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Restoration of the antibody response to sheep erythrocytes in thymectomized *Xenopus* implanted with MHC-compatible or MHC-incompatible thymus

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SUMMARY

These experiments make use of an amphibian model system for investigating the role of the thymus in T helper cell education. Clawed toads (*Xenopus laevis*), thymectomized at 7 days, are unable to mount an antibody response to thymus-dependent antigens, such as sheep red blood cells (SRBC). When thymectomized larvae are implanted with larval thymuses (either irradiated or non-irradiated), incompatible at the major histocompatibility complex (MHC) or with MHC-compatible or -incompatible 'adult' thymuses, their splenic plaque-forming cell response and serum haemolytic antibody production to SRBC are both restored, to some extent. However, levels of mercaptoethanol-resistant antibody were extremely poor in those animals implanted with MHC-incompatible 'adult' thymus. Larval thymus implants were shown, by ploidy-labelling studies, to become repopulated with host-derived lymphocytes. Whether or not these lymphocytes acquire their MHC restriction specificities in the thymus awaits clarification.

INTRODUCTION

The anuran amphibian, *Xenopus*, possesses a major histocompatibility complex (MHC) that appears to display structural and functional homologies with the mammalian MHC (Du Pasquier, Chardonnens & Miggiano, 1975; Flajnik, 1983; Flajnik, Kaufman, Hsu & Du Pasquier, 1984). As in mammals, MHC antigens in *Xenopus* appear to be involved in restriction of T-B collaborative responses. Thus carrier-primed T cells and hapten-primed B cells cooperate *in vitro* to produce 7S (IgY (Hadji-Azimi, 1979); also referred to as Ig'G' – see Bernard Bordman, Blomberg & Du Pasquier, 1981) anti-dinitrophenyl (DNP) antibody only if taken from *Xenopus laevis*/*X. gilli* clones

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(Kobel & Du Pasquier, 1975) having either one or two MHC haplotypes in common (Bernard *et al.* 1981). If the carrier-primed T cells were histo-incompatible with the DNP-primed B cells, then no 7S antibody response was observed, although occasional low affinity IgM antibody responses took place.

Xenopus can be thymectomized (Tx) very early in life, at a rudimentary stage of thymus differentiation (Horton & Manning, 1972; Tochinai & Katagiri, 1975), and subsequently are deficient in humoral antibody production to an array of T-dependent antigens (see Cribbin, 1984, for review). Therefore these animals should prove to be a useful comparative model for investigating the extent to which the thymus (rather than a peripheral site) is involved in MHC restriction of T helper cell responses. This issue remains controversial in mammals (see Singer, Hathcock & Hodes, 1982). Recently Du Pasquier & Horton (1982) have shown that Tx *Xenopus laevis*/*X. gilli* and *X. laevis*/*X. muelleri* clones implanted with genetically defined MHC-compatible or MHC-incompatible larval thymuses can respond normally to DNP-keyhole-limpet haemocyanin. The IgM antibody produced was similar in quantity, affinity and specificity to that of non-operated controls; and 7S antibody was of host spectrotype.

The experiments reported here were designed to examine the ability of MHC-incompatible larval thymus (irradiated or non-irradiated) and 'adult' thymus implants (MHC-compatible or MHC-incompatible) to restore the cellular and serum antibody response of Tx *Xenopus laevis* to sheep red blood cells (SRBC). 'Adult' thymuses were used because it is only during or after metamorphosis that 'adult-type' MHC antigens are found on *Xenopus* cells (Du Pasquier, Blomberg & Bernard, 1979; Flajnik *et al.* 1984). In order to reveal whether our thymectomy/thymus implant system would allow host cells to migrate to and develop within the foreign thymus environment, we have also investigated the origin of implant thymocytes, by ploidy-labelling experiments.

ABBREVIATIONS

2-ME	2-mercaptoethanol
MHC	major histocompatibility complex
PFC	plaque-forming cell
SRBC	sheep red blood cell
Tx	thymectomized

MATERIALS AND METHODS

Animals

Outbred adult *Xenopus laevis* were purchased commercially (Xenopus Ltd.). Inbred (G-line) adult *Xenopus laevis* were a gift from C. Katagiri (Hokkaido University, Sapporo, Japan). G-line *Xenopus* are MHC-identical (Katagiri,

1978) and have recently been designated JJ to reflect their homozygosity at the MHC (DiMarzo & Cohen, 1982); these toads display minor histocompatibility disparities (DiMarzo & Cohen, 1982) and reject skin grafts in 'sub-acute' (30–50 days) or 'chronic' (> 50 days) fashion (J. C. Arnall & J. D. Horton, in preparation). A few of the animals used as thymus donors were isogenic hybrids – *Xenopus laevis*/*Xenopus gilli*, clone LG5 (MHC haplotypes ad). The LG5 adults were a gift from L. Du Pasquier, Basel Institute for Immunology, Switzerland. *X. laevis* and LG5 offspring were produced as previously described (Horton & Manning, 1972; Kobel & Du Pasquier, 1975 respectively). Animals were kept at 23 °C until immunization.

