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Mouse models of autoimmune disease suggest that self-tolerance is maintained by unresponsive autoreactive T cells

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SUMMARY

Multiple organ-localized autoimmune diseases, such as thyroiditis and gastritis, spontaneously develop in BALB/c *nu/nu* (nude) mice receiving embryonic rat thymus grafts (TG) under their renal capsules (TG nude mice). When thyroid was grafted into the rat thymus of TG nude mice, development of autoimmune thyroiditis, but not other diseases, was completely prevented. However, when such mice received thyroid antigen plus complete Freund's adjuvant (CFA), severe autoimmune thyroiditis developed, suggesting that some thyroid-specific autoreactive T cells migrate into the periphery, but remain unresponsive. Development of autoimmune diseases, including thyroiditis, in TG nude mice was prevented by a single intraperitoneal injection of splenic $CD4^+$ cells from normal BALB/c mice and also from mice with intrathymic thyroid grafts, indicating that thyroid-specific suppressor T cells are present in normal mice and that such T cells are neither deleted nor inactivated by the intrathymic thyroid grafts, in contrast to autoreactive T cells. Thus clonal deletion in the thymus, and clonal anergy and/or ignorance in the periphery, of autoreactive cells is important to maintain immune tolerance to organ-specific antigen, but CD4 suppressor T cells may play a more important role in tolerance, and the failure of education of this population may cause autoimmune diseases in the TG nude mouse model.

INTRODUCTION

That most individuals remain free from autoimmunity suggests the existence of successful mechanisms of self-tolerance. Antigens expressed in the thymus, such as minor lymphocyte-stimulating (Mls) antigens, induce clonal deletion of the responsible T cells with the corresponding T-cell receptor (TCR) Vβ,[1–5] and any T cells escaping from this selection end up in a state of anergy in the periphery.[6–8] An additional mechanism of tolerance to extrathymic antigens was found to be due to lack of appropriate T-cell activation, i.e. ignorance.[9,10] In this paper, we use the term unresponsiveness' instead of anergy and/or ignorance, because these two states of tolerance cannot always be distinguished. We studied the effects of organ-specific antigens, introduced in sufficient quantity into the thymus, on organ-specific autoreactive T cells and suppressor T cells in a mouse model of autoimmune disease.[11]

T-cell function of athymic BALB/c *nu/nu* (nude) mice can be corrected by embryonic rat thymus grafts (TG) under their renal capsule (TG nude mice). However, multiple organ-localized autoimmune diseases, including thyroiditis and gastritis, develop spontaneously in TG nude mice at around 2 months after rat thymus grafting.[11,12] When thyroid from newborn BALB/c mice was grafted into the thymus of TG nude mice, thyroiditis, but not other autoimmune diseases, was prevented from developing, suggesting the deletion and/or unresponsiveness of thyroid-specific autoreactive T cells. On the other hand, splenic $CD4^+$ cells from adult BALB/c mice, grafted with thyroid intrathymically when newborn, were able to prevent the development not only of autoimmune thyroiditis but also of other diseases in TG nude mice, suggesting that thyroid-specific suppressor T cells[13–15] were neither deleted nor inactivated by the intrathymic thyroid graft in contrast to autoreactive T cells.

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Abbreviations: TG, thymus graft; TG nude mice, BALB/c nude mice receiving embryonic rat thymus grafts under their renal capsules.

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MATERIALS AND METHODS

Animals

BALB/c, C3H/He and female BALB/c nude (*nu/nu*) mice and timed pregnant F344 rats were purchased from Charles River Inc. (Hino, Japan).

Experimental protocol

To produce spontaneous occurrence of multiple organ-localized autoimmune diseases in nude mice, thymi harvested

from 15-day F344 rat embryos were grafted beneath the renal capsules of 4-week-old female BALB/c nude mice (TG nude mice) in which a high incidence of spontaneous multiple organ-localized autoimmune diseases develops.[11] Two weeks after the rat thymic grafts, thyroids or ovaries harvested from newborn BALB/c mice were grafted either into the thymus or near the thymus. This was done with an orally controlled micropipette introduced through a dorsal incision exposing the kidney. To produce BALB/c mice with intrathymic thyroid grafts, thyroids harvested from syngeneic newborn mice were grafted into the left lobes of thymi of newborn mice, and the right lobes removed.

