

DEVELOPMENT OF MULTIPLE ORGAN-LOCALIZED AUTOIMMUNE DISEASES IN NUDE MICE AFTER RECONSTITUTION OF T CELL FUNCTION BY RAT FETAL THYMUS GRAFT

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The T cell function of congenitally athymic nude mice and rats, as well as the severe immunodeficiency resulting from thymic dysplasia in humans, can be corrected by implantation of intact thymus or thymic epithelium (1-5). Either syngeneic or allogeneic thymic grafts are effective for reconstituting T cell functions in congenitally thymus-deficient rodents (6-10), although these functions are generally less effective than those of intact normal animals. Such reconstituted nude mice (grafted with allogeneic thymus or thymic epithelial cells) accept skin grafts from the syngeneic and the donor strain, whereas those derived from a third strain are vigorously rejected (4, 11).

In the present experiments, attempts were made to reconstitute T cell functions of nude mice by transplantation of thymic rudiments obtained from embryonic rats. The results show that these grafted mice gained T cell-mediated immune function, and accepted skin grafts from the donor rat strain. These mice, however, developed severe multiple organ-localized autoimmune diseases showing features similar to the autoimmunity observed in the mice after neonatal thymectomy, as reported previously (12-16).

Materials and Methods

Thymic Rudiment Transplantation. The rudiments of the thymuses were aseptically dissected from 15-d-old F344/DuCcj (F344) or ACI/NMs (ACI) rat embryos or 14-d-old BALB/cAJcl (BALB/c) mouse embryos (observation of vaginal plug = day 0). Female BALB/c *nu/nu* mice, 5 wk of age, (Clea Japan Inc., Tokyo, Japan) were grafted under each kidney subcapsule with two lobes of thymic rudiments. This was done with an orally controlled micropipette introduced through a dorsal incision exposing the kidney. All mice were housed in a conventional animal-care facility.

Chromosome Analysis. Chromosome analysis of lymphoid cells of thymic grafts was carried out by the air-drying method (17). The cells were harvested from 5 BALB/c *nu/nu* mice 8 wk after grafting with F344 thymic rudiments. Colchicine (0.05 mg/g body weight) was injected subcutaneously 2 h before harvest. Cells were treated with 1% sodium citrate in water for 5 min at 37°C, fixed in cold acetic acid/methanol (1:1), and then

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dried over a gas flame. The slides were stained with 2% Giemsa solution buffered with phosphate at pH 6.4. 200 cells were examined in each preparation.

Rabbit Antiserum Against Rat Species Antigen. The antisera were prepared by immunization of female rabbits with a mixture of viable thymocytes and thymic epithelial cells prepared from F344 rats. $\sim 2 \times 10^8$ cells were injected subcutaneously three times at intervals of 3 wk. 2 wk after the final immunization, the antisera were harvested. 100 μ l of the antisera were injected intraperitoneally into a normal female BALB/c mouse for in vivo absorption (18). The antisera were collected after 12 h and were used as antiserum against rat species-specific antigen.

*Membrane Immunofluorescence (IF).*¹ Five BALB/c *nu/nu* mice grafted with F344 thymic rudiments were used. For controls, three normal BALB/c and three BALB/c *nu/nu* mice, as well as F344 rats, were used. Lymphoid cells from thymus, lymph node, and grafted thymus were suspended in HBSS, and were incubated with 1:100 diluted FITC-labeled anti-Thy-1.1 or anti-Thy-1.2 mAb (Miles-Yeda LTD, Rehovot, Israel) for 20 min at 4°C and then washed in HBSS for 10 min. Lymphoid cells were also incubated with anti-rat species antigen antisera (diluted 1:50) for 20 min at 4°C, washed for 10 min, incubated for 20 min at 4°C with FITC-labeled anti-rabbit IgG (Miles-Yeda Ltd.) diluted 1:100, and then washed for 10 min. The cells thus stained were observed by a fluorescence microscope. At least 500 viable cells were counted for each sample.

Immunohistochemistry. Five F344 thymic grafts harvested from BALB/c *nu/nu* mice 8 wk after the transplantation were embedded in O.C.T. Compound (Tissue Tek II, Naperville, IL) and immediately frozen in liquid nitrogen. Thymuses were also obtained from three 12 wk old normal BALB/c mice and three normal F344 rats as control tissues. Sections were stained with FITC-labeled anti-Thy 1.2 mAb (diluted 1:100) or anti-rat species antigen antisera (diluted 1:50) by indirect IF.

PFC Assay. 13-wk-old normal BALB/c, BALB/c *nu/nu*, and BALB/c *nu/nu* with F344 thymic grafts (8 wk after grafting) were immunized intravenously with 0.2 ml of 2% SRBC. Spleens were harvested on day 7 after immunization and the PFC assay was carried out as described by Mishell and Dutton (19).

Skin Graft. All BALB/c *nu/nu* female mice at 13 wk of age (8 wk after grafting) were transplanted simultaneously with skin grafts from two different donors according to the method by Manning and Krueger (20), and one graft was always from a syngeneic female mouse or thymus donor female rat. The recipients grafted with rat thymic rudiments were observed for at least 4 mo after skin grafting.

