ACTIVITY OF HOST-DERIVED T CELLS WHICH DIFFERENTIATE IN NUDE MICE GRAFTED WITH CO-ISOGENIC OR ALLOGENEIC THYMUSES

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When a nude mouse is grafted with an allogeneic thymus from a neonatal donor, the thymus cells of donor origin, after a short period of proliferation, are replaced by cells of host origin (1, 2). These cells are typical thymocytes as they express on their plasma membrane both the θ-antigen, indicating they are T cells, and the thymus leukemia antigen (1). Also, lymph nodes and spleen are rapidly repopulated by T lymphocytes of host origin, though some transient appearance of T cells of thymus graft origin also occurs.

It was important to investigate the T-cell activity in such animals and to try to find answers to various self evident questions. Is there T-cell activity at all? Are all T-cell responses restored? Is the level of response normal? Is restoration due to T cells of thymus graft origin or of host origin?

A more critical question concerned a specific reactivity of those T cells that differentiate in the environment provided by a foreign thymus epithelium: would they recognize that epithelium as self or as nonself? Evidence obtained from allogeneic thymus grafts in neonatally thymectomized hosts suggests that the thymus graft actually sensitizes the developing host T cells (3, 4). Yet nudes grafted with CBA-T6T6 thymus accept subsequent CBA-T6T6 thymus grafts although they reject skin grafts from other strains (2). However, neonatally thymectomized mice are not necessarily comparable to nudes, they have had a thymus for the period of development and still have some peripheral T cells (5); consequently their deprivation is less.

That the sensitization is due to remaining T cells is suggested by the work of Stutman et al. (6) who found, using their system of neonatally thymectomized mice in hemiallogeneic combination, that induction of tolerance depends on the strain combination used. Furthermore, it is not known why nudes lack a thymus. Still undetected deficiencies of their immune system, other than the simple lack of T cells, might also be involved. The theoretical implications of self-recognition during embryonic development might be far more fundamental than simple immunological, i.e. immunoglobulin mediated, recognition, and these aspects merit further extensive studies.

Data presented in this paper are a first approach to the study of the T-cell reactivity in BALB/c-nu/nu mice which have been grafted with allogeneic or

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co-isogenic neonatal thymuses. Three different aspects of T-cell reactivity have been studied, which correspond to increasing degrees of specificity in the recognition structures of these cells: (*a*) the ability to respond to T-cell mitogens in vitro; (*b*) the ability to act in vivo as helper cells in the production of circulating antibodies against T-cell dependent antigens, and (*c*) the ability to act in vivo in cytotoxic reactions against foreign skin grafts.

Given evident requirements for T-cell membrane marker identification, our work on the repopulation of grafted thymuses by host-derived cells (1) was done using AKR as thymus graft donors, but as C57BL/6 newborns were more readily available, most of the allogeneic donors used in the present work were from the latter strain. In every experiment, some recipients of AKR thymuses were included to check if there was any essential difference.

Materials and Methods

Mouse Strains.—The mouse strains used were: AKR/J, CBA/J, C57BL/6JNIcr, BALB/cAnNIcr, and BALB/c-nu stock made by crossing the nude mutant to BALB/c. The mice used in this study were from the 7th or later backcross generation and grafted subcutaneously at 6–7 wk of age with thymuses from donors no more than 24 h old.

Skin Grafts.—Full thickness skin grafts were made as described by Ballantyne and Converse (7) and kept under positive pressure by an elastic bandage which was removed 10–12 days after grafting. For second set grafts to normal mice the bandage was removed earlier and the toenails of the mice were cut to prevent scratching. Skin grafts were examined daily for 2 wk following the removal of the bandage and then 2 or 3 times weekly as late rejections were always slow. Criteria for rejection were loss of the soft pink condition of the graft followed by the formation of a scab which was finally shed to disclose scar tissue.