All mice were housed in a specific pathogen-free room. These mice were killed at 5 or 10 months of age. All mice were exsanguinated through the axillary artery under ether anaesthesia, and sera from individual mice stored at −80° until used. Some 5-month-old spleens were used for adoptive transfer.

Immunofluorescence

Thyroids from adult BALB/c mice and thymi harvested from BALB/c and TG nude mice with an intrathymic thyroid graft were embedded in OTC Compound (Tissue Tek II, Naperville, IL) and immediately frozen. Cryostat sections of thyroids were fixed in acetone and incubated with serum (diluted 1/40), followed by incubation with fluorescein isothiocyanate (FITC)-labelled anti-mouse IgG (diluted 1/200) (Cappel, Organon Teknika, West Chester, PA), according to the method described previously.[15] Cryostat sections of thymi with an intrathymic thyroid graft were fixed in acetone and incubated with autoantibody to thyroid antigen obtained from a TG nude mouse with thyroiditis (diluted 1/160), washed, then incubated with rhodamine-labelled anti-mouse IgG (Cappel) and FITC-labelled anti-Thy-1.2 monoclonal antibody (mAb) (Becton Dickinson, Mountain View, CA) for staining thyroid antigen and Thy-1 simultaneously.

Serological analysis

Analysis of cell-surface antigens was performed with a FACScan (Becton Dickinson) using mAb to CD3, CD4, CD8, T-cell receptor $\alpha\beta$ (TCR$\alpha\beta$) and TCR Vβ2, 3, 5.1/5.2, 6, 7, 8.1/8.2 (Pharmingen, San Diego, CA), 8.1/8.2/8.3,[16] 9, 11, 13 and 14 (Pharmingen). Monoclonal antibodies were conjugated directly with FITC or biotin. Phycoerythrin–streptavidin (Biomeda, Foster City, CA) was used as the secondary reagent.

Spleen cell transfer

Spleens obtained from 5-month-old normal BALB/c and TG nude mice with or without intrathymic thyroid grafts were used for preparation of cell suspensions by methods described previously.[15] The presence of intrathymic thyroid grafts in TG nude mice and BALB/c mice was confirmed by cryostat sectioning before use for transfer of spleen cells. A part of the spleen cell suspensions was used for the anti-CD4 and anti-CD8 mAb (Pharmingen) treatments, by the method described previously.[12] After washing, the cells were incubated with rabbit complement (C) (diluted 1 : 15) for 45 min at 37°. Viable spleen cells (4×10^7) were injected intraperitoneally into TG nude mice that had had the rat thymi surgically removed 3 weeks after the graft. The recipient TG nude mice were killed 8 weeks after the spleen cell injection.

Immunization

Mouse thyroid extracts (1000 μg/ml) were prepared from BALB/c mice as described elsewhere.[17] 0·05 ml of the thyroid extracts or phosphate-buffered saline (PBS) and an equal volume of complete Freund's adjuvant (CFA) was injected twice into hind footpads of TG nude mice, with or without intrathymic thyroid grafts, at a 1-week interval. The mice were killed 3 weeks after the last immunization.

Immune response

For a mixed lymphocyte culture, 10^6 spleen cells of the TG nude mice, with or without intrathymic thyroid grafts, were mixed with irradiated (20 Gy, Hitachi MBR-1520R; Hitachi, Japan) spleen cells of C3H/He and incubated for 4 days at 37°, followed by a 6-hr incubation in the presence of 18·5 kBq [^{3}H]thymidine. Cells were harvested on a filter by Labo Mash (Labo Science Inc., Tokyo, Japan) and precipitated with trichloroacetic acid. The amount of [^{3}H]thymidine in the precipitates was counted with a scintillation counter (Beckman Instruments, Palo Alto, CA).

For thyroid extract stimulation, 4×10^5 spleen cells were incubated in the presence of 1 or 10 μg/ml thyroid extracts for 3 days, followed by a 16-hr incubation with the addition of [^{3}H]thymidine. The uptake of [^{3}H]thymidine was measured as in the mixed lymphocyte culture experiments.