Detection of Autoantibody. Mice grafted with rat thymic rudiments were exsanguinated through the axillary artery under ether anesthesia, and sera from individual mice were stored at -80°C until use. Sera obtained from 10 each of age-matched normal BALB/c, 3-mo- and 7-mo-old BALB/c *nu/nu* mice, and 7-mo-old BALB/c *nu/nu* mice grafted with syngeneic thymic rudiments were also tested. Cryostat sections of thyroid, stomach, liver, kidney, pancreas, heart, brain, adrenal, salivary gland, ovary, testis, prostate, and seminal vesicle of normal adult BALB/c mice were fixed with 1% formalin in PBS for 2 min and postfixed with 95% ethanol for 2 min, and used as target antigens for indirect IF. Sera diluted 20-fold were used for testing autoantibodies.

Histology. All experimental animals were autopsied. Tissues were fixed in Bouin's fixative, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Results

Fate of Thymic Graft. Thymic rudiments of 15-d-old embryonic rats appeared pestle-like with the length $\sim 600 \mu$ m and $\sim 300 \mu$ m in maximum diameter. Such rudiments from F344 or ACI rats were transplanted under kidney subcapsules of BALB/c *nu/nu* mice. At 8 wk after transplantation, the grafted thymuses generally had developed well macroscopically, with ~ 20 mg mean weight (7-50 mg) (Fig. 1a) and histologically formed lobes with cortex and medulla zones (Fig.

¹ Abbreviations used in this paper: Ab, antibody; IF, immunofluorescence.

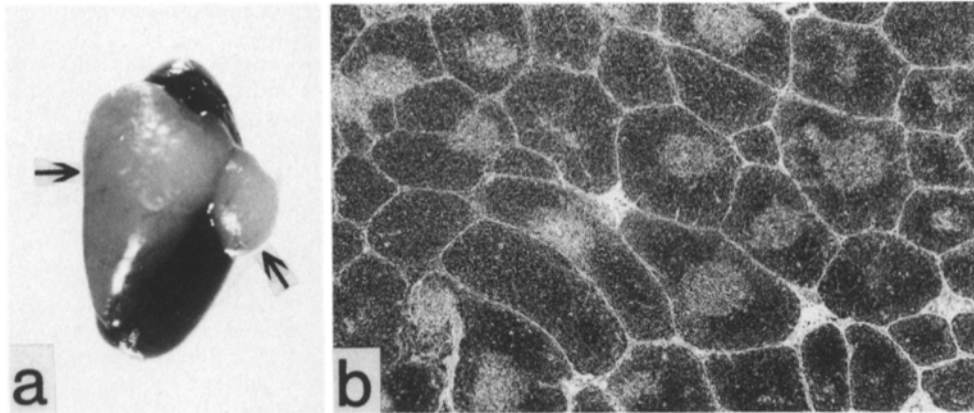


FIGURE 1. Embryonic F344 rat thymuses 8 wk after transplantation to kidney subcapsule of a BALB/c *nu/nu* mouse. (a) Macroscopic view of the thymuses (arrows) that are well developed. (b) Histology of transplanted thymus. Formation of cortex and medulla zones in lobes is clearly shown. (a) $\times 3.3$; (b) $\times 23$.

1 b). With advance of age, thymic grafts with atrophic features were recovered (about 5 mg at 13 mo of age).

Chromosome Analysis of Grafted Thymus. The modal chromosome number of lymphoid cells in the rat thymic grafts of all five nude mice was 40, and all chromosomes of these cells were telocentric, indicating that the lymphoid cells were of mouse type. No lymphoid cells with rat karyotype were recognized.

Immunohistochemical Study of Grafted Thymus. Frozen sections of F344 rat thymuses stained by the IF technique with antiserum against rat species antigen revealed a dense staining pattern throughout the thymus (Fig. 2a-1). In contrast, this antiserum did not react with frozen sections of BALB/c mouse thymuses (Fig. 2b-1). Distinct staining of the reticular meshwork structure and surrounding septal capsules was observed, when F344 thymic grafts of the *nu/nu* were reacted with the antiserum (Fig. 2c-1). Thymus sections prepared from normal mice (Fig. 2b-2) and grafted mice (Fig. 2c-2) showed quite similar staining patterns when reacted with FITC-labeled anti-Thy 1.2. This antibody, however, did not react with rat thymus sections (Fig. 2a-2).

Membrane IF Analysis of Grafted Thymus. Lymphoid cell suspensions were assayed for Thy-1.1, Thy-1.2, and rat species antigen (Table I). Almost all the cells in rat thymus grafts were Thy-1.2⁺, and the number of Thy-1.2⁺ cells apparently increased in lymph nodes of BALB/c *nu/nu* mice with grafts when compared with those without grafts. Neither rat species antigen nor Thy-1.1 were detected in the lymphoid cells from the grafted mice.

