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During frog ontogeny, PHA and Con A responsiveness of splenocytes precedes that of thymocytes

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Summary. The *in-vitro* proliferation of splenocytes and thymocytes from *Xenopus laevis-gilli* (hybrid clone LG-15) to the T cell mitogens, concanavalin A (Con A) and phytohaemagglutinin-P (PHA), were examined at specific stages of larval development (stages 51–66 of Nieuwkoop & Faber, 1967) and at 2 months post-metamorphosis. The responses of splenic lymphocytes to each mitogen were significant at all stages with stimulation indices ranging from 1·9 to 50·5 and 2·6 to 45·5 for PHA and Con A, respectively. Stage-related differences in responses of splenocytes to both mitogens suggest two waves of emergence of proliferative activity during development, divided by periods of diminished responsiveness during the metamorphic crisis.

In contrast to the responses observed with splenocytes, proliferation of thymocytes cultured with either mitogen was barely detectable, with stimulation indices ranging from 1·2 to 6·9 and 1·4 to 2·9 for PHA and Con A, respectively. These minimal responses were observed only when thymocytes were cultured at relatively high cell density (5×10^5 cells/ml); they were not improved by increased or decreased concentrations of mitogen or by increased concentrations of fetal calf serum (5 or 10%) in the medium. Co-culture of larval thymocytes with autologous splenocytes and each mitogen did not consistently increase thymocyte responses suggesting that the defect in thymocyte responsiveness is not due to lack of accessory cells. These findings suggest that if PHA- and Con A-reactive cells are present in the thymus, they are present in relatively low numbers at all stages of larval development.

The pattern of early mitogen responsiveness in the spleen at a time when the thymus is unresponsive contrasts with that observed in mammalian development in which thymocytes become responsive to mitogens in fetal stages and mitogen responsiveness appears in the spleen only around the time of birth. The apparent inactivity of larval thymocytes may reflect a population of cells that can become tolerant to those neo-self-antigens that arise during and after metamorphosis. If so, the larval amphibian thymus may provide a model to study the early events of thymocyte 'education' and differentiation in a broader time framework than is possible with fetal mammals.

Abbreviations: PHA, phytohaemagglutinin-P; Con A, concanavalin A; FCS, foetal calf serum; MLC, mixed leucocyte culture; c.p.m. counts per min.

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INTRODUCTION

Much is known about the ontogeny of murine T lymphocytes (Jordan & Robinson, 1981; Owen, 1982). Pluripotent haemopoetic stem cells arise outside the thymus and move into this organ (Moore & Owen, 1967) where they proliferate extensively and pass through several differentiative steps before they move to the periphery and differentiate into fully functional T cells. In mice, the thymus epithelium pinches off

from its site of embryonic origin, the pharyngeal pouches, at about day 13 of gestation. At this time (from day 11), large basophilic stem cells are present around and within the thymus anlagen (Moore & Owen, 1967) and a few cells expressing the differentiation antigen Thy-1 can be detected (Kamarck & Gottlieb, 1977). By 17 days of gestation, more than 95% of thymocytes express Thy-1 (Kamarck & Gottlieb, 1977; Mathieson *et al.*, 1981). Lyt1$^+$2,3$^-$ cells (the helper phenotype) can first be detected in C57BL/6 mice at 15–16 days of gestation, and they account for about 50% of thymocytes. Cells expressing Lyt2 and 3 (the suppressor/cytotoxic phenotype) are not detectable until 17 days of gestation. By day 18 the adult proportions of 10% Lyt1$^+$2,3$^-$:90% Lyt1,2,3$^+$ are established (Mathieson *et al.*, 1981). The acquisition of these differentiation antigens prior to birth is coincident with the emergence, within thymocyte populations of T cell functions such as help (Haines & Siskind, 1980; Chakravarty, 1977), cytotoxicity (Widmer & Cooper, 1979; Wood & Streilein, 1982), suppression (Lukenbach *et al.*, 1978; Ptak *et al.*, 1979) and proliferation in response to PHA, Con A, and pokeweed mitogen (PWM) (Mosier, 1974; Stobo & Paul, 1972; Harve & Manziello, 1972). In contrast, responsiveness of splenic T cells to phytomitogens emerges only after birth (Papiernik, 1976; Mosier, 1977; Adler *et al.*, 1970; Harve & Manziello, 1972) and increases to adult levels during the first 6 weeks of life.