Histology

Organs were fixed in Bouin's fixative, embedded in paraffin, sectioned, and stained with haematoxylin and eosin for histological examination.

RESULTS AND DISCUSSION

Autoimmune thyroiditis in TG nude mice can be prevented by intrathymic transplantation of thyroid

As shown in Table 1, autoimmune thyroiditis, characterized by follicular destruction with massive mononuclear lymphocyte infiltration (Fig. 1b) and circulating antibody against epithelial cells and/or thyroid colloid,[11] was observed in 52·2% and 61·1% of TG nude mice at 5 and 10 months of age, respectively. In this study, thyroid tissue obtained from syngeneic newborn mice was transplanted into rat thymi 2 weeks after they had been grafted under the renal subcapsule of TG nude mice, in order to examine whether the target organ *in situ* would escape from autoimmunity. As expected, this treatment completely prevented development of the inflammatory reaction to thyroid *in situ* (Fig. 1c) and also production of circulating antibody against thyroid (data not shown) when the mice were examined either at 5 months (0/12) or 10 months (0/10) of age. A similar experiment was also conducted with autoimmune oophoritis, which develops in 90% of TG nude mice. The lesion was similarly prevented by intrathymic transplantation of syngeneic newborn ovary (data not shown). The thyroid grafted in the rat thymus developed well histologically, forming follicles with a normal appearance and with active secretion of colloid (Fig. 1d). Expression of thyroid-specific antigen(s) in the intrathymic thyroid grafts was confirmed by immunostaining of frozen sections with autoantibody against thyroid (data not shown). Prevention of autoimmune diabetes by a similar procedure has been reported

Table 1. Prevention of autoimmune thyroiditis in TG nude mice by intrathymic transplantation of thyroid

Site of thyroid transplantation	No. of mice with Thyroiditis (grades 0–3)* At 5 months 0	1	2	3	At 10 months 0	1	2	3	Total (%)	Gastritis Total (%)
None	11	2	5	5	14	4	8	10	34/59 (57·6)	48/59 (81·4)
Intrathymic	12	0	0	0	10	0	0	0	0/22 (0)	17/22 (77·3)
Near thymus	3	0	2	3					5/8 (62·5)	7/8 (87·5)

*Thyroiditis was graded on the basis of histology. 0 = normal thyroid gland; 1 = partial lymphocyte infiltration; 2 = thyroiditis with destruction of more than 50–75% of thyroid; 3 = severe thyroiditis involving more than 75% of thyroid.