*Antibody Production Capacity in Thymus-grafted *nu/nu* Mouse.* To assess immune response against T cell-dependent antigens, mice were immunized with a single dose of SRBC. At day 7 after the immunization, spleens were removed and anti-SRBC response was studied by PFC assay (Table II). The response of untreated *nu/nu* mice was almost undetectable, whereas *nu/nu* mice with rat thymic grafts showed a considerable number of direct and indirect PFC. The

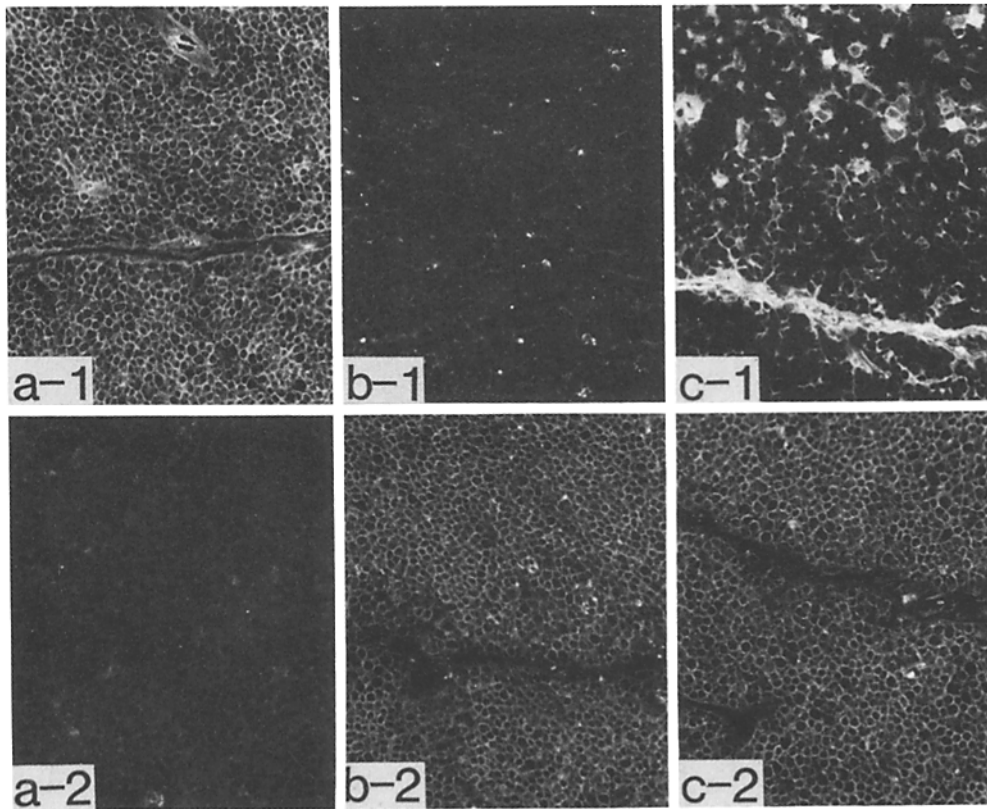


FIGURE 2. IF study of embryonic F344 rat thymus 8 wk after transplantation to kidney subcapsule of a BALB/c *nu/nu* mouse. Frozen sections of 3-mo-old F344 rat thymus (a), BALB/c mouse thymus (b), and F344 rat thymus xenograft (c) were stained with anti-rat species antigen antiserum (a-1, b-1, and c-1) and anti-Thy 1.2 antiserum (a-2, b-2, and c-2), respectively. Nonlymphoid cells in rat thymus xenograft are clearly stained with anti-rat species-specific antigen (c-1), while thymocytes are Thy-1.2 antigen-positive (c-2). (a, b, and c) $\times 200$.

TABLE I
Surface Antigen Phenotype of Lymphoid Cells of BALB/c nu/nu Mice Grafted with Embryonic F344 Thymic Rudiments Determined by IF

Animals (n)	Thymus or grafted thymus			Lymph node		
	Thy-1.1*	Thy-1.2*	Rat antigen [‡]	Thy-1.1	Thy-1.2	Rat antigen
	% [§]	%	%	%	%	%
BALB/c <i>nu/nu</i> grafted with F344 thymus (5)	0 [§]	>98	0	0	35.8 \pm 5.6	0
Normal BALB/c (3)	0	>98	0	0	65.5 \pm 9.0	0
BALB/c <i>nu/nu</i> (3)	—	—	—	0	8.9 \pm 1.7	0
Normal F344 (3)	70.0 \pm 4.3	0	100	0	0	100

* FITC-labeled mAb.

[‡] Rabbit anti-rat thymus antiserum absorbed in vivo in mouse.

[§] Percent positive cells (mean \pm SE).

TABLE II
Anti-SRBC Response of BALB/c nu/nu Mice Grafted with Embryonic F344 Thymic Rudiments Studied by PFC Assay

Experiment	Mice (n)	Number of PFC (mean \pm SE)/10 ⁶ spleen cells		
		Direct assay (D)	Indirect assay (I)	I - D
1	BALB/c nu/nu grafted with F344 thymus (4)	22.0 \pm 9.1	42.5 \pm 14.3	18.1 \pm 7.3
	Normal BALB/c (3)	24.0 \pm 8.8	61.7 \pm 20.6	37.8 \pm 9.3
	BALB/c nu/nu (3)	0.2 \pm 0.1	0.3 \pm 0.1	0.1 \pm 0.1
2	BALB/c nu/nu grafted with F344 thymus (4)	22.8 \pm 5.8	39.7 \pm 9.0	17.2 \pm 8.3
	Normal BALB/c (3)	15.4 \pm 2.4	94.5 \pm 19.7	79.1 \pm 17.1
	BALB/c nu/nu (3)	0.7 \pm 0.2	1.2 \pm 0.5	0.6 \pm 0.3

TABLE III
Survival of Skin Grafts from Various Donors in BALB/c nu/nu Mice Grafted with Embryonic F344 or ACI Thymic Rudiments

Number of recipients	Thymus donor	Skin donor	Graft survival
3	—	BALB/c (H-2 ^d)	>4 mo
3	—	C3H/He (H-2 ^k)	>4 mo
3	—	F334 (RT1-A ^l)	>4 mo
3	—	ACI (RT1-A ^a)	>4 mo
6	F344	BALB/c	>5 mo
6	F344	C3H/He	<12 d
6	F344	F334	>5 mo
6	F344	ACI	<12 d
6	ACI	BALB/c	>5 mo
6	ACI	C3H/He	<12 d
6	ACI	F334	<12 d
6	ACI	ACI	>5 mo

response observed by indirect PFC assay in the *nu/nu* mice with thymus grafts was up to 50% of that in normal BALB/c mice.

