Tolerance Induction in Thymectomized, Adult Xenopus by Co-Transplantation of Thymus and Tolerated Skin Graft

Authors: Tozaki, Shizuka, Ono, Masatada, and Tochinai, Shin

Source: Zoological Science, 17(9) : 1267-1273

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.17.1267

ZOOLOGICAL SCIENCE **17**: 1267–1273 (2000)

Tolerance Induction in Thymectomized, Adult *Xenopus* by Co-Transplantation of Thymus and Tolerated Skin Graft

Shizuka Tozaki*, Masatada Ono and Shin Tochinai

Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan

ABSTRACT—In *Xenopus*, semi-xenogeneic JB skins render donor-specific tolerance to perimetamorphic JJ larvae, whereas the same grafts are never accepted by adults. To clarify the mechanisms underlying tolerance induction, we tried to find a method of inducing tolerance in adults. First, we reconstituted early-thymectomized (E-Txd) adults with larval or adult thymi. All JB skin grafts transplanted to E-Txd adults that had been reconstituted with larval thymi were rejected, while almost all of the E-Txd larvae that had been reconstituted with larval or adult thymi were rendered tolerant. Second, we transplanted tolerated JB (tol-JB) skin, i.e., JB skin that reportedly possessed a suppressive activity (Ono and Tochinai, 1995), to late-thymectomized adults and found that those adults were rendered tolerant. Third, when tol-JB skin and larval or adult thymi were simultaneously transplanted to E-Txd animals, many of the E-Txd adults were rendered tolerant. The overall results indicate that donor-specific tolerance can be induced in thymectomized JJ adults by co-transplanting either a larval or an adult thymus and a tol-JB skin graft.

INTRODUCTION

Most studies on the ontogeny of immunity in amphibians have used the South African clawed frog *Xenopus laevis*, whose immune system is quite similar to that of mammals (Flajnik *et al*., 1984; Hsu and Du Pasquier, 1984; Schwager and Hadji-Azimi, 1985; Hsu *et al*., 1985; Kaufman *et al*., 1985a, b). *Xenopus* serves as a model for studying self-tolerance. Allogeneic or semi-xenogeneic adult skin grafts readily tolerize perimetamorphic *Xenopus* larvae (Chardonnens and Du Pasquier, 1973; DiMarzo and Cohen, 1982a, b; Tochinai, 1993), whereas skin grafts of the same genetic combinations transplanted to adults are always rejected. Since splenic lymphocytes obtained from animals that tolerated skin grafts retain their proliferative responsiveness to the skin donor antigens both *in vitro* and *in vivo* (Flajnik *et al*., 1985; Arnall and Horton, 1987; Sakuraoka and Tochinai, 1993), and since tolerance is barely inducible in metamorphosing larvae that have been thymectomized just before skin grafting (Barlow and Cohen, 1983; Tochinai, 1993), it is thought that tolerance induction in *Xenopus* larvae may result from a thymus-derived suppressive activity rather than from clonal deletion or anergy of immunocompetent T cells.

Although it is known that immunological unresponsiveness to “self” components is ascribable to clonal deletion or anergy of specific clones of immunocompetent T cells, this cannot explain the observation that the adoptive transfer of cells from immunologically tolerant animals reduces the immune response to the same antigen in naive recipient animals (Nakamura *et al*., 1987; Dorf *et al*., 1992). In addition, the concept that suppressor cells play an important role in tolerance induction has been suggested from recent issues examining the types of responses of polarized Th1 and Th2 (reviewed by Bloom *et al*., 1992 and Hayday, 1995). Du Pasquier and Bernard (1980) reported that when thymocytes from metamorphosing *Xenopus* were transferred into isogeneic adults, this delayed the rejection of skin grafts that differed from the adult host by minor histocompatibility antigens. Furthermore, Ono and Tochinai (1995) reported that the tolerated JB (tol-JB) skin (i.e., JB skin that had been transplanted to metamorphosing JJ larvae and accepted for 4–5 weeks after grafting) could induce tolerance even in late-thymectomized larvae. They concluded that this tolerance induction was due to suppressive activity in tol-JB skin grafts. They also reported that lymphocytes obtained from tol-JB skin exhibited the same suppressive activity and that about 30% of the lymphocytes obtained from the tol-JB skin were stained with XT-1 (*Xenopus* T cell specific monoclonal antibody; Nagata, 1988). These data support the hypothesis that thymus-derived cells suppress the immune response in metamorphosing larvae.