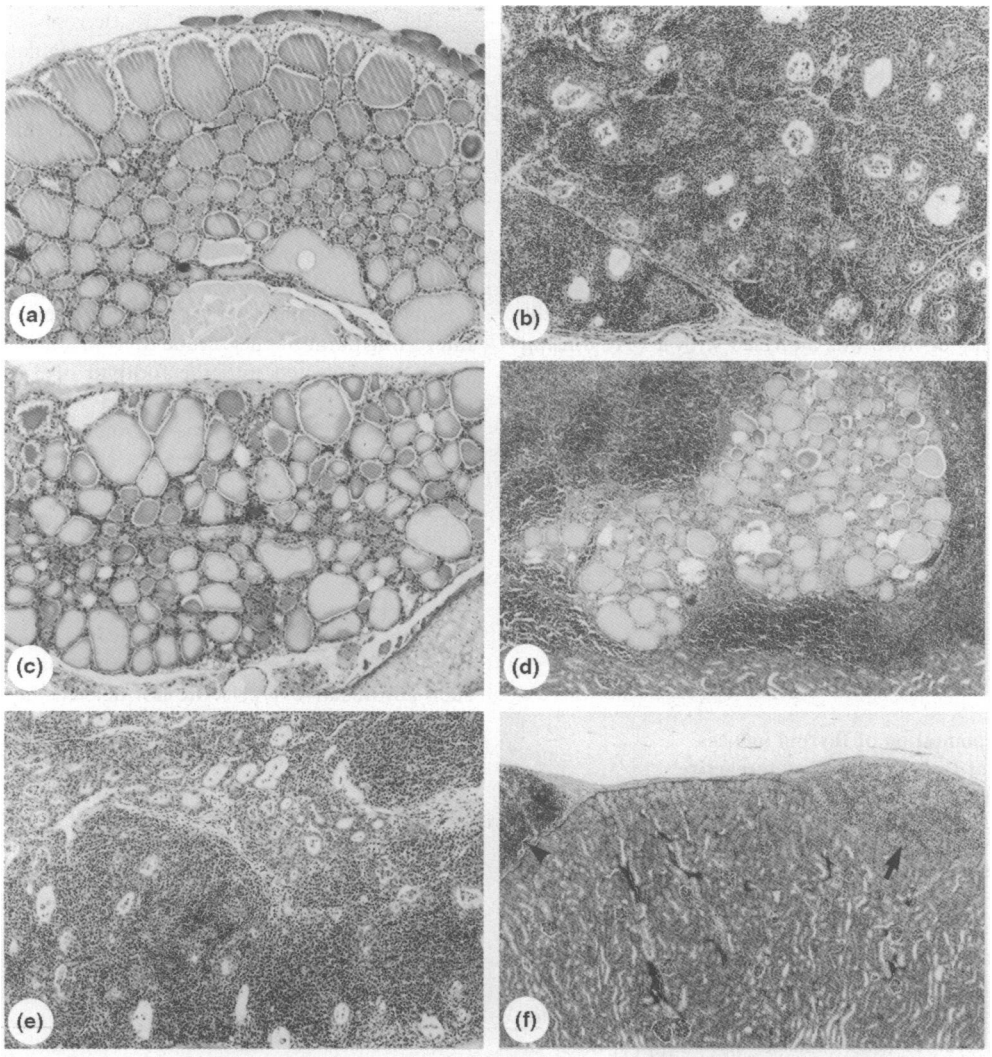

Figure 1. (a) Section of a thyroid from a 10-month-old normal BALB/c mouse (×79). (b) Section of a thyroid from a 10-month-old TG nude mouse (×79). Note destruction of follicles with massive infiltration of mononuclear cells. Histological grade 3 (see Table 1). (c and d) Sections of a thyroid *in situ* (c) (×79) and a thyroid transplanted intrathymically into a rat thymus graft (d) (×49) of a 10-month-old TG nude mouse (Table 1). Note lack of an inflammatory reaction and normal structure of follicles with colloidal secretion in both cases. (e and f) Section of a thyroid *in situ* (e) (×79) and a thyroid (arrow) transplanted under the renal capsule near a rat thymic graft (arrow head) (f) (×38) of a TG nude mouse (Table 1). Note destruction of follicles with severe inflammatory reaction in both thyroids.

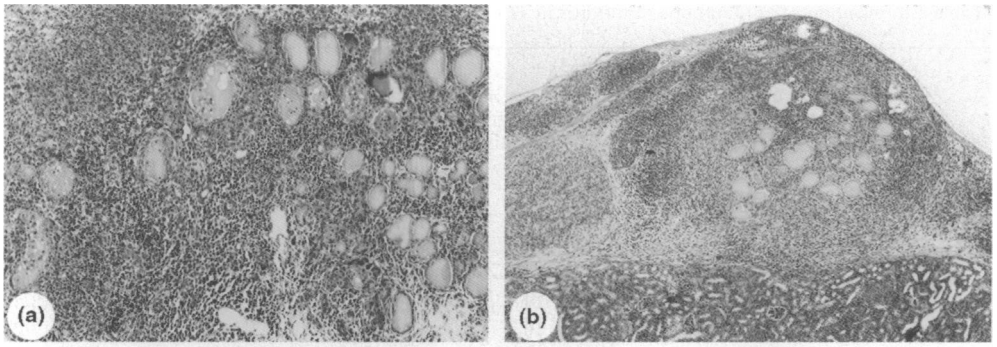

Figure 2. Section of a thyroid *in situ* (a) (×79) of a TG nude mouse and its intrathymic thyroid graft (b) (×38) after immunization with thyroid antigens emulsified in CFA (Table 2). Note severe inflammatory reaction in both thyroids. Histological grade 3.

in the BioBreeding rat[18,19] and in the multi-dose streptozotocin mouse model.[20] Thyroid implantation under the renal capsule near the rat thymi of TG nude mice, however, showed no preventive effect on development of thyroiditis, i.e. a severe inflammatory reaction was observed in both the thyroid *in situ* (Fig. 1e) and the thyroid graft (Fig. 1f) in five of eight TG nude mice thus treated (Table 1).