Immunization Schedules.—To test the response to thymus-dependent antigens, 5×10^8 sheep red blood cells (SRBC)[1] or 10^{10} active T4 bacteriophage were injected weekly for 3 wk. The animals were then bled and the antibody assayed by hemagglutination or phage inactivation (8), as appropriate. Nude mice injected with allogeneic thymus cells or occasionally even untreated nude mice make hemagglutinating antibody against SRBC after one injection, but the response is less, not greater, following boosting (9) and after three injections no hemagglutinating antibody can be detected in such animals. The schedule for immunization with T4 was later found to be suboptimal; however, the same schedule was used for all the mice used in this study.

Immunofluorescence.—A preceding paper (1) has described the immunofluorescence techniques used to detect the various lymphocyte membrane antigens (θAKR, θC3H, TL, and Ig), as well as all details concerning the characteristics of the specific antisera used, and our methods for fluorescence microscopy.

Assay for Lectin Mitogenicity.—Concanavalin A (Con A, Sigma Chemical Co., St. Louis, Mo.), phytohaemagglutinin (PHA P, Difco Laboratories, Detroit, Mich.) and pokeweed mitogen (PWM, Gibco, Grand Island, N. Y.) were used without further purification. Their mitogenic activity was assayed according to Andersson et al. (10), i.e., by cultivating samples of 10^6 cells in 1 ml medium RPMI 1640 medium (Microbiological Associates Inc., Bethesda, Md.), 0,03M Hepes (*N*-2-Hydroxyethylpiperazine-*N*-2 ethanesulfonic acid, Calbiochem, Los Angeles, Calif.), 10% fetal calf serum (Gibco), 2mM glutamin, 100 IU/ml of penicillin-streptomycin Flow laboratories Ltd., Irvine, Scotland), in Falcon plastic tubes (no. 2058 Falcon Plastics,) Div. of Bioquest, Oxnard, Calif.), in the presence of various lectin doses. After 48 h of culture [^{3}H]thymidine was added (1 μC per culture) and the cells were kept in culture for another

[1] *Abbreviations used in this paper:* Con A, Concanavalin A; PHA, phytohaemagglutinin; PWM, pokeweed mitogen; SRBC, sheep red blood cell.

24 h. Then the cells were harvested, collected on Millipore filters (Millipore Corp., Bedford, Mass.), washed, precipitated with TCA, and the incorporated radioactivity was recorded.

RESULTS

A considerable number (150–200) of nu/nu mice backcrossed at least seven times to BALB/c were used for these studies and at least a quarter of the mice died or became sick during the course of the experiments and could not be used. We preferred to look for various parameters of T-cell related functions with relatively few mice for each of them, rather than to use all the animals for a given restricted field of T-cell reactivity. Therefore, only clear-cut facts have been considered and marginal responses have been disregarded.

In Vitro Responses to T-Cell Mitogens.—Two mitogens widely accepted as acting primarily on T cells were used, i.e., soluble Con A and PHA (11). PWM was also assayed as it has recently been found that its B-cell mitogenicity requires the presence of some T cells (12). These mitogens have been routinely used in our laboratory for other experiments and all three were always found ineffective in stimulating spleen or lymph node cells from nude mice. On the other hand, all three could stimulate spleen and lymph node cells from normal BALB/c or other mouse strains, and Con A could also stimulate thymus cells. The response was dose-dependent showing, for Con A, a maximum between 2.5 and 10 μg of lectin/ml culture, for PHA a maximum between 5 and 25 μg of lectin/ml culture, and for PWM a plateau established at about 10–50 μg of lectin/ml culture. Therefore, when enough cells were available a whole spectrum of Con A doses (1, 3.125, 6.25, 12.5, 25, and 50 μg/ml) and of PHA and PWM doses (1, 6.25, 12.5, 25, 50, and 100 μg/ml) was routinely used.

When nudes grafted with neonatal allogeneic thymuses were treated in the same way, they gave a response intermediate between BALB/c mice and untreated nu/nu. A qualitative representation of our observations is given in Table I and one experiment is explained in extenso below.