In the present study, attempts have been made to identify how thymus-dependent suppression is involved in tolerance induction in *Xenopus*. To avoid possible effects of the endogenous thymus on tolerance induction, we used thymec-

* Corresponding author: Tel. +81-0123-36-8119.
E-mail: stozaki@hht.ac.jp
† Present Address: Department of Orthoptics, Hokkaido College of High Technology; Megumino Kita-2, 12-1, Eniwa 061-1396, Japan

tomized JJ larvae or adults as hosts for judging donor-specific tolerance. First, we examined possible effects of transplanting perimetamorphic thymuses to JJ adults which had been thymectomized at st. 45–46 (early-thymectomized: E-Txd) on the induction of tolerance for semi-xenogeneic JB skin grafts. Second, to examine the suppressive activity in tol-JB skin grafts, which has been demonstrated previously by Ono and Tochinai (1995), we transplanted adult JB skin grafts to JJ larvae to first induce tolerance and then transplanted the tolerated JB skin grafts (i.e., tol-JB skin grafts) to JJ animals which had been thymectomized at st. 54–55 (late-thymectomized: L-Txd). Third, tol-JB skin grafts and thymi were simultaneously transplanted to E-Txd adults which were not rendered tolerant by transplantation of the thymus only, in order to clarify the mechanism of suppressive activity in tol-JB skin grafts. We succeeded in inducing tolerance in thymectomized JJ adults by grafting tol-JB skin grafts and thymi simultaneously. Possible mechanisms of tolerance induction in adult *Xenopus* are discussed.

MATERIALS AND METHODS

Experimental animals

The animals used in the present study included inbred (there is no graft rejection or MLR between J individuals) J strain (JJ, MHC haplotype, *j/j*), outbred wild-type *Xenopus laevis*, and a colony of *Xenopus borealis* (BB, MHC haplotype, *b/b*). All the animals were bred and maintained in our laboratory. The BB animals used in the present study do not reject skin grafts from one another, which shows their genetic homogeneity. Artificial insemination was performed to produce inter-specific hybrids (JB, MHC haplotype, *j/b*) between *X. laevis* eggs and *X. borealis* sperm. All larvae and metamorphosed animals were reared at 23°C in dechlorinated tap water. Developing animals were staged according to the normal table of Nieuwkoop and Faber (1956).

Experimental design

Three experiments were conducted to evaluate possible contributions of the thymus and/or tolerated JB (tol-JB) skin grafts to the development of tolerance induction in *Xenopus* (Fig. 1).

Experiment I: Thymi, either from JJ larvae or adults, and naive adult JB skin grafts were simultaneously transplanted into JJ larvae (st. 54–55) or adults (4-month-old), which had been thymectomized at st. 45–46 (early-thymectomized: E-Txd). JJ spleen (either larval or adult) and naive JB skin graftings were also performed. The transplanted JB skin grafts were observed frequently for 130 days after the grafting to determine whether tolerance was induced.

Experiment II: To examine the suppressive activity in tol-JB skin grafts, we transplanted JB skin grafts into JJ larvae (primary hosts) to first induce tolerance (Tochinai, 1993). Then, the JB skin grafts that had been accepted for 4–5 weeks (called tol-JB skin) were transplanted into JJ animals (secondary hosts) at different developmental stages. These secondary hosts had been thymectomized at st. 54–55 (late-thymectomized: L-Txd).

Experiment III: The tol-JB skin grafts and thymi (either larval or adult) were simultaneously transplanted into E-Txd adults (secondary hosts) which were not rendered tolerant by transplantation of the thymus only, in order to determine the mechanism of suppressive activity in the tol-JB skin grafts.

To evaluate donor-specific tolerance induction, 30 days after the first-set JB or tol-JB skin grafting, second-set JB skin grafts and third-party allografts (from wild-type adults, third party) were transplanted into all animals used in the experiment. JB skin graft rejection takes about 20 days when JJ adults are used as hosts (Nakamura *et al.*, 1987). In this study, we observed skin graft survival for at least 100 days for allografts and second-set JB skin grafts. Therefore, the survival of first-set JB skin grafts was observed for at least 130 days.

Tissue Grafting

Skin grafting: A square piece (4.0 mm^2) of adult JB ventral skin was grafted onto JJ adults or metamorphosing JJ larvae as described previously (Obara *et al.*, 1983; Nakamura *et al.*, 1987). It was determined that the size of the skin grafts was enough to induce tolerance of semi-xenogeneic skin grafts in perimetamorphic larvae (Barlow and Cohen, 1983; Sakuraoka and Tochinai, 1993; Tochinai, 1993; Ono and Tochinai, 1995). The viability of the grafted skin was observed frequently, in animals without anesthesia, under a dissecting microscope. Rejection was monitored by the percentage of the area occupied by destroyed white pigment cells, and the day when all the pigment cells were destroyed was defined as the "end point." Mean rejection time (MRT) was calculated by dividing sum of rejection days (the days taken for reaching "end point") by the number of experimental animals.