In TG nude mice with intrathymic thyroid grafts, inflammatory reactions in organs other than the thyroid were recognized at similar incidences as in TG nude mice. For example, gastritis developed in 81·4% and 77·3%, respectively, of TG nude mice and those with intrathymic thyroid grafts at incidences (Table 1). The surface phenotype of spleen cells in these two groups of mice was analysed with a FACS using the following mAb: CD3, CD4, CD8, TCR$\alpha\beta$ and TCR Vβ2, 3, 5.1/5.2, 6, 7, 8.1/8.2, 8.1/8.2/8.3, 9, 11, 13 and 14. There were, however, no apparent differences between TG nude mice with and without intrathymic thyroid grafts (data not shown).

The mixed lymphocyte culture assay against allogeneic spleen cells also revealed comparable results in both groups of mice (data not shown). Thus, the preventive effect of autoimmune diseases by intrathymic thyroid graft is limited to thyroiditis, i.e. this phenomenon is probably related to a thyroid-specific immune response, but not to a systemic alteration in immune function.

Intrathymic transplantation of thyroid induces unresponsiveness of thyroid-specific autoreactive T cells in TG nude mice

The results described above suggested several possible effects of the thyroid grafts within rat thymi on the immune system. The first is clonal deletion[1–5] of thyroid-specific autoreactive T cells by sufficient stimulation with thyroid-specific antigens, because thyroid-specific antigen(s) were well expressed in the intrathymic thyroid grafts, as shown in Fig. 3a.

The second is clonal anergy[6–8] or ignorance[9,10] of thyroid-specific autoreactive T cells, caused by inappropriate or insufficient stimulation by thyroid-specific antigens, respectively, during the peripheralization of the T cells. It is generally thought that naive T cells do not readily circulate through non-lymphoid organs,[21] in which case some of the thyroid-specific T cells would encounter the antigen(s) of intrathymic thyroid grafts without appropriate costimulatory signals or insufficient amount of antigen(s). As shown in Fig. 1d and Fig. 3b, the finding that few lymphocytes were found in the thyroid graft may suggest such a possibility.

The third is formation of thyroid-specific suppressor T cells[14,15] with an ability to inhibit or regulate thyroid-specific autoreactive T cells.

The first possibility was not clearly demonstrated, although not completely eliminated, by the analysis of Vβ usage of the T-cell population alone, as described above. The finding that spleen cells of TG nude mice with intrathymic thyroid grafts did not show an *in vitro* proliferative response to crude thyroid antigens, in contrast to the high response of spleen cells of TG nude mice with thyroiditis (data not shown), supports the possibility of clonal deletion or clonal anergy. To examine this interesting point further, crude thyroid antigens emulsified with CFA were injected into the footpad of TG nude mice with intrathymic thyroid grafts. After 3 weeks, an inflammatory response was observed in the thyroids *in situ* (Fig. 2a) and the intrathymic thyroid grafts (Fig. 2b) of all mice (Table 2), indicating that some autoreactive T cells were present in the periphery, but probably unresponsive due to anergy, although clonal ignorance was also possible. These results taken together suggest either that clonal deletion by the organ-specific peptides of such autoreactive T cells in the thymus is not complete, so that the undeleted population leaves the thymus for the periphery and remains in anergy and/or ignorance, or that such autoreactive T cells are not deleted, but functionally are skewed in the thymus, and leave for the periphery and remain unresponsive with a T-helper type 2 (Th2) dominant response. In the following experiments, we studied the third possibility. As shown in Table 3, all of the autoimmune diseases in the TG nude mice were shown to be prevented by injection of $CD4^+$, but not $CD8^+$, spleen cells obtained from normal mice. Autoimmune thyroiditis as well as other lesions in the TG nude mice were not prevented by injection of spleen cells from TG nude mice with intrathymic thyroid grafts, indicating that thyroid antigen-specific suppressor T cells were not induced in the donor TG nude.