In the frog, *Xenopus laevis*, the complete development of immune responsiveness to adult levels takes approximately 3–4 months. During the larval stages, frogs attain the capacity to produce IgM and IgY antibodies to T-dependent antigens (Kidder, Ruben & Stevens, 1973; Du Pasquier & Haimovich, 1976), to reject skin allografts (Horton, 1969; DiMarzo & Cohen, 1982), and to respond to allogeneic cells in mixed leucocyte culture (MLC) (Du Pasquier & Weiss, 1973; Du Pasquier, Blomberg & Bernard, 1979; Cohen *et al.*, 1983). Larval development is terminated by metamorphic crisis that is coincident with a decrease in the number of lymphoid cells in thymus, liver, and spleen (Du Pasquier & Weiss, 1973; Cohen *et al.*, 1983). After metamorphosis, lymphocyte numbers rapidly increase and adult-type responsiveness characterized by production of high affinity IgM and IgY antibodies (Du Pasquier, 1982) and ability to reject rather than tolerate minor histocompatibility locus disparate as well as major histocompatibility complex disparate skin grafts (DiMarzo & Cohen, 1982) emerges. Proliferative responses of thymocytes from outbred larval *Xenopus* to the T cell mitogens, Con A and PHA, were studied by Williams *et al.* (1983) in experiments limited by the number of cells that could be obtained. No consistent proliferative response to PHA was observed at any larval stage. Thymocyte responses emerged during post-metamorphic life between 3 and 5 months of age and reached a maximal level at 9 months of age. A small response to Con A was observed at larval stages 56–57, but was absent at stages 58–59, and increased gradually after metamorphosis through the 1st year of life. Larval spleen cell responses could not be studied because individual animals have too few cells. In the present study we pooled cells from larval isogeneic (cloned) *X. laevis-gilli* frogs (Kobel & Du Pasquier, 1975) to be able to examine the responses of larval spleen cells to Con A and PHA and to reinvestigate the apparent inactivity of larval thymocytes. We were interested in whether a splenic T cell response would emerge in the absence of a thymocyte response and whether subsets of T cells responsive to one or the other mitogen would be detected at a different time in ontogeny, thereby suggesting differential emergence of functional subsets.

MATERIALS AND METHODS

Frogs

Isogeneic larvae were obtained by induced ovulation of *X. laevis-gilli* hybrid clones (LG-15) according to published procedures (Kobel & Du Pasquier, 1975). They were reared at about 24° at a density of about 10 larvae/4 l in dechlorinated tap water and fed nettle powder 3 times weekly. After metamorphosis, the frogs were fed *Tubifex* and ground beef heart.

In vitro *lymphocyte culture*

The spleen and bilateral thymuses were removed aseptically from larvae of specific developmental stages (Nieuwkoop & Faber, 1967) and dissociated separately with fine forceps in sterile depression slides containing about 300 μl of Leibovitz (L-15) culture medium (GIBCO, Grand Island, NY) diluted to amphibian tonicity and supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, $1{\cdot}25 \times 10^{-2}$ M sodium bicarbonate, 5×10^{-5} M 2-mercaptoethanol and 1% heat-inactivated foetal calf serum (GIBCO) (Green & Cohen, 1979). Lymphocytes from about four to seven genetically identical larvae of the same stage were pooled for each experiment, and each

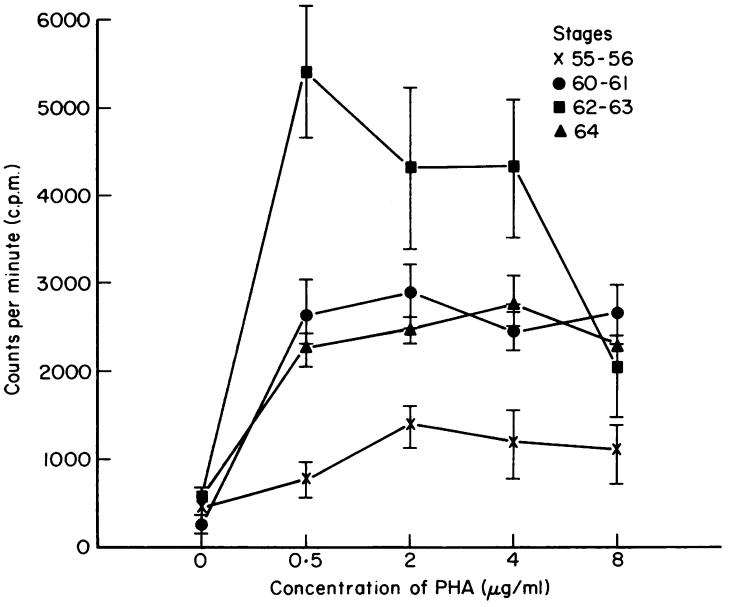

Fig. 1. Dose response of larval splenocytes from stages 55–56 (×——×), 60–61 (●——●), 62–63 (■——■), and 64 (▲——▲) to various concentrations of PHA. Data are expressed as average c.p.m. ± SEM of [^{3}H]TdR uptake by 1×10^5 splenocytes/ml.

experiment was repeated at least 3 times for every stage examined. Spleen cells were cultured at a density of 1×10^5 cells/ml and thymocytes at a density of 5×10^5 cells/ml (except where otherwise indicated in tables or figure legends) in 96-well round bottom microtitre plates (COSTAR, Cambridge, MA) in a total volume of 200 μl. Based on dose–response curves of larval splenocytes from several developmental stages for PHA (Fig. 1) and Con A (Fig. 2), 2 μg/ml in culture was selected as the optimal concentration for each mitogen unless otherwise indicated in tables or figure legends. Both mitogens were obtained from DIFCO (Detroit, MI). The cultures were assayed for mitogen-induced [^{3}H]-thymidine ([^{3}H]TdR) incorporation according to published procedures (Green & Cohen, 1979). Briefly, after incubation at 26° for 48 hr in a 5% CO_2-95% air atmosphere, the cultures were pulsed with 1 μCi of [^{3}H]TdR (5 μCi/ml, specific activity = 2 Ci/mmol) (New England Nuclear, Boston, MA) and incubated for an additional 24 hr before harvesting with an Otto Hiller precipitator (Otto Hiller Co., Madison, WI).