In the experiment represented in Fig. 1 and Tables II and III we looked at

TABLE I

Ability to Respond to T-Cell Mitogen: Nudes, Normal BALB/c, and Allogeneic Thymus-Grafted Nudes

Organ	Lectin	Nudes	BALB/c	Thymus-grafted nudes
Thymus	Con A		Poor	Poor
	PHA		None	None
	PWM		None	None
Lymph Nodes	Con A	None	Good	Good
	PHA	None	Good	Marginal
	PWM	None	Good	Marginal
Spleen	Con A	None	Good	Variable
	PHA	None	Good	Marginal
	PWM	None	Variable	Variable

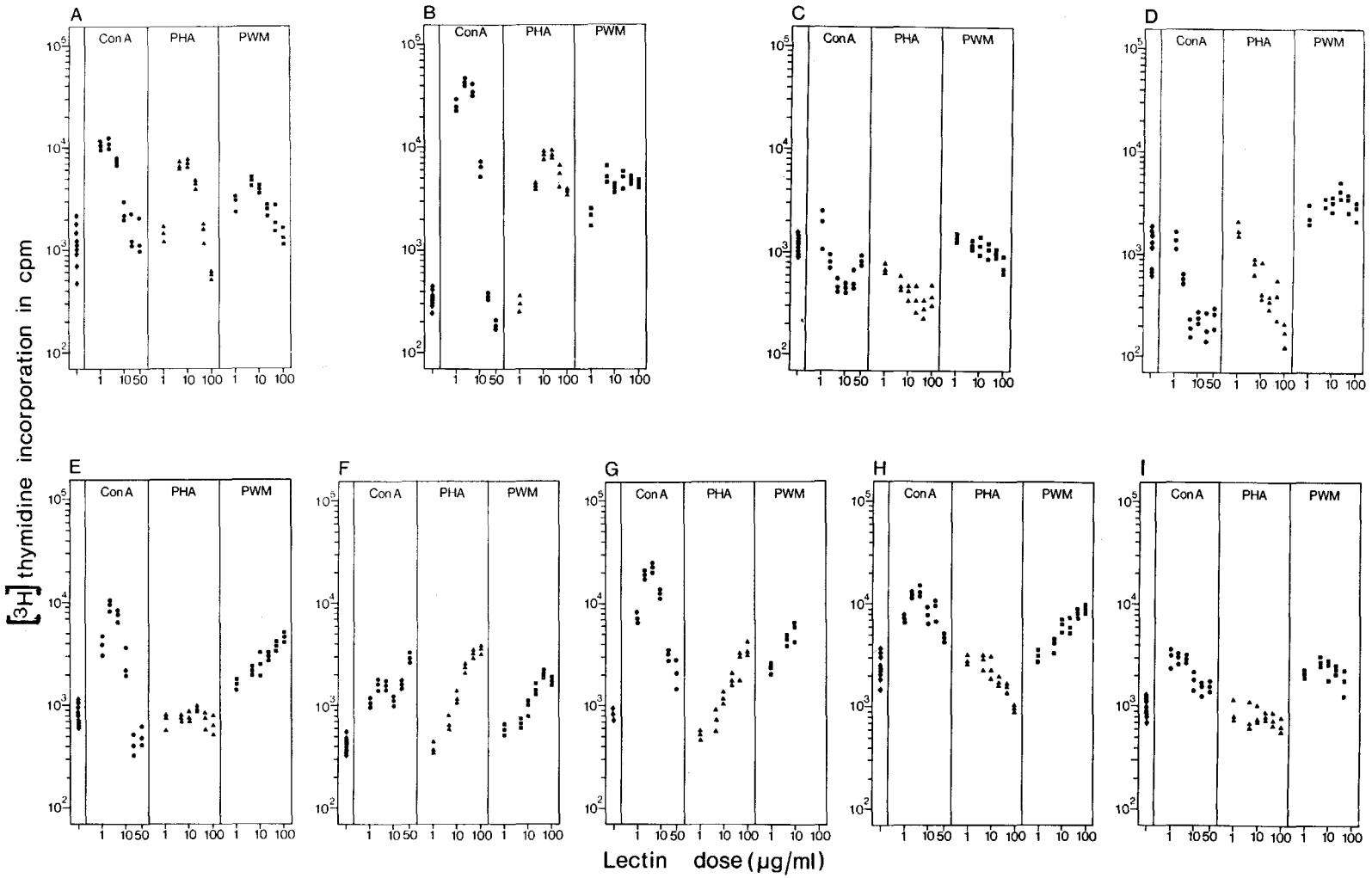

FIG. 1. Stimulation by Con A, PHA, and PWM of spleen cells from normal BALB/c (A and B), untreated nude (C and D), and BALB/c-nu grafted with C57BL/6 or AKR thymus (E-I) (see text). Background incorporation is shown in the left hand column. Normal BALB/c respond to all three mitogens while untreated nudes do not and grafted nudes respond consistently only to Con A.