Skin Grafting. BALB/c *nu/nu* mice bearing xenogeneic thymus grafts from F344 or ACI rats were grafted with skin from syngeneic mice, allogeneic C3H/He mice, thymus donor rats, or rats of other than donor strains. It is clear from Table III that skin grafts from both syngeneic mice and thymic donor rats were perfectly accepted and regular hair growth was observed until sacrifice at 7 mo of age. In contrast, all the skin grafts from C3H/He mice or rats of other than donor strains were rejected within 12 d.

Histological Study. T cell areas of spleen and lymph nodes in all the grafted *nu/nu* mice were considerably reconstituted at 3 mo of age. In all grafted *nu/nu* mice, no obvious pathological features were recognized in liver, kidney, lung, or several other organs. However, in thyroid, stomach, salivary gland (sublingual

TABLE IV
*Development of Organ-localized Inflammatory Lesions and Detection of Auto-antibodies in
 BALB/c nu/nu Mice Grafted with Embryonic F344 Rat Thymus*

Number of mice examined	Age when killed*	State of the disease	Mice with autoimmune diseases (%)					
			Thyroid	Salivary gland	Stomach	Adrenal gland	Ovary or Testis	Prostate
12 female	3 mo	A [‡]	4 (33.3)	7 (58.3)	10 (83.3)	4 (33.3)	8 (66.7)	—
		B [§]	1 (8.3)	3 (25.0)	1 (8.3)	1 (8.3)	4 (33.3)	—
		C [¶]	3 (25.0)	0	0	1 (8.3)	0	—
10 male		A	3 (30.0)	3 (30.0)	6 (60.0)	1 (10.0)	0	3 (30.0)
		B	0	1 (10.0)	0	0	0	0
		C	0	0	1 (10.0)	2 (20.0)	0	0
14 female	7 mo	A	7 (50.0)	9 (64.3)	13 (92.9)	3 (21.4)	8 (57.1)	—
		B	2 (14.3)	4 (28.6)	0	1 (7.1)	5 (35.7)	—
		C	0	1 (7.1)	0	2 (14.3)	0	—
10 male		A	6 (60.0)	6 (60.0)	8 (80.0)	1 (10.0)	2 (20.0)	4 (40.0)
		B	0	2 (20.0)	0	0	1 (10.0)	1 (10.0)
		C	0	0	0	3 (30.0)	0	0
16 male	14 mo	A	6 (43.8)	8 (50.0)	12 (75.0)	3 (18.8)	4 (25.0)	9 (56.3)
		B	0	3 (18.8)	0	0	0	0
		C	0	0	0	0	1 (6.3)	0

* BALB/c nu/nu mice were grafted at 5 wk old.

[‡] Lesion + Ab; mouse with both an affected organ and an autoantibody against component of the organ.

[§] Lesion only; mouse with an affected organ.

[¶] Ab only; mouse with an autoantibody.

gland and submaxillary gland), adrenal, ovary, testis, and prostate (median lobe [i.e., coagulating gland] and dorsolateral lobe) inflammatory lesions were noticed, characterized by mononuclear cell infiltration. As shown in Table IV, incidence and severity of these lesions gradually increased with the advance of age, and they were observed more often in females than males. In the majority of mice, multiple organs were affected. Thyroiditis (Fig. 3, *a* and *b*) (incidence at 7 mo: 64.3% in female, 60% in male), sialoadenitis (Fig. 4*a*) (92.9% in female, 80% in male), and prostatitis (50%) were characterized by obstruction or damage of the epithelial cells, with massive cell infiltration. Destruction of the follicular (thyroid) (Fig. 3*b*), acinous (salivary gland) (Fig. 4*a*), and ductal (prostate) architecture, and formation of lymphoid follicles were frequently observed. Severe cases of thyroiditis showed a pattern comparable to human Hashimoto's disease. Oophoritis (92.8%) showed complete loss of follicles with mild mononuclear cell infiltration. Gastritis (92.9% in female, 80% in male) (Fig. 5, *a* and *b*) was characterized by depletion of parietal and chief cells with varying degrees of lymphocyte infiltration along the thickened muscularis mucosa, which increased with age and resulted in formation of large folds. Adrenitis (28.5% in female, 10.0% in male) (Fig. 6*a*) and orchitis (30.0%) were also observed in these mice.