Organ grafting: Thymus or spleen grafting was performed according to the technique described by Tochinai (1993). A pair of thymi or a spleen was subcutaneously transplanted into JJ adults and metamorphosing JJ larvae. Adult thymi or spleens were obtained from frogs that were about 4-month-old (2 months after metamorphosis), and larval ones were obtained from st. 54–55 larvae.

Thymectomy

For early thymectomy, st. 45–46 JJ larvae (4–5 days after fertilization) were thymectomized by cauterization using a needle connected to a high frequency electroscalpel apparatus, as described previously (Horton and Manning, 1972; Arnall and Horton, 1987). Absence of the thymus was confirmed by external observations of larvae at st. 50–55. For late thymectomy, thymi of st. 54 JJ larvae were cauterized in the same manner as that used for early thymectomy.

Statistical Analysis

Student *t*-test was used to check the significance among the MRTs of transplanted JB skin grafts.

RESULTS

Tolerance induction by grafting thymus or spleen to early-thymectomized JJ animals

To understand the mechanism of the thymus in induction of tolerance to semi-xenogeneic JB skin grafts, we tried to induce tolerance in JJ adults by perimetamorphic thymus transplantation (Experiment I, see Fig. 1). We simultaneously transplanted both a JB skin graft and a pair of thymi from JJ larvae or adults into JJ larvae (st. 54–55) or adults (4-month-old) that had been thymectomized at st. 45–46 (early-thymectomized: E-Txd). Because E-Txd animals cannot reject either semi-xenogeneic JB nor xenogeneic BB skin grafts (Horton *et al.*, 1992), we were able to exclude from consideration the effects of the endogenous thymus and examine the effects of the transplanted thymus on induction of tolerance to simultaneously transplanted JB skin grafts. To check whether donor-specific tolerance was induced, we also transplanted both allografts and second-set JB skin grafts 30 days after the first transplantation. The animals were deemed as tolerant only