Thymectomy

Thymectomy was carried out using the microcautery method of Horton & Manning (1972). Thymuses were removed when larvae were 7 days old (stage 47). Larvae were checked for absence of thymus regeneration before metamorphosis and also at post-mortem.

Thymus implantation

Single thymus implants were transplanted to thymectomized larvae when 4–6 weeks old (stages 54–57); implants were placed under the skin, medial to the eye. The donor thymuses used were either from larvae of similar stage to the host, or suitably sized fragments of thymuses (usually half a thymus) from 4- to 5-month-old toadlets:– these toadlet thymuses are referred to as 'adult'. All reconstitutions with larval thymuses were performed using MHC-incompatible thymuses, some of which were irradiated before use: they were given a dose of either 1000 rads (dose rate 200 rads/min) or 5000 rads (1000 rads/min) by exposure to a cobalt-60 source. Adult thymuses were either MHC-compatible or MHC-incompatible. Some larval thymus implants were examined histologically at 4 and 30 days after transplantation. For this study alone Tx animals were implanted (on opposite sides of the head) with both a control and an irradiated (either 1000 or 5000 rads) thymus. This allowed direct comparison of the effect of irradiation on thymus implant development, avoiding individual host differences.

Animals used in the MHC-incompatible reconstitution experiments include: (i) outbred thymectomized hosts reconstituted with thymus from outbred non-siblings; (ii) outbred thymectomized hosts reconstituted with thymus from inbred G-line animals; and (iii) inbred G-line thymectomized hosts reconstituted with thymus from cloned LG5 animals (see Tables 1–4). Where outbred/outbred combinations were used, the donor and host were F_1's of different parentage and differed from each other by at least one, and probably by two MHC alleles, as controls (from the same families as used here) were shown always to reject each other's skin grafts in an 'acute' fashion (18–21 days at 24–26 °C – data not shown). The same is true for the outbred/G-line

combinations. G-line (jj) and LG5 (ad) reject each other's skin grafts in 21–23 days (J. C. Arnall & J. D. Horton, in preparation). For the MHC-compatible reconstitution experiments, both Tx hosts and thymus donors were inbred, G-line animals.

Origin of implant lymphocytes

Triploid, Tx larvae were implanted with diploid thymuses and thymic lymphocytes were assayed for ploidy at 6–8 months of age. Triploid (3N) *Xenopus* were produced by the cold-shock technique of Kawahara (1978). Ploidy of lymphocytes was assessed by silver staining of nucleoli (Olert, 1979).

Antigen preparation and immunization

Sterile sheep red blood cells (SRBC) (Tissue Culture Services) were washed three times and resuspended in saline. 6- to 12-month-old toadlets were immunized by injection of 0·05 ml 10 % SRBC/gm body wt., intraperitoneally. Toadlets received either a single SRBC injection and were tested for plaque-forming cells (PFC) 6 days later, or three injections given 3 days apart (the 'multiple-injection' schedule: see Results) and tested for splenic PFC and serum antibody production 2 weeks after the final injection. Immunized animals were kept at 26 °C.

PFC assay

Spleens were removed aseptically from MS222 (Sandoz)-anaesthetized animals. Spleen cell suspensions were prepared, chilled on ice, as described in detail elsewhere (Cribbin, 1984) using amphibian strength L15 medium supplemented with 1 % heat-inactivated foetal calf serum (Flow). Splenocytes were adjusted to a maximal concentration of 5×10^6 lymphocytes/ml. Samples (160 μl) of splenocytes were mixed with 12 μl 25 % SRBC, and 40 μl 1: 10 (SRBC-absorbed) guinea pig serum (Welcome) as a source of complement. Assay mixtures were allowed to warm to room temperature and then gently pipetted into 90 μl (double microscope slide) chambers. Two to four chambers were set up per sample. After 2 h incubation at 30 °C, PFC were counted – only when a central *Xenopus* lymphocyte could be seen in the plaque was a PFC scored. PFC were expressed as the number/10^6 leucocytes.