TABLE II

*Stimulation by Con A, PHA, and PWM of Lymph Node Cells**

Mitogen	Dose	Animals						
		A	B	C	D	E & F	G & H	I
	μg/ml							
—	0	2.72 (1.17)	2.53 (1.07)	2.57 (1.25)	3.33 (1.06)	3.44 (1.09)	3.94 (1.03)	2.86 (1.10)
CON A	1	4.42 (1.02)	4.52 (1.05)	2.75 (1.08)	3.26 (1.00)	4.08 (1.06)	4.47 (1.02)	3.41 (1.02)
	3.125	4.59 (1.09)	4.91 (1.05)	2.53 (1.15)	2.88 (1.19)	4.21 (1.08)	4.65 (1.06)	3.06 (1.06)
	6.25	4.37 (1.02)	5.01 (1.06)	2.56 (1.05)	2.42 (1.09)	3.95 (1.10)	4.60 (1.08)	2.71 (1.03)
	12.5	3.11 (1.51)	4.45 (1.03)	2.60 (1.28)	2.47 (1.18)	3.74 (1.07)	4.11 (1.06)	2.73 (1.16)
	25	2.68 (1.13)	2.50 (1.32)	2.30 (1.15)	2.45 (1.09)	2.62 (1.27)	2.97 (1.07)	2.61 (1.15)
	50	2.48 (1.10)	2.29 (1.19)	2.39 (1.24)	2.59 (1.07)	2.24 (1.06)	2.79 (1.08)	2.48 (1.22)
PHA	1	2.71 (1.05)	2.68 (1.06)		3.18 (1.05)	3.36 (1.02)	3.86 (1.03)	2.84 (1.05)
	6.25	3.87 (1.07)	4.01 (1.11)		2.87 (1.07)	3.17 (1.02)	4.27 (1.17)	2.81 (1.26)
	12.5	3.98 (1.02)	4.28 (1.04)	2.46 (1.10)	2.71 (1.03)	3.15 (1.07)	4.12 (1.10)	2.45 (1.19)
	25	3.95 (1.18)	4.38 (1.02)		2.63 (1.10)	2.96 (1.05)	4.01 (1.10)	2.31 (1.08)
	50	3.60 (1.13)	4.36 (1.12)		2.49 (1.27)	2.57 (1.08)	3.77 (1.08)	2.32 (1.08)
	100	3.10 (1.17)	4.25 (1.03)		2.52 (1.09)	2.46 (1.13)	3.30 (1.22)	2.51 (1.20)
PWM	1	3.38 (1.05)	3.13 (1.14)		3.35 (1.06)		3.97 (1.03)	3.20 (1.10)
	6.25	3.74 (1.08)	3.61 (1.03)	2.41 (1.05)	3.39 (1.06)		4.19 (1.04)	3.32 (1.11)
	12.5	3.86 (1.05)	3.73 (1.05)	2.44 (1.11)	3.43 (1.03)	4.01 (1.04)	4.24 (1.01)	3.29 (1.23)
	25	3.80 (1.01)	3.85 (1.02)		3.45 (1.02)	4.03 (1.05)	4.23 (1.04)	2.98 (1.05)
	50	3.89 (1.12)	4.00 (1.02)		3.45 (1.02)		4.26 (1.03)	2.89 (1.06)
	100	3.79 (1.06)	4.01 (1.06)		3.49 (1.04)		4.29 (1.03)	2.60 (1.16)

* [^{3}H]thymidine uptake is expressed in logarithmic units as mean (standard error).

A and B, normal BALB/c; C and D, untreated nudes; E – I, BALB/c-nu grafted with C57BL/6 or AKR thymus (see text).