Thyroid-specific suppressor T cells in normal mice are neither deleted nor inactivated by intrathymic transplantation of thyroid

Next we examined the possibility that organ-specific suppressor T cells present in normal mice[15,22] are deleted and/or enter a state of unresponsiveness when the corresponding organs are

Table 2. The effects of thyroid extract injection with CFA on the development of autoimmune thyroiditis in TG nude mice

Mice	Immunization	Mice with thyroiditis (grades 0–3)*				
		0	1	2	3	Total
TG nude mice	PBS + CFA	5	1	2	2	5/10
TG nude mice	Antigen + CFA	0	0	2	8	10/10
TG nude mice with intrathymid thyroid graft	PBS + CFA	10	0	0	0	0/10
TG nude mice with intrathymic thyroid graft	Antigen + CFA	0	2	3	5	10/10

* For grading of lesions, see Table 1.

grafted intrathymically. Thyroids from newborn female BALB/c mice were grafted intrathymically in newborn syngeneic normal female mice. The grafted thyroids developed well, expressing thyroid-specific antigen(s) in the thymus (Fig. 3). Spleen cells from the mice were injected into TG nude mice to examine whether or not they had the ability to prevent development of autoimmune thyroiditis. As shown in Table 3, no autoimmune diseases, including thyroiditis, were found to develop in the recipients, indicating that thyroid-specific suppressor T cells were neither deleted in the thymus nor inactivated, unlike thyroid-specific autoreactive T cells.

Our data show that organ-specific autoreactive T cells, but not organ-specific suppressor T cells, are inactivated or deleted when the corresponding antigens are expressed in the thymus of normal as well as of TG nude mice. Those autoreactive T cells that are functionally skewed or escaped from clonal deletion in the thymus leave for the periphery and remain in a state of unresponsiveness due to anergy, ignorance and/or Th1 to Th2 switch. In many cases, they could be activated by antigen plus adjuvant (Table 2), but they did not show *in vitro* proliferation when cultured with thyroid antigen extract. It is now very important to analyse the cause of unresponsiveness in this population, although separation of such a population experimentally is very difficult. One approach may be the establishment and characterization of autoreactive T-cell lines from TG nude mice with thyroiditis and from those with thyroid engraftment after antigen stimulation, which is now in progress in our laboratories.

The fact that multiple organ-localized autoimmune diseases develop in the TG nude mice is probably due to the failure of education of organ-specific suppressor T cells and/or the failure of total deletion or complete inactivation of organ-specific autoreactive T cells in the grafted rat thymus. The grafted rat thymus showed a normal architecture composed of donor epithelia and host lymphocytes.[11] Thymic dendritic cells, macrophages and blood vessels were also of the host type

Table 3. Prevention of autoimmune thyroiditis and gastritis in TG nuce mice with injection of spleen cells from various donor mice

Cell source and treatment	No. of mice with Thyroiditis (grades 0–3)*					
	0	1	2	3	Total	Gastritis
Normal BALB/c mice						
Non-treated	10	0	0	0	0/10	0/10
Anti-CD4 + C	4	1	2	3	6/10	7/10
Anti-CD8 + C	10	0	0	0	0/10	0/10
TG nude mice						
non-treated	0	1	2	5	8/8	8/8
BALB/c mice with intrathymic thyroid graft						
Non-treated	12	0	0	0	0/12	0/12
Anti-CD4 + C	2	0	1	2	3/5	4/5
Anti-CD8 + C	6	0	0	0	0/6	0/6
TG nude mice with intrathymic thyroid graft						
Non-treated	3	2	2	3	7/10	10/10

* For grading of lesions, see Table 1.