Serology

Blood was collected by cardiac puncture and serum removed. 25 μl of serum were serially diluted in V-well microtitration plates (Flow) in mammalian-strength phosphate-buffered saline (PBS) or 0·1 M-2-mercaptoethanol (2-ME) diluted in PBS. 2-ME-treated plates were incubated at 37 °C for 1 h: this removes IgM antibody activity, but leaves IgY antibody intact (Turner & Manning, 1974). 5 μl 5 % SRBC and 20 μl 1: 10 guinea pig complement

(absorbed with SRBC) were then added. The contents of the wells were mixed by gentle agitation and the plates then incubated for 2 h at room temperature. The maximum dilution of serum which caused lysis of the SRBC was taken as the titre of haemolytic antibody, and was expressed as $-\log_2$ titre.

Statistics

Antibody responses were compared using Student's t-test.

RESULTS

(a) *Background cellular and serum antibody levels*

Controls, Tx and Tx animals reconstituted with a larval allothymus showed neither background cellular production of antibody nor 2-Me-resistant serum antibody (see Table 1). Three out of eight controls, two out of three Tx and two out of two thymus-implanted Tx animals did give a positive background total serum antibody titre; levels of antibody were comparable ($P > 0{\cdot}1$) between groups.

(b) *Cellular antibody production in animals immunized with a single injection of SRBC*

The 6-day splenic PFC responses given by control, Tx and Tx animals implanted with MHC-incompatible larval thymus are shown in Table 2. Control animals gave a response of 215 ± 180 PFC/10^6 leucocytes (mean ± S.D.) whereas Tx animals did not respond to SRBC. However, implantation

Table 1. *Background cellular and serum antibody production to SRBC in non-immunized control, thymectomized, and thymectmized animals reconstituted with MHC-incompatible larval thymus*

	Control	Tx	Tx + larval thymus*
PFC/10^6 spleen leucocytes	00000	00	00
	$\bar{x} = 0$	$\bar{x} = 0$	$\bar{x} = 0$
Total serum antibody titre ($-\log_2$)	04300 003	044	23
	$\bar{x} = 1{\cdot}8 \pm 1{\cdot}3$	$\bar{x} = 2{\cdot}7 \pm 2{\cdot}3$	$\bar{x} = 2{\cdot}5 \pm 0{\cdot}7$
2-Mercaptoethanol-resistant antibody titre ($-\log_2$)	000 00	00	00
	$\bar{x} = 0$	$\bar{x} = 0$	$\bar{x} = 0$

Results are expressed as mean ± S.D. where appropriate.
Animals used were outbred *X. laevis*. *Restored with inbred *X. laevis* G-line thymuses.
All animals were aged 6–8 months.

Table 2. *Cellular antibody production to SRBC in control, thymectomized, and thymectomized animals reconstituted with MHC-incompatible larval thymus, after a single injection of antigen*

	Control	Tx	Tx + larval thymus*
PFC/10^6 spleen leucocytes	26 210 165 457	0000	40 30 241 121
	$\bar{x} = 215 \pm 180$	$\bar{x} = 0$	$\bar{x} = 108 \pm 98$

Results are expressed as mean ± S.D. where appropriate.
The assay was performed 6 days after the injection of antigen.
Animals used were outbred *X. laevis*. *Reconstituted with outbred (non-sibling) *X. laevis* thymuses.
All animals were aged 7 months.

of an MHC-incompatible larval thymus restored the abilty of Tx animals to produce plaques against SRBC. The number of PFC obtained was 108 ± 98 PFC/10^6 leucocytes which was not significantly different from the control response ($P > 0{\cdot}1$). (Serum antibody production was not tested in animals receiving a single SRBC injection.)

(c) *Cellular and serum antibody production in animals given a multiple SRBC injection schedule*

The responses of control, Tx and Tx animals implanted with non-irradiated larval or irradiated larval MHC-incompatible thymus are shown in Table 3, and the response of Tx animals reconstituted with MHC-compatible or MHC-incompatible adult thymus are shown in Table 4.

Controls

Control animals all responded to SRBC, with a cellular antibody level of 85 ± 43 PFC/10^6 leucocytes and a total serum antibody titre of 7·4 ± 1·2. After treatment of the immune sera with 2-ME, the IgY antibody titre was 4·0 ± 2·1.

Thymectomized

Tx animals did not respond to SRBC. None of the nine animals tested gave any anti-SRBC PFC. Six out of nine animals did have anti-SRBC serum antibody, but the mean titre of 3·1 ± 2·5 was similar ($P > 0{\cdot}1$) to the titre given by non-immunized Tx animals (see Table 1). This value of 3·1 was significantly lower than the titre displayed by immunized controls ($P < 0{\cdot}001$). The immunized Tx animals produced no 2-ME-resistant antibody.