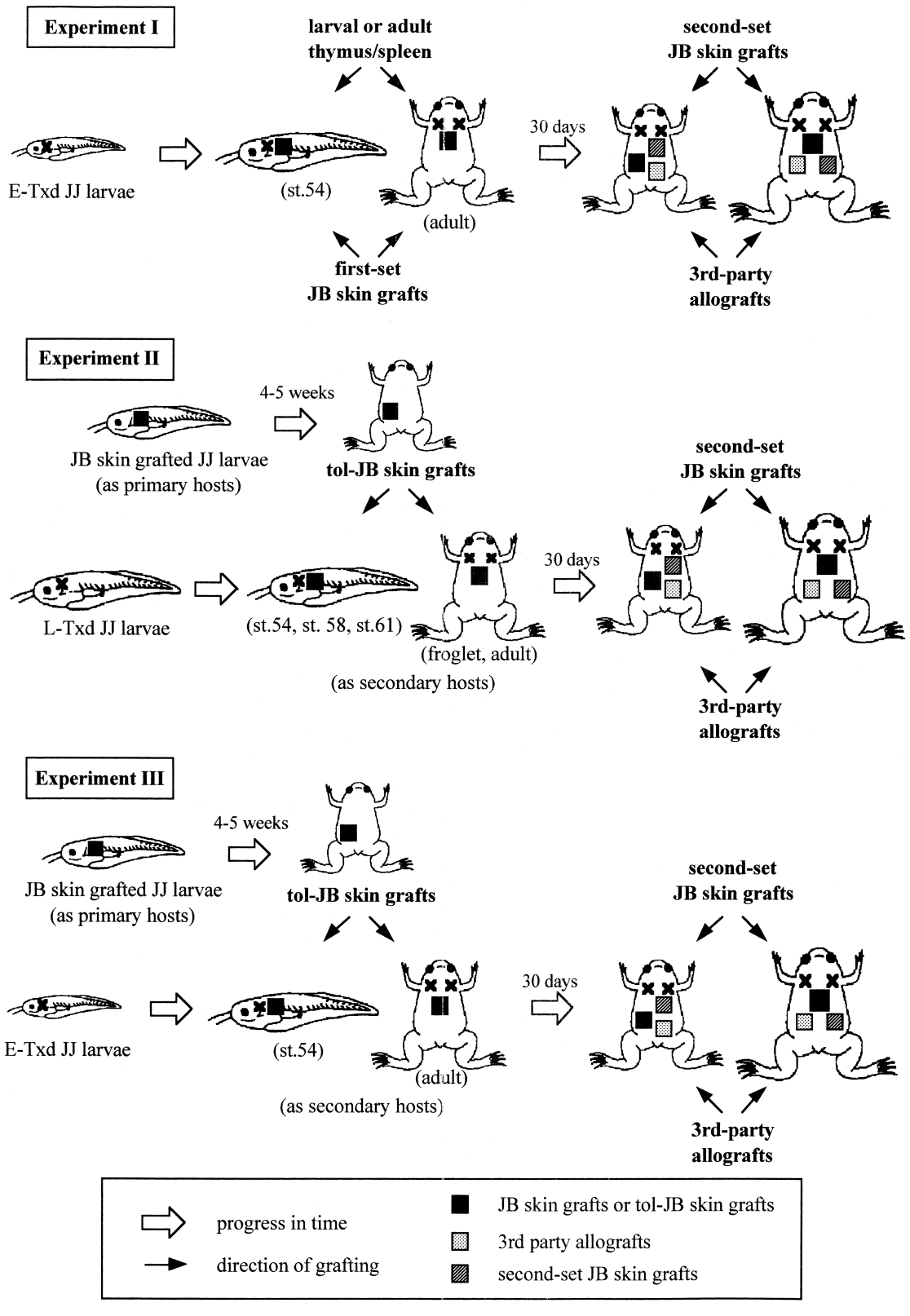

Fig. 1. Experimental design to identify how thymus-dependent suppression is involved in tolerance induction in *Xenopus*. In Experiment I, E-Txd JJ larvae or adults were transplanted with a JJ thymus or spleen and with first-set JB adult skin. In Experiment II, L-Txd JJ animals (secondary hosts) at different developmental stages were transplanted with tolerated JB (tol-JB) skins, which had been obtained by grafting JB skins to JJ larvae (primary hosts). In Experiment III, E-Txd JJ larvae or adults (secondary hosts) were co-transplanted with a JJ thymus and a tol-JB skin graft. For evaluating donor specificity, all animals used in the experiment were grafted with second-set JB skin grafts and allografts 30 days after the first-set JB or tol-JB skin grafting.

Table 1. Tolerance induction by grafting thymus or spleen to E-Txd animals

Type of grafted organ	Stages at transplantation			
	E-Txd adults		E-Txd larvae	
	% tolerant[1)]	MRT of first-set JB[2)]	% tolerant	MRT of first-set JB
adult thymus	20 (1/5)	37	100 (4/4)	(+)
larval thymus	0 (0/3)	44	67 (4/6)	34
adult spleen	0 (0/3)	20	0 (0/10)	20
larval spleen	0 (0/3)	44	0 (0/4)	63
none[3)]	0 (0/10)	(–)	0 (0/10)	(–)

Naive JB skin grafts were transplanted to E-Txd adults (4-month-old) and larvae (st. 54–55). JJ thymus (adult or larval) or JJ spleen (adult or larval) grafting was performed at the same time as skin grafting. Percentages of tolerance induction for each transplantation and MRT (mean rejection time) of first-set JB skin grafts are shown.

[1)] In parenthesis, the numbers of animals which accepted both first- and second-set JB skin grafts but rejected allografts/number of experimental animals, are presented.

[2)] MRT in days in non-tolerant animals.

[3)] E-Txd animals without any grafted organ were used as controls.

(+): Skin grafts except for allografts were not rejected at all.

(–): None of the skin grafts (first- and second-set JB skin grafts and allografts) were rejected at all.

when they accepted both first- and second-set JB skin grafts but rejected the third-party allograft.

Table 1 shows the number of E-Txd animals that were rendered tolerant. None of the skin grafts (first- and second-set JB skin grafts and third-party allografts) were rejected when they were transplanted into either E-Txd larvae or E-Txd adults, and this result suggested that the E-Txd animals had a lower level of immunoreactivity. All JB skin grafts transplanted to larval thymus-grafted E-Txd adults were rejected (% tolerant, 0/3). These larval thymus-grafted E-Txd adults rejected the first-set JB skin grafts later than non-thymectomized adults. The mean rejection time (MRT) of first-set JB skin grafts in larval thymus-grafted E-Txd adults was 44 days, whereas that in non-thymectomized JJ adults was about 20 days (Nakamura *et al.*, 1987). On the contrary, all adult thymus-grafted E-Txd larvae accepted both first- and second-set JB skin grafts but rejected allografts (% tolerant, 4/4). The MRT of third-party allografts was 26 days (data not shown). Because allograft rejection by larvae at st. 64-65 takes about 15 days (Obara *et al.*, 1983), a slight delay in allograft rejection was confirmed in adult thymus-grafted E-Txd larvae, which developed to st. 64–65 when second-set JB skin grafts and allografts were transplanted. When JJ spleen (either from larvae or adults) instead of JJ thymus was transplanted into E-Txd JJ animals, all skin grafts were rejected.

Tolerance induction by grafting tol-JB skin to late-thymectomized JJ animals

To examine the possible suppressive activity in tol-JB skin grafts, we transplanted tol-JB skin into late-thymectomized (L-Txd, thymectomized at st. 54–55) animals (Experiment II, see Fig. 1). Thirty days after tol-JB skin transplantation, we transplanted both JB skin grafts and allografts to check for donor-specific tolerance induction. Because our preliminary study suggested that tolerance to JB skin grafts rarely became inducible in later stages (Obara *et al.*, 1983; Cohen *et al.*, 1985), we used st. 54, st. 58, and st. 61 larvae, froglets (adults immediately after metamorphosis), and adults (4-month-old) as hosts. We also transplanted naive JB skin grafts into L-Txd animals as controls.