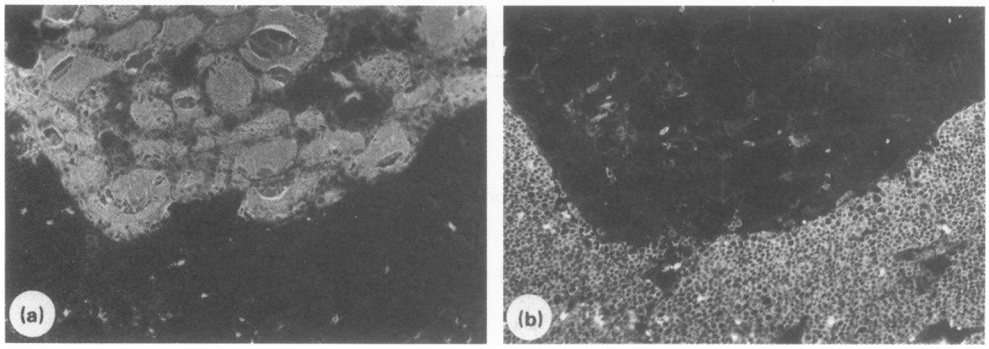

Figure 3. A frozen section of the thymus with a thyroid graft of a normal BALB/c mouse was stained simultaneously with autoantibody to thyroid antigen(s) (rhodamine) (a) and anti-Thy-1.2 antibody (FITC) (b) (both ×116). Note apparent expression of thyroid antigen(s) in the graft (a).

(unpublished observations). Positively selected $CD4^+$ cells from TG nude mice transferred similar lesions to naive syngeneic nude mice,[22] suggesting that effector cells mediating autoimmunity are restricted by host mouse major histocompatibility complex (MHC). In addition, the finding that T-cell lines specific to retinal antigen established from TG nude mice with uveoretinitis vigorously respond to the antigen when cultured with antigen-presenting cells (APC) from syngeneic mouse but not thymic donor rat (unpublished observations) also shows host MHC restriction.

In the mouse, thymectomy 2–4 days after birth causes localized autoimmune lesion in some organs and tissues.[23] The incidence of lesion is greatly reduced when thymectomies are performed at day 0 or 7.[23] In the TG nude model, it is conceivable that an inappropriate interaction between xenogeneic thymic epithelial cells and host T-cell precursors may induce autoimmunity; rat thymi can reconstitute autoreactive T cells, but cannot produce suppressor T cells properly, probably because maturation or education of suppressor T cells requires a more refined epithelial T-cell interaction in the thymus. We speculate that the capability of rat thymus to educate mouse pre-T cells is similar to that of the thymus of a mouse up to day 2–4 after birth.

Thus dysfunction or deletion of organ-specific suppressor T cells and the presence of organ-specific autoreactive T cells in a state of unresponsiveness are extremely high risk factors for individuals. It is possible that suppressor T cells play an important role in maintaining the unresponsiveness of autoreactive T cells in the periphery, although no evidence for this hypothesis is available at present. Recently, we demonstrated that activation of organ-specific suppressor T cells is achieved in the periphery by organ-specific antigens produced from mature organs.[24] It is now very important to study whether such a suppressor T-cell population can be generated and/or activated in autoimmune patients by appropriate manipulation of the immune system for the treatment of the disease.