Thymectomized animals implanted with a larval MHC-incompatible thymus

An MHC-incompatible larval thymus implant restored the ability of Tx animals to respond to SRBC. However, the splenic cellular antibody response

Table 3. *Cellular and serum antibody production to SRBC in control, thymectomized, and thymectomized animals reconstituted with a larval thymus, after multiple antigen injection*

	Control	Tx	Tx + larval thymus	Tx + irradiated larval thymus	
				1000 rads	5000 rads
PFC/10^6 spleen leucocytes	105 26 107 114 88 133 105 136 109 32 14 54	000 000 000	83* 71* 11* 6* 2* 17† 25† 85†	106† 85† 69†	198† 139† 18†
	$\bar{x} = 85 \pm 4{\cdot}3$	$\bar{x} = 0$	$\bar{x} = 38 \pm 26$	$\bar{x} = 87 \pm 19$	$\bar{x} = 118 \pm 92$
Total serum antibody titre ($-\log_2$)	10 7 8 6 6 7 7 7 8 8	344 500 066	10* 7* 7* 6* 6* 5† 10† 8†	8† 6† 7†	7† 5† 8†
	$\bar{x} = 7{\cdot}4 \pm 1{\cdot}2$	$\bar{x} = 3{\cdot}1 \pm 2{\cdot}5$	$\bar{x} = 7{\cdot}4 \pm 1{\cdot}8$	$\bar{x} = 7{\cdot}0 \pm 1{\cdot}0$	$\bar{x} = 6{\cdot}7 \pm 1{\cdot}5$
2-Mercaptoethanol-resistant antibody titre ($-\log_2$)	7 6 0 4 3 4 4 4	000 000 0	5* 4* 4* 2* 4* 2†	0† 2† 2†	2† 2† 0†
	$\bar{x} = 4{\cdot}0 \pm 2{\cdot}1$	$\bar{x} = 0$	$\bar{x} = 3{\cdot}5 \pm 1{\cdot}2$	$\bar{x} = 1{\cdot}3 \pm 1{\cdot}2$	$\bar{x} = 1{\cdot}3 \pm 1{\cdot}2$

Results are expressed as mean ± S.D. where appropriate.
Animals were given three injections of antigen, 3 days apart and the assays was performed 2 weeks after the final injection.
Control and thymectomized (non-reconstituted) animals were either outbred or inbred (G-line) *X. laevis*.
Reconstituted animals were thymectomized (Tx) outbred *X. laevis*, implanted with outbred *X. laevis* thymuses (*) or inbred *X. laevis* G-line thymuses (†).
All animals were aged 7–12 months.

was only 38 ± 36 PFC/10^6 leucocytes, which was significantly different from the control level ($P < 0{\cdot}02$). The total serum antibody titre obtained was $7{\cdot}4 \pm 1{\cdot}8$ and the 2-ME-resistant antibody titre was $3{\cdot}5 \pm 1{\cdot}2$. These values were not significantly different from the control levels ($P > 0{\cdot}1$).

Thymectomized animals implanted with an irradiated larval MHC-incompatible thymus

Irradiated MHC-incompatible larval thymus implants were able to restore the ability of Tx animals to mount a PFC response and total serum antibody response to SRBC; thymuses given 1000 or 5000 rads were equally effective. The cellular antibody response (87 ± 19 and 118 ± 92 PFC) and total serum antibody titres ($7{\cdot}0 \pm 1$ and $6{\cdot}7 \pm 1{\cdot}5$) are comparable to control levels, in both 1000 and 5000 rad thymus-reconstituted animals respectively ($P > 0{\cdot}1$). The 2-ME-resistant antibody titres for animals reconstituted with irradiated thymuses appeared lower than control levels, but this difference was not significant ($P > 0{\cdot}05$, but $< 0{\cdot}1$). However, more experiments are needed to confirm that 2-ME-resistant antibody titres are restored to control levels.