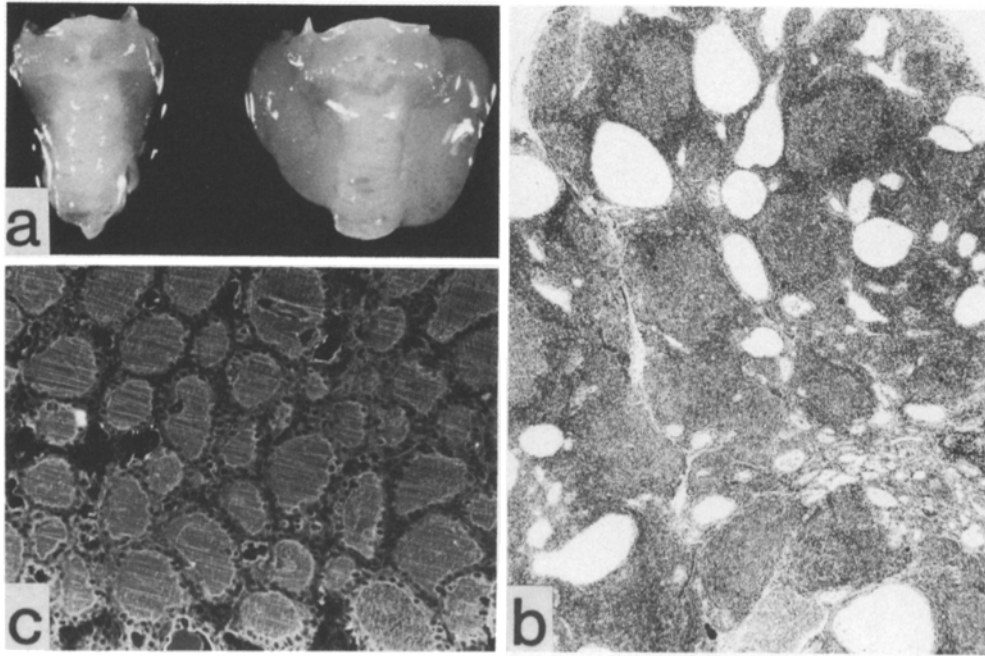


FIGURE 3. Thyroiditis and demonstration of the corresponding autoantibody. (a) Enlarged thyroid glands found in a 7-mo-old BALB/c *nu/nu* female mouse grafted with embryonic F344 rat thymus (right). Age-matched control thyroid gland (left). (b) Histological section of diseased thyroid (shown in Fig. 3 a, right). Destruction of follicular architecture and formation of lymphoid follicles are seen. (c) Detection of antiepithelia and anticolloid antibodies in thyroiditis by IF. (a) $\times 8$, (b) $\times 23$, (c) $\times 65$.

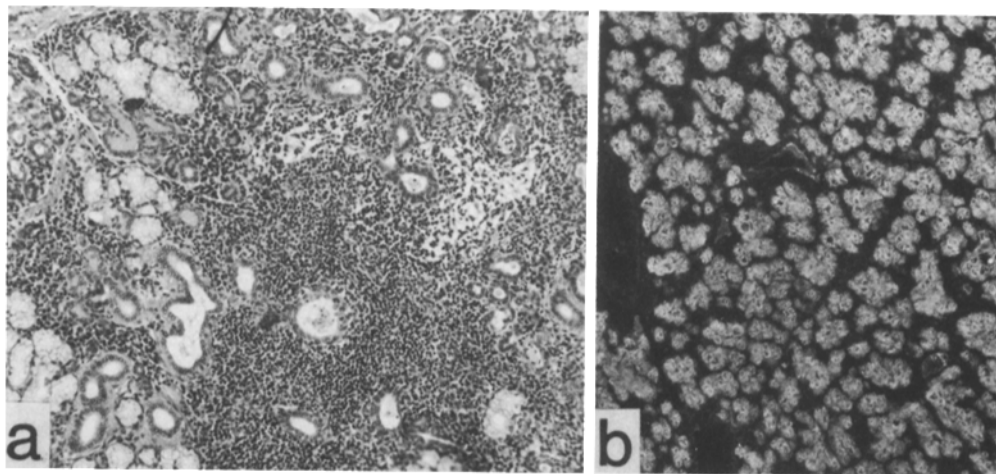


FIGURE 4. Sialoadenitis and demonstration of the corresponding autoantibody. (a) Lesion found in a sublingual gland of a 7-mo-old BALB/c *nu/nu* mouse grafted with embryonic F344 rat thymus. Destruction of acinous architecture and extensive infiltration of mononuclear cells were noticed. (b) Detection of antimucous cell antibody detected in sialoadenitis of sublingual gland by IF. (a) $\times 100$; (b) $\times 65$.