Normal mice respond to all three mitogens, untreated nudes do not respond and the grafted nudes show intermediate responses.

the mitogen stimulation for the whole series of mice at the same time, i.e., under exactly the same conditions of culture, and with exactly the same lectin preparations. The effect on spleen, lymph node, and thymus was checked for the whole range of lectin doses when enough cells were available.

The mice used for this experiment were: four normal BALB/c, four untreated nudes, four BALB/c-nu/nu which had been grafted with C57BL/6 thymuses 55 and 56 days previously, and one BALB/c-nu which had been grafted with an AKR thymus 45 days previously.

TABLE III

*Stimulation by Con A, PHA, and PWM of Thymus Cells**

Mitogen	Dose in	Animals						
		A	B	C	D	E & F	G & H	I
	μg/ml							
—	0	2.38 (1.18)	2.53 (1.07)			2.61 (1.18)	2.31 (1.21)	2.54 (1.34)
CON A	1	2.98 (1.29)	4.52 (1.05)	Athymic				
	3.125	3.09 (1.24)	4.91 (1.05)			3.58 (1.12)	3.14 (1.12)	
	6.25	2.84 (1.10)	5.01 (1.06)			3.62 (1.15)	2.99 (1.11)	
	12.5	2.80 (1.11)	4.45 (1.03)					
	25	2.75 (1.06)	2.50 (1.32)					
	50	2.67 (1.10)	2.29 (1.19)					
PHA	1	2.49 (1.36)	2.68 (1.06)					3.05 (1.06)
	6.25	2.69 (1.03)	4.01 (1.11)			2.97 (1.12)	2.73 (1.04)	2.45 (1.17)
	12.5	2.52 (1.08)	4.28 (1.04)			2.81 (1.14)	2.48 (1.08)	2.26 (1.10)
	25	2.51 (1.04)	4.38 (1.02)			2.72 (1.06)		2.16 (1.31)
	50	2.44 (1.10)	4.36 (1.12)					2.20 (1.49)
	100	2.41 (1.13)	4.25 (1.03)					2.41 (1.05)
PWM	1	2.40 (1.07)	3.13 (1.14)					
	6.25	2.53 (1.25)	3.61 (1.03)				2.20 (1.10)	
	12.5	2.70 (1.14)	3.73 (1.05)			2.62 (1.06)	2.12 (1.18)	
	25	2.58 (1.17)	3.85 (1.02)			2.79 (1.09)	2.02 (1.05)	
	50	2.28 (1.08)	4.00 (1.02)					
	100	2.58 (1.12)	4.01 (1.06)					

* See Table II for explanations. Although limited numbers of cells from grafted nudes were available the responses were closer to the normal controls than was found for spleen and lymph node cells.

Cells from pairs of normal mice were pooled to give two pools of normal cells from spleen, two pools from lymph nodes, and two pools from thymus (A and B in Fig. 1 and Tables II and III). Similarly, cells from pairs of untreated nudes were pooled to give two pools of nude cells from spleen and two pools from lymph nodes (C and D). The spleens of all five grafted nudes were treated separately (E-I) but for lymph node and thymus cultures, two pools of cells were made from the four mice with C57BL/6 grafts.

There were three cell cultures per mitogen per cell. Since single animals or pools of cells from only two animals were used the background values were more variable than those usually obtained with cells from larger pools. Therefore, in

Fig. 1, we present the raw data for the number of cpm of [^{3}H]thymidine incorporated by cells in the individual cultures.

Fig. 1 shows the response of spleen cells. Normal BALB/c controls responded well to all three mitogens, while the only effect in untreated nudes was an inhibition, increasing with increasing Con A or PHA doses. The thymus-grafted nudes show a fairly good response to Con A and a variable response to PWM; only two of them were stimulated by PHA, but curiously enough by higher doses than usual. The thymus cells from BALB/c and thymus-grafted nudes gave only a poor response to Con A, and no response to PHA and PWM (Table III). Nude lymph node cells (Table II) were totally unreactive to all three mitogens, while BALB/c showed high incorporation values. Thymus-grafted nudes could be well stimulated by Con A only, while the effect of PHA and PWM was really too low to be considered as significant. Compared to the four mice grafted with C57BL/6 thymus 55–56 days before, the mouse grafted with AKR (45 days after grafting only) looked less responsive to mitogens. Immunofluorescence examination of its thymus, spleen, and lymph node cells showed that in this particular mouse, the repopulation of the lymphoid organs by host-derived T cells had not proceeded as far as previously reported for 45 days (1) although the thymus was fully repopulated (Table IV).