Table 4. *Cellular and serum antibody production to SRBC in thymectomized animals reconstituted with MHC-compatible or MHC-incompatible 'adult' thymus, after multiple antigen injections*

	MHC-compatible	MHC-incompatible
PFC/10^6 spleen leucocytes	176 197 25 313 75 35 141 $\bar{x} = 137 \pm 102$	20* 12* 58* 39* 27† 1† 32† 28† 4* $\bar{x} = 25 \pm 18$
Total serum antibody titre ($-\log_2$)	4 10 7 $\bar{x} = 7{\cdot}0 \pm 3{\cdot}0$	6* 4* 6* 9* 4† 5† 4† 4† $\bar{x} = 5{\cdot}3 \pm 1{\cdot}8$
2-Mercaptoethanol-resistant antibody titre ($-\log_2$)	0 4 3 $\bar{x} = 2{\cdot}3 \pm 2{\cdot}1$	3* 0* 0* 3* 0† 0† 0† 0† $\bar{x} = 0{\cdot}8 \pm 1{\cdot}4$

Results are expressed as mean ± S.D.
Animals were given three injections of antigen, 3 days apart and the assay was performed 2 weeks after the final injection. For control and Tx data see Table 3.
Both thymectomized host and donor thymus were inbred *X. laevis* G-line in the MHC-compatible combinations.
*Animals used were thymectomized outbred *X. laevis* reconstituted with inbred *X. laevis* G-line thymuses.
†Animals used were thymectomized inbred *X. laevis* G-line, reconstituted with *X. laevis*/gilli clone LG5.
All animals were aged 9–12 months.

Thymectomized animals implanted with an 'adult' thymus

Both MHC-compatible and MHC-incompatible 'adult' thymus implants were able to restore SRBC responses of Tx animals, but to different extents. Animals implanted with an MHC-compatible thymus gave a cellular antibody response of 137 ± 102 PFC and a total serum antibody titre of 7 ± 3. Two out of three animals produced 2-ME-resistant antibody, with a mean level of $2{\cdot}3 \pm 2{\cdot}1$. Reconstitution with an MHC-incompatible thymus enabled Tx animals to mount a cellular antibody response of 25 ± 18 PFC, and a mean total serum antibody titre of $5{\cdot}3 \pm 1{\cdot}8$. Only two out of eight animals were able to produce 2-ME-resistant antibody, with a mean level of $0{\cdot}8 \pm 1{\cdot}4$. All the responses given by animals reconstituted with MHC-compatible thymus were comparable to control levels ($P > 0{\cdot}1$). In contrast, in the animals given an 'adult' MHC-incompatible thymus, the cellular antibody response and both total and 2-ME-resistant serum antibody titres were all significantly lower than controls ($P < 0{\cdot}001$, $P < 0{\cdot}01$ and $P < 0{\cdot}01$ respectively).