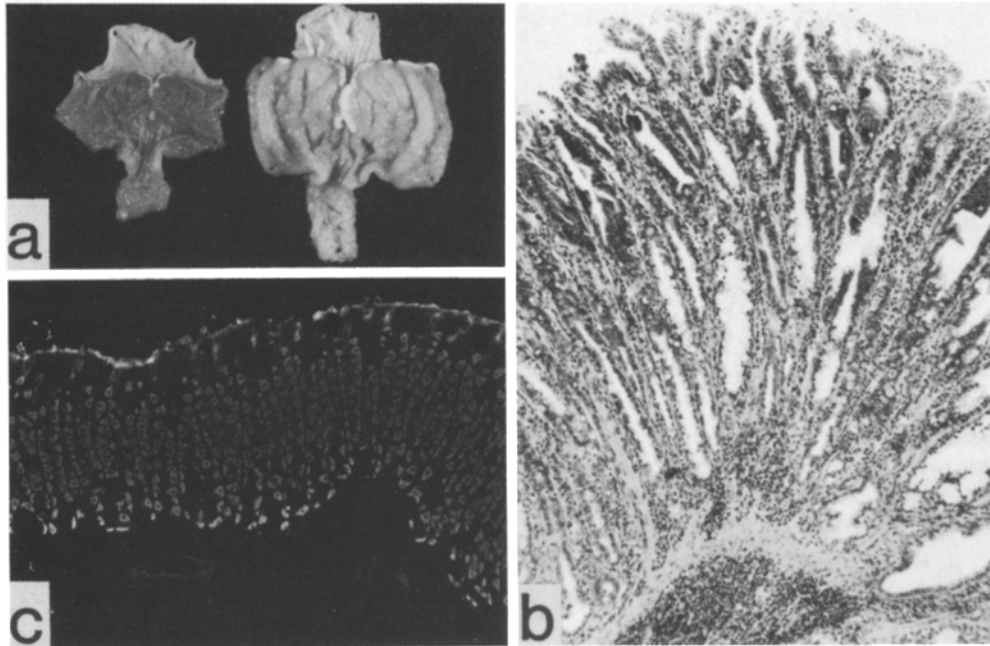


FIGURE 5. Gastritis and demonstration of the corresponding autoantibody. (a) Giant folds of glandular stomach found in a 7-mo-old BALB/c *nu/nu* female mouse grafted with embryonic F344 rat thymus (*right*). Age-matched control stomach (*left*). (b) Histological section of diseased stomach (shown in Fig. 4a, *right*). Depletion of parietal and chief cells and hyperplasia of mucous and endocrine cells were noticed, with severe mononuclear cell infiltration around thickened muscularis mucosa. (c) Detection of antiparietal cell antibody in gastritis by IF. (a) $\times 1$, (b) $\times 100$, (c) $\times 65$.

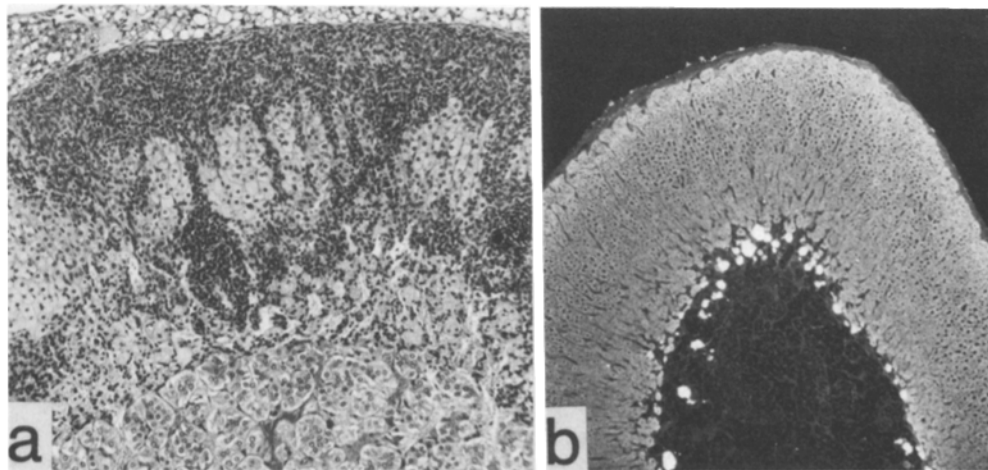


FIGURE 6. Adrenitis and demonstration of the corresponding autoantibody. (a) Lesion found in an adrenal gland of a 3-mo-old BALB/c *nu/nu* female mouse grafted with embryonic F344 rat thymus. Infiltration of numerous mononuclear cells was observed in cortex area. (b) Detection of antiadrenal cortex antibody in adrenitis by IF. The antibody reacted also with steroid-producing cells in ovary and Leydig cells in testis. (a) $\times 100$; (b) $\times 65$.

No such abnormalities were observed in normal, *nu/nu* mice and *nu/nu* mice with syngeneic thymus grafts.

Detection of Circulating Autoantibody. As shown in Table IV, circulating antibodies of IgG type were detected by indirect IF test in the sera of *nu/nu* recipients, and a good correlation was generally observed between the specificity of the antibodies detected and the organs affected in each mouse. Antibodies against thyroid epithelia and colloid (Fig. 3c) were detected in the sera of mice with thyroiditis. Circulating antibodies against epithelial cells of salivary gland (Fig. 4b) and prostate were also detected in the sera of mice with sialoadenitis and prostatitis, respectively. An antibody against parietal cells (Fig. 5c) was detected in the sera of mice with gastritis. Detection of antibodies against steroid-producing cells such as adrenal cortex (Fig. 6b) and interstitial cells of ovary was well correlated with adrenalitis. Three types of antibodies, i.e. against ooplasm, zona pellucida, or steroid-producing cells were detected in the sera of mice with oophoritis. An antibody against acrosome of mature sperm was detected in the sera of mice with orchitis. In contrast, such autoantibodies were not detected in the sera of normal, *nu/nu* mice, and *nu/nu* mice with syngeneic thymus grafts. Antinuclear antibodies, however, were observed in the sera of a proportion of old control *nu/nu* mice and thymus-grafted mice.