Response to T-Cell Dependent Antigens.—Nudes grafted with allogeneic thymuses were injected with T-cell dependent antigens, either SRBC or T4 bacteriophage; serum antibody levels were tested by hemagglutination or phage inactivation.

Some mice were immunized within 3 wk of thymus grafting, i.e., while donor-derived cells were still present in the thymus and may be present in the spleen and lymph nodes. Others were immunized 4 wk or more after thymus grafting when donor-derived cells could no longer be found in the thymus and the T cells of the peripheral lymphoid organs were mostly host-derived.

The number of mice and the range of responses are given in Table V for the mice with thymus grafts, for normal and nude controls, and for a group of nudes grafted with C57BL/6 neonatal spleens. It has already been shown that, if BALB/c-nu/nu are injected with a normal thymus cell suspension from C57BL/6 or BALB/c donors and submitted to a similar immunization schedule, only the recipients of BALB/c cells are able to respond (13).

TABLE IV

Percent of Cells Having Various Membrane Markers as Detected by Direct Immunofluorescence from the Nude Mouse Grafted with AKR Thymus 45 Days Previously

	TL	θ-AKR	θ-C3H	Ig
Thymus	57.2	<0.3	99.63	0.44
Spleen		<0.2	27.0	67.6
Lymph nodes		<0.2	14.5	81.6

The untreated nudes and nudes grafted with neonatal spleens produced no detectable antibody. Most of the thymus-grafted mice were able to respond when the antigen was given more than 4 wk after grafting although the antibody levels were lower than those of normal mice and comparable to those found with BALB/c thymus cell suspensions. When the antigen was given within 3 wk of grafting there was no response to T4 and, in the group with C57BL/6 thymuses only 2/11 responded to SRBC while 10/11 of the mice with BALB/c thymuses responded. This difference may depend on the ability of the donor lymphocytes to cooperate with the host B cells.

TABLE V

Response to T-Cell Dependent Antigens

	Time after thymus grafting	SRBC antigen		T4 antigen	
		No. responding	Range of response* hemagglutination titer	No. responding	Range of response* (K value for phage inactivation)
nu/nu controls	—	0/4	—	0/4	—
BALB/c controls	—	6/6	2^{-7}–2^{-10}	5/5	13.4–90.0
BALB/c-nu/nu with BALB/c thymus	Within 3 wk	10/11	2^{-4}–2^{-6}	0/5	—
	After 4 wk	12/14	2^{-4}–2^{-11}	4/5	0.7–14.7
BALB/c-nu/nu with C57BL/6 thymus	Within 3 wk	2/11	2^{-3}–2^{-6}	0/5	—
	After 4 wk	8/10	2^{-5}–2^{-10}	6/9	0.4–39.0
BALB/c-nu/nu with C57BL/6 spleen	After 4 wk	0/2	—	0/4	—
BALB/c-nu/nu with AKR thymus	After 4 wk	4/5	2^{-5}–2^{-9}	4/4	1.1–9.6

* A hemagglutinin titer of less than 2^{-3} or a K value of less than 0.2 was regarded as negative.

Among the 10 mice that failed to respond to T4 when injected within 3 wk of thymus grafting, three out of five recipients of BALB/c thymuses and two out of five recipients of C57BL/6 thymuses, were challenged again 10 wk later. The BALB/c grafted nudes then responded but the C57BL/6 grafted nudes did not.

Response to Skin Grafts.—Mice bearing thymus grafts were then grafted with skin from the thymus donor strain or from an unrelated strain. Skin grafts were made at least 6 wk after thymus grafting when the thymus would have been completely repopulated by host-derived cells. It is clear from Table VI that the rejection times are much slower than those of normal controls and were also very variable. As an additional control BALB/c-nu/nu with C57BL/6 thymuses

were grafted with BALB/c skin. Obviously these grafts should be accepted since cells capable of rejecting the graft should kill the host.