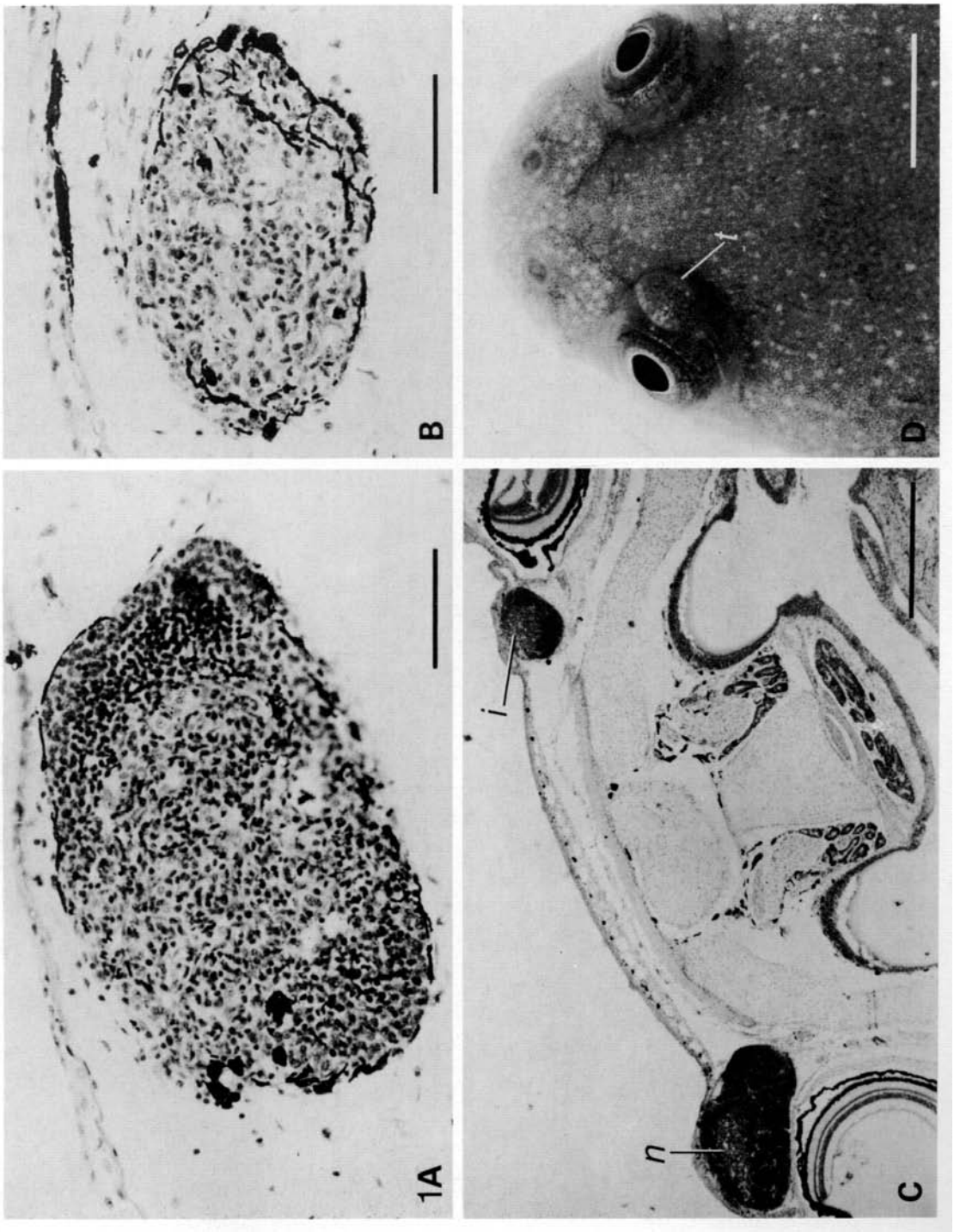
(d) *Observations on thymus implants*

Histology

Non-irradiated larval allothymus implants appeared normal in structure within 4 days of implantation (Fig. 1A). In contrast, both 1000 rad and 5000 rad (Fig. 1B) irradiated implants were smaller and predominantly epithelial, with only a few scattered lymphocytes. The difference in size between control and irradiated thymuses was pronounced at 30 days post-implantation (Fig. 1C) when animals have begun postmetamorphic life. However, both 1000 and 5000 rad thymuses did contain lymphocytes and displayed cortex/medulla differentiation. Although histological studies were not continued here after 30 days, external observations revealed that non-irradiated implants remained readily detectable under the skin, medial to the eye (Fig. 1D). (Long-term retention of normal histology of larval allothymus implants has been shown by Horton & Horton, 1975.) Some 1000 rad-treated implants were just visible at postmortem, whereas others had disappeared, as had all the 5000 rad-irradiated thymuses. Both MHC-compatible and -incompatible 'adult' thymus implants remained readily visible throughout the experiments.

Host cell immigration

Table 5 shows the results of ploidy-labelling experiments investigating the origin of lymphocytes in larval (non-irradiated) allothymus implants. In 3N control animals $61 \pm 7\%$ (mean $\pm$ S.D.) of thymic lymphocytes were recorded with three nucleoli, whereas in 2N controls only 4% were recorded with three nucleoli; now $76 \pm 9\%$ had just two nucleoli. In triploid Tx animals implanted with *diploid* thymuses, thymus implant lymphocyte nucleolar number appeared directly comparable to *triploid* controls; $63 \pm 8\%$ were recorded as having three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nucleoli and under 30% were recorded with two nucleoli. Thus the implants have been heavily infiltrated by host-derived cells. No observations were made here on irradiated larval or adult thymus implants.

DISCUSSION

Non-irradiated, MHC-incompatible larval thymus implants display a normal histology and become repopulated by host lymphocytes, although precise, quantitative, measurements of the extent of this repopulation cannot be made with the silver-staining technique used (see below). Anti-SRBC antibody responses of thymectomized animals given such a thymus are restored, although the splenic PFC response following multiple SRBC injections was lower than in controls. Restoration was not due simply to a non-specific 'allogeneic effect', since non-SRBC-injected Tx toadlets reconstituted with larval allothymus displayed only background anti-SRBC reactivity. Experiments with nude mice have shown that an implanted, neonatal, allothymus also becomes repopulated with host cells (Loor & Kindred, 1973). However, the ability of such 'athymic' mice to mount an antibody response to SRBC is only partially restored (Kindred & Loor, 1974). In *Xenopus* irradiated, MHC-incompatible, larval thymus implants initially contained few lymphocytes, but became lymphoid within a few weeks, although the origin of these thymocytes has yet to be examined. Irradiated implants remain very much smaller than non-irradiated implants and tend to disappear after metamorphosis: thus long-term lymphoid repopulation appears not to occur. In nude mice, irradiated, allogeneic perinatal thymus implants remain small and largely epithelial (Loor & Hägg, 1977). Such irradiated thymus implants fail to restore responses of nude mice to T cell-dependent antigens (Kindred, 1978), whereas in *Xenopus*, irradiated implants restored antibody responses to SRBC.