As shown in Table 2, all L-Txd animals rejected both second-set JB skin grafts and allografts when they were transplanted with naive first-set JB skin grafts. In contrast, a high percentage of tolerance was induced in tol-JB skin-grafted L-Txd larvae at st. 54 (% tolerant, 6/7). Likewise, about half or more of the tol-JB skin-grafted L-Txd larvae at st. 58, and st. 61, froglets, and even adults, were rendered tolerant. The MRTs for second-set JB skin grafts, which were grafted 30 days after the tol-JB skin transplantation, were 15 days for st. 54 larvae, 46 days for st. 58 larvae, 76 days for st. 61 larvae, 73 days for froglets and 45 days for adults. A significant delay in JB skin graft rejection was confirmed in tol-JB skin-grafted L-Txd animals, except for st. 54 larvae ($p<0.005$ for st.58 larvae and $p<0.05$ for adults, student *t*-test).

Tolerance induction by co-transplantation of a thymus and a tol-JB skin graft into early-thymectomized JJ animals

As shown in Experiment II, transplantation of the tol-JB skin, which contained a suppressive activity, induced tolerance even in L-Txd adults. In order to determine the mechanism of the suppressor activity, Experiment III was conducted (Fig. 1), in which the tol-JB skin grafts and a pair of thymi (obtained from either adults or larvae) were transplanted simultaneously into E-Txd adults, which were not rendered tolerant by transplantation with the thymus only. The only difference from Experiment I was that tol-JB skin was grafted instead of JB skin. Thirty days later, JB skin grafts and

Table 2. Tolerance induction by transplanting tolerated JB skin or naive JB skin to L-Txd animals

Type of grafted skin	Stages of hosts	% tolerant[1)]	MRT of second-set JB[2)]
tol-JB	larvae (st. 54)	86 (6/7)	15
	larvae (st. 58)	67 (4/6)	46*
	larvae (st. 61)	50 (2/4)	76
	froglets	67 (6/9)	73
	adults	50 (3/6)	45**
naive JB	larvae (st. 54)	0 (0/7)	34
	larvae (st. 58)	0 (0/5)	28
	adults	0 (0/6)	19

Tol-JB skin or naive JB skin was transplanted into L-Txd animals at each stage.
Percentages of tolerance induction and MRT (mean rejection time) of the second-set JB skin grafts transplanted 30 days after tol-JB skin grafting are shown.

[1)] In parenthesis, the numbers of animals which accepted both first- and second-set JB skin grafts but rejected allografts/number of experimental animals, are presented.

[2)] MRT in days in non-tolerant animals.

* Significant differences were ovserved between tol-JB skin and naive JB skin grafted larvae at st. 58 ($p<0.005$).

** Significant differences were ovserved between tol-JB skin and naive JB skin grafted adults ($p<0.05$).

Table 3. Tolerance induction in E-Txd animals by co-transplantation of thymus and tol-JB skin

Type of grafted organ	Stages at transplantation			
	E-Txd adults		E-Txd larvae	
	% tolerant[1)]	MRT of second-set JB[2)]	% tolerant	MRT of second-set JB
adult thymus	20 (1/5)	37	100 (4/4)	(+)
adult thymus	63 (5/8)	22	100 (9/9)	(+)
larval thymus	25 (2/8)	22	100 (7/7)	(+)
none	0 (0/10)	23	88 (7/8)	29

Tol-JB skin grafts were transplanted to E-Txd adults (4-month-old) and larvae (st. 54-55). JJ thymus (adult or larval) grafting was performed at the same time as skin grafting. Percentages of tolerance induction for each transplantation and MRT (mean rejection time) of second-set JB skin grafts transplanted 30 days after the tol-JB skin grafting are shown.

[1)] In parenthesis, the numbers of animals which accepted both first- and second-set JB skin grafts but rejected allografts/number of experimental animals, are presented.

[2)] MRT in days in non-tolerant animals.

(+): Skin grafts except for allografts were not rejected at all.

allografts were also transplanted.

As shown in Table 3, all E-Txd larvae and some of the E-Txd adults grafted with both tol-JB skin grafts and a pair of thymi (either from larvae or adults) were rendered tolerant. The MRT for second-set JB skin grafts was 22 days for both adult and larval thymus-transplanted E-Txd adults. All of the E-Txd adults and 1 in 8 of the E-Txd larvae that had not undergone transplantation with the thymus rejected tol-JB skin (MRT: 35 days and 108 days, respectively) and also rejected both second-set JB skin grafts (MRT: 23 days and 29 days, respectively) and allografts (MRT: 37 days and 78 days, respectively; data not shown).