Second set grafts were rejected faster than first grafts except in the striking case of mice which had received a C57BL/6 thymus and later on C57BL/6 skin grafts. In this group the first grafts were rejected at times comparable to the rejection times of C57BL/6 skin grafts on mice bearing BALB/c thymus grafts but the second grafts always survived. Two of these mice had received the first C57BL/6 skin graft 23 wk after the C57BL/6 thymus graft, but this

TABLE VI

Rejection of Skin Grafts

	Skin donor	First graft		Second graft	
		No. rejected	Time (days)	No. rejected	Time (days)
Nude control	C57BL/6	0/3	50+	—	—
	CBA	0/5	50+	—	—
BALB/c control	CBA	10/10	12–15	10/10	8–12
	C57BL/6	10/10	14–16	10/10	8–12
BALB/c-nu/nu with BALB/c thymus	CBA	5/5	11–17	3/3	10–12
	C57BL/6	7/8	12–31	7/7	10–13
BALB/c-nu/nu with C57BL/6 thymus	BALB/c	0/6	50+	—	—
	CBA	10/11	12–20	5/5	11–15
	C57BL/6	14/15	12–20	0/8	50+
BALB/c-nu/nu with C57BL/6 spleen	C57BL/6	0/6	50+	—	—
BALB/c-nu/nu with AKR thymus	CBA	4/4	13–21	4/4	13–18
	C57BL/6	2/2	14–44	2/2	13–15
	AKR	6/6	11–40	4/4	12–23

did not affect the rejection pattern: the first grafts were rejected and the second were accepted.

DISCUSSION

These data show that nude mice grafted with neonatal thymuses display only a partial reconstitution of the immune response, though data previously published have shown that they develop normal numbers of T cells (1). We can summarize our observations as follows: (*a*) The restoration of the reactivity of T-cell mitogens is only partial: thymus, spleen, and lymph node cells can be fairly well stimulated by Con A, but the mitogenic response to PHA and PWM is poor or absent; (*b*) The restoration of the reactivity to T-cell dependent antigens (T4 and SRBC) is also incomplete and serum antibody levels are lower

than those obtained in normal mice; and (*c*) The restoration of the reactivity to skin grafts is partial in the sense that rejection is slower than with normal mice, and also more variable. Moreover, the pattern of rejection of skin grafts from donor and unrelated strains is intriguing.

An interesting point which has appeared from very preliminary work using the freeze fracture technique concerns membrane particles. These are distributed homogeneously in untreated nudes while in normal mice a large proportion of cells show clusters of membrane particles (14) and it has been suggested that cells with clusters of particles are mature T cells (15). A nude mouse which had been reconstituted with an AKR thymus graft and which had normal numbers of θ-positive cells in spleen and lymph nodes nevertheless lacked cells with clusters of particles.

Findings of intermediate responses in nude mice grafted with neonatal thymuses have been made by Wortis et al. (16) for skin graft rejection time and peripheral white cell counts, by Pritchard and Micklem for response to oxazolone (17), and by Pritchard et al. for serum γA levels (18), although the latter report normal γG and γM levels.

Various reasons could be put forward to explain this. It is possible that the T-cell activity is completely restored but that nude mice have some other deficiency that is not made good by thymus grafting. We have no data bearing directly on this question but the B cells and macrophages behave in a similar way to those of normal mice with regard to uptake of antigen, to the presence and distribution of membrane-bound immunoglobulin, to lectin-binding sites and to the process of their capping and endocytosis (footnote 2; Loor, unpublished observations). Further, the data for skin graft rejection by nude mice reconstituted with thymus grafts is closely comparable to that of neonatally thymectomized mice reconstituted with thymus grafts (3, 19) and in this case there is no reason to suspect any deficiency other than lack of thymus.