MHC-incompatible adult thymus implants (in contrast to MHC-compatible ones) fail to restore humoral responses to T-dependent antigens above background levels in nude mice (Radov, Sussdorf & McCann, 1975) and adult thymus grafts are inferior (with respect to restoring immune function) to neonatal thymuses in neonatally-thymectomized mice (discussed by Loor & Hägg, 1977). In *Xenopus* 'adult' alloimplants seem partially to restore both the cellular antibody response in the spleen and 2-ME-sensitive serum antibody

Fig. 1. (A & B) Sections through centre of non-irradiated (A) and 5000 rad irradiated (B) thymus implants in the same Tx host 4 days postimplantation. Non-irradiated thymus displays typical cortex and medulla. Note the smaller number of lymphocytes in irradiated implant. Stain: H & E. Scale bar = 100 μm. (C) Section through centre of non-irradiated (n) and 1000 rad irradiated (i) thymus implants in a Tx host 30 days post-implantation. Note pronounced size difference, although both implants contain lymphocytes and cortex/ medulla differentiation. Stain: H & E. Scale bar = 600 μm. (D) A 14-week-old Tx toadlet with larval thymus alloimplant (t) medial to left eye. Scale bar = 2 mm.

Table 5. *Infiltration of larval allothymus implants by host lymphocytes*

Animals	No.	Mean percentage (± S.D.) thymic lymphocytes recorded with One nucleolus	Two nucleoli	Three nucleoli	Uncertain number of nucleoli
Triploid Control (Family 14)	15	1 ± 1	35 ± 10	61 ± 7	3 ± 1
Diploid Control (Family 16)	6	14 ± 5	76 ± 9	4 ± 4	6 ± 3
Triploid Tx (Family 14) implanted with Diploid Thymus (Family 16)	6	2 ± 2	29 ± 6	63 ± 8	5 ± 3

Nucleolar number assessed by $AgNO_3$ staining. At least 200 thymocytes counted in each animal.
Ploidy of thymus donors and Tx hosts used was checked (blood cells silver stained) prior to use.
Triploid hosts = Family 14 (outbred XL $♀^{14} \times ♂^{14}$). Diploid donors of larval thymus = Family 16 (outbred XL $♀^{16} \times ♂^{16}$).
Assays at 6–8 months.

production. However, 2-ME-resistant antibody (presumably IgY) was not recorded in the majority of Tx animals given MHC-incompatible 'adult' thymus. Future thymus reconstitution experiments with *Xenopus* will concentrate on the use of 'adult' thymus implants and determine whether there are real differences in IgY antibody production between (genetically defined) Tx animals given MHC-compatible and MHC-incompatible (2 haplotype-disparate) thymus implants. Monoclonal antibodies against *Xenopus* IgY are now available, and these will be used in an enzyme-linked immunosorbent assay or radio-immunoassay.

Overall, our restoration experiments using SRBC, and others (Du Pasquier & Horton, 1982) using an antigen known to require MHC-restricted T-B collaboration *in vitro* (Bernard *et al.* 1981), would tend to lead to the conclusion that host-derived T cells, that have developed in the foreign thymus, can repopulate the periphery and there collaborate perfectly well with host-derived B lymphocytes (and antigen-presenting cells) in primary *in vivo* antibody responses. The amphibian thymus may, at first glance, seem not to be centrally involved in self-restriction of helper T cells. However, the involvement of donor-derived cells in effecting the antibody responses observed in our experiments cannot be ruled out, particularly where non-irradiated thymus implants were used. In this respect Nagata & Cohen (1984) have recently found, using flow cytometry on mithramycin-stained cells, either no donor-derived cells or up to 46 % or 32 % in thymus or spleen respectively (the

proportion depending on the host/donor combinations used) 6–13 months after Tx *Xenopus* were implanted (as toadlets) with 'adult' MHC-compatible or MHC-incompatible thymuses. (Interestingly, these authors reveal that MHC-incompatible thymuses also fully restore the splenic PFC response to SRBC).