DISCUSSION

To clarify perimetamorphic changes in the properties of the thymus, we transplanted a pair of larval or adult thymi into E-Txd animals which were known to have no ability to reject either semi-xenogeneic JB or xenogeneic BB skin grafts (Horton *et al.*, 1992). This inability of these animals enabled us to exclude from consideration the effects of the endogenous thymus in Experiment I. If some activities in the larval thymus are necessary for tolerance induction, E-Txd adults reconstituted with a pair of larval thymi will be rendered tolerant. As shown in Table 1, most of the adult or larval thymus-grafted E-Txd adults rejected the JB skin, whereas almost all of the adult or larval thymus-grafted E-Txd larvae were rendered tolerant. One adult thymus-grafted E-Txd adult accepted both first- and second-set JB skin grafts but rejected third-party allograft. Such acceptance of JB skin grafts was not reappeared at all in the other adult thymus-grafted E-Txd adults, even when we took the animals which were excluded from the data because they died before the end of observed period (130 days) into consideration. Therefore, adult-thymus

grafted E-Txd adults were deemed "not rendered tolerance." Similar results were obtained from preliminary experiments in which cell suspensions of thymi were injected into E-Txd animals. In these experiments, all the JB skin grafts transplanted into larval thymocytes-injected E-Txd adults were rejected, whereas some of the adult thymocytes-injected E-Txd larvae (2 out of 3 experimental animals) were rendered tolerant. These findings suggest that even the adult thymus has a suppressive effect on skin graft rejection.

Du Pasquier and Bernard (1980) reported that the transfer of thymocytes from metamorphosing *Xenopus* into isogeneic adults significantly prolonged the survival of skin grafts which differed from the adult host in terms of minor histocompatibility antigens. The reason why their injection of thymocytes only delayed the graft rejection might have been because the suppression by transferred thymocytes was interfered with, at least in part, by a variety of immunoreactive cells in the non-thymectomized hosts. The E-Txd animals that were used in this study as hosts allowed us to exclude from consideration the effects of the endogenous thymus, thereby providing us with a simpler experimental system.

Ono and Tochinai (1995) induced tolerance in L-Txd larvae by transplanting both JB skin grafts and tol-JB skin grafts simultaneously, and their study suggested dhat tol-JB skin grafts have a suppressive effect. Properties of tol-JB skin grafts were thus analyzed by transplanting tol-JB skin into L-Txd animals at different developmental stages in Experiment II. More than half of the tol-JB skin-grafted L-Txd adults showed donor-specific tolerance to JB skin transplanted 30 days after tol-JB skin grafting, whereas tolerance induction did not occur in naive JB skin-grafted L-Txd adults (Table 2). This result suggests that the tol-JB skin graft exerts an activity which suppresses JB skin graft rejection. Because the percentage of tolerant frogs gradually decreased with the development of the hosts (from 86% at st. 54 to 50% in adults), the suppressor activity of tol-JB skin grafts may be influenced by age, stage and/or other unknown factors of the host. On one hand, results of Experiment III suggest the possibility that the thymus contributes to the suppressor activity in tol-JB skin grafts. When tol-JB skin grafts were transplanted into E-Txd JJ adults, all the transplanted skin grafts (tol-JB skin, JB skin and allograft) were rejected, whereas when a tol-JB skin graft was simultaneously transplanted with a pair of thymi (either larval or adult) into E-Txd adults, many of them were rendered tolerant (Table 3). This suggests that some thymus-derived cells are necessary for inducing and/or maintaining tolerance. Furthermore, the percentage of tolerance in adult thymus-grafted E-Txd adults was higher than that in larval thymus-grafted E-Txd adults. The reason for this result might be because adult thymus had more thymocytes than larval one. These results allow us to speculate that the tol-JB skin grafts exert an activity, by themselves or probably in combination with an activity of the thymus, to suppress JB skin graft rejection and effect donor-specific tolerance. From the results of Experiments I and III, we can tentatively conclude that the suppressor activity is generated when the thymus (either larval or adult) is placed in a perimetamorphic larval body and that the suppressor activity can be induced even in adults by co-transplantation of a thymus (either larval or adult) and tol-JB skin grafts. The reason for perimetamorphic tolerance induction may not be because the suppressor activity is down-regulated in the adult thymus but because the functional suppressor activity is generated or potentiated in the larval period.