The possibility must be considered that the host-derived cells which differentiate in the grafted thymus are not physiologically active and that the reconstitution is due to a residual population of donor thymus cells. We were unable to find such a population in BALB/c-nu grafted with AKR thymuses using immunofluorescence to detect AKR cells. However, Pritchard and Micklem did find such a population using CBA-T6T6 thymus grafts. The most obvious argument that host-derived cells are responsible for the immune responses found, is that if the responses are due to donor cells they should be greatest soon after grafting when more donor cells can be detected. In fact the contrary is found, responses are very poor at first and become better after the thymus and peripheral organs have been repopulated. Moreover, a neonatal spleen graft which could be expected to provide a small number of donor type cells is totally

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[2] Loor, F. and G. Roelants. The dynamic state of the macrophage plasma membrane. I. Attachment and fate of immunoglobulin, antigen, and lectins. Manuscript in preparation.

ineffective. Furthermore, since it has already been shown (13, 20) that C57BL/6 thymus cell suspensions are ineffective in long-term restoration, it would be very difficult, with this hypothesis, to explain why C57BL/6 thymus grafts are almost as effective as BALB/c thymus grafts. It is also difficult to explain how C57BL/6 thymus cells could be responsible for the rejection of C57BL/6 skin grafts or AKR cells for the rejection of AKR skin grafts. In fact this result alone would seem to make the hypothesis of residual donor cell activity untenable.

There remains the possibility that the time relations between the grafted neonatal thymus and the cells of the host are out of step, thus leading either to maturation of less cells or to maturation of less types of cells. The latter would be possible if the repertoire of T-cell precursors is reduced as the animal grows older. This is really a whole range of possibilities, since the absence of a thymus during development and the subsequent grafting of a neonatal thymus into an adult animal could lead to many abnormalities. The simplest explanation would be that in the adult animal there are less T-cell precursors available thus leading to a purely quantitative deficiency in the thymus-grafted nudes. Certainly, the grafted thymus never becomes as large as the thymus of a normal 3–5 wk old mouse. Nevertheless, we could find a normal proportion of θ-positive cells in the spleen and lymph nodes of the few animals examined 8 wk after thymus grafting (1). Here the results of the mitogen stimulation may provide a clue, and the obvious lack of response to PHA suggests that some qualitative deficiencies might also be involved. Stobo and Paul (21) state that "spleen cells from 2-, 3-, and 4-wk old animals have achieved a greater proportion of their adult level of Con A responsiveness than of their adult level of PHA responsiveness". Thus, the reconstituted nudes may be simply exhibiting retarded development. However, the same authors (22) also claim that one subset of thymus-derived cells can be stimulated only by Con A while a second population can be stimulated by both Con A and PHA. Thus, our results could also be explained as due to a deficiency in this second population. However, it should be emphasized here that our results were all from 48 h mitogen stimulated cell cultures allowed to incorporate [^{3}H]thymidine for a further 24 h. It is possible that the nude cells grow more slowly in vitro and that a different pattern would be found a few days later. If this were so, it would not invalidate the results obtained at 2 days of culture, but would add another possible dimension to the picture.

The development of BALB/c type cells within a C57BL/6 type thymus is another type of developmental abnormality. What is really striking is that the animals bearing a C57BL/6 thymus are not originally tolerant to C57BL/6 skin but develop tolerance after a first skin graft has been made, but animals bearing an AKR thymus do not become tolerant to AKR skin. Further work will be necessary to determine whether this is due to serum factors such as blocking or enhancing antibodies, or to cellular factors such as a specific elimination of cells which could be active against the graft or as generation of suppressor cells.

SUMMARY

If nude mice are grafted with a neonatal thymus, host type precursor cells develop within the graft thymus and after about 6 wk the T-cell population of the thymus, spleen, and lymph nodes is of host type. However, immunological responsiveness produced in nude mice in this manner is incomplete: (*a*) the ability to react to T-cell mitogens in vitro is greater than in untreated nudes but lower than in normal mice; (*b*) the response to T-cell dependent antigens is less than normal; and (*c*) the rejection of skin grafts is slower than in normal animals. Whether host precursor cells which differentiate in an allogeneic thymus are able to reject skin grafts from thymus donor strain appears to depend on the strain combination used.

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