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REFERENCES

1. Kappler J.W., Roehm N. & Marrack P. (1987) T cell tolerance by clonal elimination in the thymus. *Cell* **49,** 273.
2. Marrack P., Lo D., Brinster R. *et al.* (1988) The effect of thymus environment on T cell development and tolerance. *Cell* **53,** 627.
3. Kisielow P., Bluthmann H., Staerz U.D., Steinmetz M. & von Boehmer H. (1988) Tolerance in T-cell receptor transgenic mice involves deletion of nonmature $CD4^+8^+$ thymocytes. *Nature* **333,** 742.
4. Pullen A.M., Marrack P. & Kappler J.W. (1988) The T-cell repertoire is heavily influenced by tolerance to polymorphic self-antigens. *Nature* **335,** 796.
5. Sha W.C., Nelson C.A., Newberry R.D., Kranz D.M., Russell J.H. & Loh D.Y. (1988) Positive and negative selection of an antigen receptor on T cells in transgenic mice. *Nature* **336,** 73.
6. Rammensee H.-G., Kroschewski R. & Frangoulis B. (1989) Clonal anergy induced in mature $V\beta6^+$ T lymphocytes on immunizing *Mls*-1^b mice with Mls-1^a expressing cells. *Nature* **339,** 541.
7. Ramsdell F., Lantz T. & Fowlkes B.J. (1989) A nondeletional mechanism of thymic self tolerance. *Science* **246,** 1038.
8. Burkly L.C. Lo D., Kanagawa O., Brinster R.L. & Flavell R.A. (1989) T-cell tolerance by clonal anergy in transgenic mice with nonlymphoid expression of MHC class II I-E. *Nature* **342,** 564.
9. Ohashi P.S., Oehen S., Buerki K. *et al.* (1991) Ablation of tolerance and induction of diabetes by virus infection in viral antigen transgenic mice. *Cell* **65,** 305.
10. Oldstone M.B.A., Nerenberg M., Southern P., Price J. & Lewicki H. (1991) Virus infection triggers insulin-dependent diabetes mellitus in a transgenic model: role of anti-self (virus) immune response. *Cell* **65,** 319.
11. Taguchi O., Takahashi T., Seto M., Namikawa R., Matsuyama M. & Nishizuka Y. (1986) Development of multiple organ-localized autoimmune diseases in nude mice after reconstitution of T cell function by rat fetal thymus graft. *J Exp Med* **164,** 60.
12. Ichikawa T., Taguchi O., Takahashi T. *et al.* (1991) Spontaneous development of autoimmune uveoretinitis in nude mice following reconstitution with embryonic rat thymus. *Clin Exp Immunol* **86,** 112.
13. Cunningham A.J. (1975) Active suppressor mechanism maintaining tolerance to some self components. *Nature* **254,** 143.
14. Kojima A., Tanaka-Kojima Y., Sakakura T. & Nishizuka Y. (1976) Prevention of postthymectomy autoimmune thyroiditis in mice. *Lab Invest* 34, 601.
15. Taguchi O. & Nishizuka Y. (1987) Self tolerance and localized

autoimmunity. Mouse models of autoimmune disease that suggest tissue-specific suppressor T cells are involved in self tolerance. *J Exp Med* **165,** 146.

16. STAERZ U.D., RAMMANSEE , H.- G., BENEDETTO J.D. & BEVAN M. (1985) Characterization of a murine monoclonal antibody specific for an allotypic determinant on T cell antigen receptor. *J Immunol* **134,** 3994.
17. TWAROG F.J. & ROSE N.R. (1968) The production of thyroid autoantibodies in mice. *J Immunol* **101,** 242.
18. KOEVARY S.B. & BLOMBERG M. (1992) Prevention of diabetes in BB/Wor rats by intrathymic islet injection. *J Clin Invest* **89,** 512.
19. POSSELT A.M., CLYDE F.B., FRIEDMAN A.L. & NAJI A. (1992) Prevention of autoimmune diabetes in the BB rat by intrathymic islet transplantation at birth. *Science* **256,** 1321.
20. HEROLD K., MONTAG A.G. & BUCKINGHAM F. (1992) Induction of tolerance to autoimmune diabetes with islet antigens. *J Exp Med* **176,** 1107.
21. MACKAY C. (1993) Horming of naive, memory and effector lymphocytes. *Curr Opin Immunol* **5,** 423.
22. IKEDA H., TAGUCHI O., TAKAHASHI T., ITOH G. & NISHIZUKA Y. (1988) L3T4 effector cells in multiple organ-localized autoimmune disease in nude mice grafted with embryonic rat thymus. *J Exp Med* **168,** 2397.
23. TAGUCHI O., TAKAHASHI T. & NISHIZUKA Y. (1990) Self-tolerance and localized autoimmunity. *Curr Opin Immunol* **2,** 576.
24. TAGUCHI O., KONTANI K., IKEDA H., KEZUKA T. *et al.* (1994) Tissue-specific suppressor T cells involved in self-tolerance are activated extrathymically by self-antigens. *Immunology* **82,** 365.