As mentioned above, when we transplanted tol-JB skin grafts into E-Txd adults, all the grafted skin, tol-JB skin grafts, JB skin grafts and allografts were rejected (Table 3). This result was surprising because E-Txd animals have repeatedly been shown to have very few thymocytes (Horton, 1997) and therefore, cannot reject either JB or even totally xenogeneic BB skin grafts (Horton *et al.*, 1992). In addition, it is hard to explain why L-Txd adults, which are known to have some thymus-derived cells (Gravenor *et al.*, 1995) and to show slightly depressed mitogen responses (Rollins-Smith *et al.*, 1996), were rendered tolerant by transplantation with tol-JB skin (Table 2). From these observations, we conclude that the tol-JB skin grafts contain several types of cell populations derived from the primary host (JJ larvae transplanted with JB skin grafts that were tolerated for 4–5 weeks): 1) cells which suppress the JB skin-graft rejection (Ono and Tochinai, 1995); 2) cells which are primed to destroy JB skin grafts; and 3) other immunoreactive cells such as passenger antigen presenting cells (APCs). NK-like cells, which were reported to develop in the spleens of E-Txd adults but were absent from the spleens of E-Txd larvae (Horton *et al.*, 1998), may also have played a role when skin grafts were transplanted to E-Txd adults. At present, however, we cannot directly examine the contribution of these cell populations in tolerance induction due to the lack of appropriate cellular markers in *Xenopus*. Further studies are undoubtedly necessary to identify the cell populations which are contained in the tol-JB skin grafts.

Because tolerance was never induced in naive JJ adults that received tol-JB skin, not only suppression but also a transient depression of the immune system may be involved in tolerance induction. Several lines of evidence suggest that adults have "stronger" immunoreactivities than larvae. For example, the larval antibody repertoire is smaller than that of adults (Hsu and Du Pasquier, 1992); most of the adult-type MHC class I positive cells do not appear until larvae reach st. 57 (Flajnik *et al.*, 1986; Flajnik and Du Pasquier, 1988); and cells with an NK-like activity develop in the spleens of E-Txd adults but are absent from the spleens of E-Txd larvae (Horton *et al.*, 1998). Furthermore, it is possible that the immunoreactivity of thymectomized (both E-Txd and L-Txd) adults, even those reconstituted with a thymus, is lower than that of non-thymectomized adults at the time of tol-JB skin transplantation, because it takes a few days for the transplanted thymus to become vascularized (Horton, 1997). Thus, differential prosperity and decline of suppressor cells, effector cells and/or APCs and their expansion, and impaired immune responsiveness might be required for tolerance induction.

We were able to induce tolerance in thymectomized adults by co-transplantation of a thymus and a tol-JB skin graft. We

believe that suppression is one of the important mechanisms which allows animals to metamorphose smoothly by inducing tolerance of adult-type antigens. However, it is not likely that suppression is the only mechanism involved in inducing tolerance of adult-type antigens. A delicate balance between immunity and suppression may determine the fate of tolerance induction (reviewed in Goodnow, 1996). Although we are far from clarifying how such a delicate balance is controlled, the experimental system reported here provides a valuable model for analyzing mechanisms of tolerance induction to newly appearing antigens in immunocompetent animals.

ACKNOWLEDGEMENTS

We would like to thank Dr. Masami Wakahara (Hokkaido University) for a critical reading of this manuscript.

REFERENCES

Arnall JC and Horton JD (1987) *In vivo* studies on allotolerance perimetamorphically induced in control and thymectomized *Xenopus*. Immunol 62: 315–319

Barlow EH and Cohen N (1983) The thymus dependency of transplantation allotolerance in the metamorphosing frog *Xenopus laevis*. Transplantation 35: 612–619

Bloom B, Salgame P, Diamond B (1992) Revisiting and revising suppressor T cells. Immunol Today 13: 131–136

Chardonnens X and Du Pasquier L (1973) Induction of skin allograft tolerance during metamorphosis of the toad *Xenopus laevis*: a possible model for studying generation of self tolerance to histocompatibility antigens. Eur J Immunol 3: 569–573

Cohen N, DiMarzo S, Rollins-Smith L, Barlow E, Vanderschmidt-Parsons S (1985) The ontogeny of allo-tolerance and self-tolerance in larval *Xenopus laevis*. In "Metamorphosis" Ed by Balls M, Clarendon Press, Oxford, pp 388–419

DiMarzo SJ and Cohen N (1982a) Immunogenetic aspects of *in vivo* allotolerance induction during the ontogeny of *Xenopus laevis*. Immunogenetics 16: 103–116

DiMarzo SJ and Cohen N (1982b) An *in vivo* study of the ontogeny of alloreactivity in the frog, *Xenopus laevis*. Immunology 45: 39–48

Dorf M, Kuchoroo V, Collins M (1992) Suppressor T cells: some answers but more questions. Immunol Today 13: 241–243

Du Pasquier L and Bernard CCA (1980) Active suppression of the allogeneic histocompatibility reactions during the metamorphosis of the clawed toad *Xenopus*. Differentiation 16: 1–7