The experiments with irradiated thymus reported here and elsewhere (Du Pasquier & Horton, 1982) were set up to remove the possibility of donor B cell contamination, as both T and B lymphocytes exist in the *Xenopus* thymus (Williams, Cribbin, Zettergren & Horton, 1983; Hsu, Julius & Du Pasquier, 1983). B cell activity in *Xenopus* is eliminated by a dose of 500 rads *in vitro* (Blomberg, Bernard & Du Pasquier, 1980) and so the irradiated thymuses used here should be free of B cells. The possibility that radiation-resistant (see Bernard *et al.* 1981) donor-derived helper T cells, rather than host-derived T cells, might be involved in the immune response of restored *Xenopus* still cannot be excluded. Moreover, involvement of radiation-sensitive donor-derived suppressor T cells could account for the higher mean level of splenic PFC's recorded in Tx animals implanted with irradiated implants than in animals given non-irradiated thymus – see Table 3. Although a dose of 3000 rads results in organ-culture thymuses becoming virtually devoid of lymphocytes within 2 weeks (J. H. Russ, personal communication), on the other hand Cribbin (1984) has evidence that 3000 rad-irradiated lymphocytes (from spleen/peripheral blood lymphocyte mixtures) of one genotype can, when injected alongside immunizing SRBC into a thymectomized host of another genotype, cooperate with the B cells of that host to effect a normal 6 day primary anti-SRBC PFC response. Whether or not a long-term restoration (i.e. comparable to the interval used here between thymus implantation and SRBC injection) occurs was not resolved in Cribbin's experiments. (A long-lasting T-B cooperation does not occur in anti-SRBC responses of nude mice injected with MHC-incompatible thymocytes or splenocytes (Kindred & Weiler, 1972).) The outcome of such experiments in *Xenopus* should allow a clearer interpretation of the red cell studies with MHC-incompatible thymus-reconstituted animals reported here. Lack of involvement of donor implant lymphocytes in restoration is suggested by our unpublished observations that neither larval spleen nor larval liver alloimplants reconstitute anti-SRBC responses of early thymectomized *Xenopus*.

The failure to demonstrate a role of the thymus in MHC restriction of T cells could conceivably be obscured in these thymectomy-reimplantation studies by early seeding of immature, but already host-MHC-restricted, T-axis lymphocytes, prior to removal of the thymus at 7 days of age. These lymphocytes could then mature into functional T cells under the hormonal influence (see Dardenne, Tournefier, Charlemagne & Bach, 1973) of the thymus implant: extra-thymic differentiation of T precursor cells could well be the restorative mechanism in animals given irradiated thymus implants, where

implants failed to grow well. Experiments have recently been performed by Flajnik, Du Pasquier & Cohen (1984) which address this issue. They have used chimaeric *Xenopus* in which the anterior 'half' of an embryo is joined to a posterior 'half' of an MHC-disparate embryo. The anterior portion contains the thymic anlagen of one MHC haplotype and the posterior portion contains the haemopoietic stem cell source of a different MHC type. Hence during ontogeny of these chimaeras, lymphocyte precursors entering the thymus exclusively differentiate in an MHC-incompatible thymus epithelial environent. It was found that cellular antibody responses to SRBC were normal in some chimaeric combinations, whereas they did not occur in others. Chimaeric animals were still able to produce both IgM and IgY antibodies to DNP-keyhole-limpet haemocyanin, but the IgY response showed delayed kinetics and lower antibody titres. For the moment it appears that the thymus may play some part in the education of *Xenopus* helper T cells, but its influence is by no means critical.

In none of the experiments investigating the role of the *Xenopus* thymus in MHC restriction is it known whether the host T cells developing are biased towards interactions with cells of the thymus donor type rather than with those of the genotype of the Tx host or stem cell source in the chimaeric animal. Work in this laboratory has begun to examine this issue (see Lallone, 1984) by studying the potential of T cells from control animals and thymus-implanted Tx *Xenopus* to cooperate with B cells of various MHC types in a primary *in vitro* antibody assay (to avoid possible alteration in T helper cell bias induced by *in vivo* priming). Finally, it will be important to consider more closely the actual MHC-restricting elements (epithelium, etc.) within the amphibian thymus. It has been suggested that self-MHC restriction is learned in mammalian thymus by interaction of developing T cells with MHC products on thymic antigen-presenting cells; the latter are able to migrate into the thymus from the bone marrow (Longo & Schwartz, 1980). Thus host-derived, MHC-restricting, antigen-presenting cells may well migrate into an MHC-incompatible (amphibian) thymus, along with lymphoid stem cells, and so partially obscure the organ's role in T cell education.

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