Discussion

Rat thymus rudiments, when grafted under the renal capsule of BALB/c nude mice, developed normally and formed cortex and medulla structures, apparently showing normal age changes. It was evident by chromosome analysis and membrane antigen assays that T precursor cells of the host migrated into the rat thymus graft and expressed Thy-1.2 antigen, and further, that a certain population of T cells moved into the peripheral lymphoid organs. Rat lymphoid cells did not persist in the grafts in the xenogeneic combination described here. This was slightly different from the previous report in which significant numbers of donor type thymocytes were recognized within the graft for a relatively longer time, when allogeneic thymus of mice was grafted (9). This difference may be due to species differences of the grafts, or more probably to the age of the grafts; i.e., embryonic rudiments with a very small number of thymocytes were used in the present experiment, in contrast to newborn allogeneic thymus containing a larger number of lymphoid cells.

The capacity of immune responses of the recipients estimated by the antibody response by indirect PFC assay was approximately half of that in control BALB/c mice, which indicated that helper T cell function was considerably recovered by xenogeneic thymic graft. The recovery rate is similar to that of *nu/nu* mice reconstituted by graft of allogeneic thymus or thymic epithelium (4, 7, 9). Skin grafting was also carried out to examine the state of immunological tolerance of the grafted mice. The results obtained were generally consistent with the observation of allograft tolerance following reconstitution of nude mice by allogeneic thymus (4, 11). It was noted, however, that the grafted mice accepted skin from the donor strain of rat, but rejected that of other strains, indicating that the T cell function is competent enough to tell the difference in allohistocompatibility of xenogeneic species.

Previous attempts have been made by others to restore T cell function in immunodeficient rodents with thymic epithelium after depleting thymocytes in the graft by organ culture under various conditions (21–23), which necessitates a significant amount of work. In contrast, the use of xenogeneic whole thymus graft as described here is rather simple and can reconstitute considerable T cell function. In addition, rat thymus-grafted *nu/nu* mice will provide us with valuable experimental materials for analysis of cell to cell interactions involved in antibody formation and T cell-mediated immunity.

The nude mice grafted with rat thymic rudiments generally survived more than 1 yr without any severe infectious diseases under a conventional environment. Histological examination, however, revealed a high incidence of inflammatory reactions in several organs, such as thyroid, stomach, salivary gland, and ovary, corresponding with the presence of the organ-specific autoantibody, strongly indicating that these lesions are caused by autoimmunity. In terms of the spectrum of organs affected, histopathology, and development of the autoantibodies to corresponding tissue or organ, this autoimmune disease model shows patterns quite similar to organ-specific autoimmune diseases in humans and to postthymectomy autoimmune diseases, as previously described (12–16). In the latter model, neonatal thymectomy during the critical period, 2–4 d after birth, induced organ-specific autoimmune disease without any exogenous sensitization by antigens. The disease could be completely prevented by inoculation of a certain number of T cells from normal adult mice (15, 24, 25). By analyzing the cellular processes involved in development of these autoimmune diseases, we obtained results that suggest that neonatal thymectomy abrogates the Lyt-1 T cell subset. These cells suppress any self-reactive cells directed toward autoantigen, which implies that these regulatory T cells normally peripheralize via the thymus after the day of thymectomy, while the latter self-reactive T cell type is generated by the day of thymectomy. It was shown recently by Sakaguchi et al. that reconstitution of BALB/c nude mice with anti-Lyt-1-treated spleen cells, without any other manipulation, caused organ-specific autoimmune diseases (26), quite similar to neonatal thymectomy autoimmune diseases. These reports strongly suggest that autoimmunity observed in nude mice grafted with xenogeneic thymus is also caused by a similar dysfunction of regulatory T cells, although further analysis is required to substantiate this suggestion.

Summary

Restoration of T cell function of athymic BALB/c *nu/nu* mice was investigated after transplantation of xenogeneic thymic rudiments from 15-d-old embryonic rats into kidney subcapsule. The rudiments developed well and formed a proper thymus structure composed of donor epithelia and host lymphocytes. Examination of antibody responses to SRBC revealed that approximately half the normal number of indirect PFCs were observed. Skin grafts from syngeneic BALB/c mice and thymic donor rat strains were accepted, whereas those from allogeneic mice and the rats of other than donor strains were vigorously rejected. Thymus-grafted nude mice under a conventional environment survived without any evident infectious diseases. Histological and immunofluorescence studies, however, showed a high incidence of multiple organ-localized autoimmune diseases

in thyroid, salivary gland, stomach, adrenal, prostate, ovary, and testis in mice that produced the corresponding autoantibodies.

These results together suggested that rat thymic grafts reconstituted T cell functions of *nu/nu* mice to a considerable degree, but that organ-localized autoimmune diseases developed, probably because certain auto-antigens of the recipients were recognized by the newly reconstituted host immunity.