Flajnik MF, Kaufman JF, Riegert P, Du Pasquier L (1984) Identification of class I major histocompatibility complex encoded molecules in the amphibian *Xenopus*. Immunogenetics 20: 433–442

Flajnik MF, Du Pasquier L, Cohen N (1985) Immune responses of thymus/lymphocyte embryonic chimeras: studies on tolerance and major histocompatibility complex restriction in *Xenopus*. Eur J Immunol 15: 540–547

Flajnik MF, Kaufman JF, Hsu E, Manes M, Parisot R, Du Pasquier L (1986) Major histocompatibility complex-encoded class I molecules are absent in immunologically competent *Xenopus* before metamorphosis. J Immunol 137: 3891–3899

Flajnik MF, Du Pasquier L (1988) MHC class I antigens as surface markers of adult erythrocytes during the metamorphosis of *Xenopus*. Devel Biol 128: 198–206

Goodnow CC (1996) Balancing immunity and tolerance: deleting and tuning lymphocyte repertoires. Proc Natl Acad Sci 93: 2264–2271

Gravenor I, Horton TL, Ritchie P, Flint E, Horton JD (1995) Ontogeny and thymus-dependence of T cell surface antigens in *Xenopus*: flow cytometric studies on monoclonal antibody-stained thymus and spleen. Devel Comp Immunol 19: 507–523

Hayday A (1995) Is antigen-specific suppression now unsuppressed? Curr Biol 5: 47–50

Horton JD and Manning MJ (1972) Response to skin allografts in *Xenopus laevis* following thymectomy at early stages of lymphoid organ maturation. Transplantation 14: 141–154

Horton JD, Horton TL, Ritchie P, Varley A (1992) Skin xenograft rejection in *Xenopus*-immunohistology and effect of thymectomy. Transplantation 53: 473–476

Horton JD (1997) Thymectomy and transplantation in *Xenopus*. In "Immunology Methods Manual" Ed by Lefkovits I, Academic Press, New York, pp 2396–2406

Horton TL, Ritchie P, Watson MD, Horton JD (1998) Natural cytotoxicity towards allogeneic tumor targets in *Xenopus* mediated by diverse splenocyte populations. Devel Comp Immunol 22: 217–230

Hsu E and Du Pasquier L (1984) Studies on *Xenopus* immunoglobulins using monoclonal antibodies. Mol Immunol 21: 257–270

Hsu E, Flajnik MF, Du Pasquier L (1985) A third immunoglobulin class in amphibians. J Immunol 135: 1998–2004

Hsu E and Du Pasquier L (1992) Changes in the amphibian antibody repertoire are correlated with metamorphosis and not with age or size. Devel Immunol 2: 1–6

Kaufman JF, Flajnik MF, Du Pasquier L, Riegert P (1985a) *Xenopus* MHC class II molecules, I. Identification and structural characterization. J Immunol 134: 3248–3257

Kaufman JF, Flajnik MF, Du Pasquier L (1985b) *Xenopus* MHC class II molecules, II. polymorphism as determined by two-dimensional gel electrophoresis. J Immunol 134: 3258–3264

Nagata S (1988) T cell-specific antigen in *Xenopus* identified with a mouse monoclonal antibody: biochemical characterization and species distribution. Zool Sci 5: 77–83

Nakamura T, Maeno M, Tochinai S, Katagiri C (1987) Tolerance induced by grafting semi-allogeneic adult skin to larval *Xenopus laevis*: possible involvement of specific suppressor cell activity. Differentiation 35: 108–114

Nieuwkoop PD and Faber J (1956) "Normal table of *Xenopus laevis* (Daudin)" 2nd ed, North Holland Publisher, Amsterdam

Obara N, Kawahara H, Katagiri C (1983) Response to skin grafts exchanged among siblings of larval and adult gynogenetic diploids in *Xenopus laevis*. Transplantation 36: 91–95

Ono M and Tochinai S (1995) Demonstration of cells possessing tolerance-inducing activity in *Xenopus laevis* rendered tolerant perimetamorphically. Transplantation 60: 66–70

Rollins-Smith LA, Needham DAP, Davis AT, Blair PJ (1996) Late thymectomy in *Xenopus* tadpoles reveals a population of T cells that persists through metamorphosis. Devel Comp Immunol 20: 165–174

Sakuraoka J and Tochinai S (1993) Demonstration of cells involved in rejection of tolerogenic grafts in tolerant *Xenopus*. Devel Comp Immunol 17: 439–447

Schwager J and Hadji-Azimi I (1985) Anti-immunoglobulin M induces both B-lymphocyte proliferation and differentiation in *Xenopus laevis*. Differentiation 30: 29–34

Tochinai S (1993) Strictly thymus-dependent tolerance induction in immunologically competent *Xenopus laevis*. Zool Sci 10: 855–858

(Received June 19, 2000 / Accepted July 22, 2000)