33$, $P < .01$).

[b] ± standard deviation.

between these hypotheses requires information on the true fate of the 3-week-old thymus implant, *i.e.,* whether in fact it was destroyed by the recipient or whether its cells had emigrated from the graft site to engage in GVH activity.

Summary. It has been reported that antibody-forming capacity in athymic (nude) mice can be restored only by transplantation of thymus cells from histocompatible donors. In experiments reported here, nude mice received, 4 weeks prior to the injection of sheep erythrocytes, solid thymus grafts from neonatal or 3-week-old allogeneic donors. Restoration of antibody-forming capacity and persistence of transplants at the graft site were observed in animals receiving neonatal but not in animals receiving 3-week-old thymus tissue.

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