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References

1. Pantelouris, E. M. 1971. Observations on the immunobiology of 'nude' mice. *Immunology*. 20:247.
2. Wortis, H. H., S. Nehlsen, and J. J. Owen. 1971. Abnormal development of the thymus in "nude" mice. *J. Exp. Med.* 134:681.
3. Schuurman, H. J., J. G. Vos, R. Broekhuizen, C. J. W. M. Brandt, and L. Kater. 1985. In vivo biological effect of allogeneic cultured thymic epithelium on thymus-dependent immunity in athymic nude rats. *Scand. J. Immunol.* 21:21.
4. Hong, R., H. Schulte-Wissermann, E. Jarrett-Toth, S. D. Horowitz, and D. D. Manning. 1979. Transplantation of cultured thymic fragments. II. Results in nude mice. *J. Exp. Med.* 149:398.
5. Hong, R., M. Santosham, H. Schulte-Wissermann, S. Horowitz, S. H. Hsu, and J. A. Winkelstein. 1976. Reconstitution of B and T lymphocyte function in severe combined immunodeficiency disease after transplantation with thymic epithelium. *Lancet*. 2:1270.
6. Loor, F., and B. Kindred. 1973. Differentiation of T-cell precursors in nude mice demonstrated by immunofluorescence of T-cell membrane markers. *J. Exp. Med.* 138:1044.
7. Kindred, B., and F. Loor. 1974. Activity of host-derived T cells which differentiate in nude mice grafted with co-isogenic or allogeneic thymuses. *J. Exp. Med.* 139:1215.
8. Kindred, B. 1976. Lymphocytes which differentiate in an allogeneic thymus. I. Response to MLC determinants and skin grafts from the thymus donor strain. *Cell. Immunol.* 25:189.
9. Loor, F., B. Kindred, and L.-B. Hagg. 1976. Incomplete restoration of T-cell reactivity in nude mice after neonatal allogeneic thymus grafting. *Cell. Immunol.* 26:29.
10. Splitter, G. A., T. C. McGuire, and W. C. Davis. 1977. The differentiation of bone marrow cells to functional T lymphocytes following implantation of thymus grafts and thymic stroma in nude and ATxBM mice. *Cell. Immunol.* 34:93.
11. Isaak, D. D. 1978. Fate of skin grafts from different inbred strains on nude mice bearing allogeneic thymus grafts. *J. Reticuloendothel. Soc.* 23:231.
12. Kojima, A., Y. Tanaka-Kojima, T. Sakakura, and Y. Nishizuka. 1976. Spontaneous development of autoimmune thyroiditis in neonatally thymectomized mice. *Lab. Invest.* 34:550.
13. Kojima, A., O. Taguchi, and Y. Nishizuka. 1980. Experimental production of possible autoimmune gastritis followed by macrocytic anemia in athymic nude mice. *Lab. Invest.* 42:387.
14. Taguchi, O., Y. Nishizuka, T. Sakakura, and A. Kojima. 1980. Autoimmune ooph-

- oritis in thymectomized mice: detection of circulating antibodies against oocytes. *Clin. Exp. Immunol.* 40:540.
15. Taguchi, O., and Y. Nishizuka. 1981. Experimental autoimmune orchitis after neonatal thymectomy in the mouse. *Clin. Exp. Immunol.* 46:425.
 16. Taguchi, O., A. Kojima, and Y. Nishizuka. 1985. Experimental autoimmune prostatitis after neonatal thymectomy in the mouse. *Clin. Exp. Immunol.* 60:123.
 17. Kurita, Y., T. Sugiyama, and Y. Nishizuka. 1968. Cytogenetic studies on rat leukemia induced by pulse doses of 7,12-dimethylbenz(a)anthracene. *Cancer Res.* 28:1738.
 18. Shigeno, N., U. Hammerling, C. Arpels, E. A. Boyse, and L. J. Old. 1968. Preparation of lymphocyte-specific antibody from anti-lymphocyte serum. *Lancet.* 2:320.
 19. Mishell, R. I., and R. W. Dutton. 1967. Immunization of dissociated spleen cell cultures from normal mice. *J. Exp. Med.* 126:423.
 20. Manning, D. D., and G. G. Krueger. 1974. Use of cyanoacrylate cement in skin grafting congenitally athymic (nude) mice. An improved technique. *Transplantation* (Baltimore). 18:380.
 21. Hays, E. F., and T. R. Beardsley. 1984. Immunologic effects of human thymic stromal grafts and cell lines. *Clin. Immunol. Immunopathol.* 33:381.
 22. Jordan, R. K., J. H. Robinson, N. A. Hopkinson, K. C. House, and A. L. Bentley. 1985. Thymic epithelium and the induction of transplantation tolerance in nude mice. *Nature (Lond.)* 314:454.
 23. Jordan, R. K., A. L. Bentley, G. A. Perry, and D. A. Crouse. 1985. Thymic epithelium. I. Lymphoid-free organ cultures grafted in syngeneic intact mice. *J. Immunol.* 134:2155.
 24. Kojima, A., Y. Tanaka-Kojima, T. Sakakura, and Y. Nishizuka. 1976. Prevention of postthymectomy autoimmune thyroiditis. *Lab. Invest.* 34:601.
 25. Sakaguchi, S., T. Takahashi, Y. Nishizuka. 1982. Study on cellular events in post-thymectomy autoimmune oophoritis in mice. II. Requirement of Lyt-1 cells in normal female mice for the prevention of oophoritis. *J. Exp. Med.* 156:1577.
 26. Sakaguchi, S., K. Fukuma, K. Kuribayashi, and T. Masuda. 1985. Organ-specific autoimmune diseases induced in mice by elimination of T cell subset. I. Evidence for the active participation of T cells in natural self-tolerance; deficit of a T cell subset as a possible cause of autoimmune disease. *J. Exp. Med.* 161:72.