Quantification of the response

For each pool of cells tested, an average of background counts per minute (c.p.m.) for cells cultured in medium alone, an average of stimulated c.p.m. for cells cultured with mitogen, and a ratio of the two (stimulation index = stimulated c.p.m./background c.p.m.) was computed. Usually five replicate wells that contained cells alone or cells plus mitogen were plated. Data for each experiment were expressed as mean c.p.m. ± SEM, and background c.p.m. and stimulated c.p.m. were compared by a two-tailed Student's *t*-test.

Normalization of the data

To compare mitogen responses of cells from larvae of different developmental stages, data gathered from multiple experiments on animals of the same stage (conducted in the same way but at different times) were normalized so that they could be pooled for statistical comparisons. For each developmental stage, the mean background c.p.m. from individual experiments were averaged to obtain a normalization value for that stage. This normalization value was divided by the background c.p.m. for each experiment to obtain a normalization constant for each experiment. All raw c.p.m. (counts obtained from an individual well) for each experiment were multiplied by the normalization constant determined for that experiment. The normalized data from a given experiment were combined with the normalized data from all experiments at that

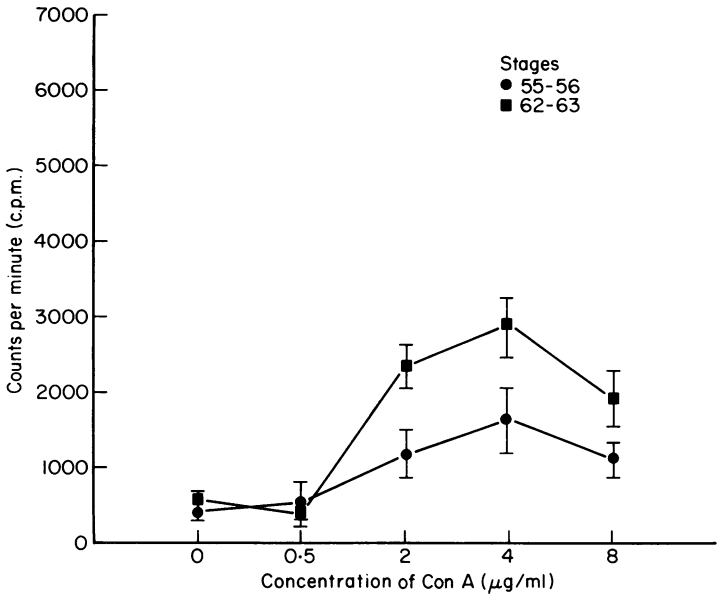

Fig. 2. Dose response of larval splenocytes from stages 55–56 (●——●) and 62–63 (■——■) to various concentrations of Con A. Data are expressed as mean c.p.m. ± SEM of [^{3}H]TdR uptake by 10^5 splenocytes/ml.

stage to obtain a grand average background c.p.m. and a grand average stimulated c.p.m. These normalized values were compared by a two-tailed Student's *t*-test after transformation to the $\log_{10}$ values. Data in Figs 3 and 4 are plotted as stage *vs* normalized stimulated CPM minus normalized background CPM. Data in Figs 1 and 2 and Tables 1–5 were not normalized.

Enumeration of splenic and thymic lymphocytes

In each experiment, pooled viable (trypan-blue exclusion) thymocytes or splenocytes were counted by standard haemocytometer methods. The total number was divided by the number of larvae in each pool to determine an average number of thymocytes or splenocytes per frog. To determine the average number of thymocytes or splenocytes per frog at each stage, these mean values were averaged and the SE computed for all experiments at each stage (usually three to five experiments at each stage).

RESULTS

The larval spleen becomes lymphoid at stage 50 (Manning & Horton, 1969). Already by stage 51 (the earliest stage we examined), the proliferative response of splenocytes to both mitogens was highly significant, with stimulation indices ranging from 7 to 15. Responses to both mitogens increased during the next 2–3 weeks of development to stages 55–56 where maximum responses (S.I. = 35–40) were recorded. Responsiveness then declined to its lowest point at stage 59, recovered to another high level at stages 60–61 and declined slightly to the level observed at 2 months post-metamorphosis (Fig. 3). Responses to both mitogens were approximately equal throughout larval development. Representative experiments at each of four stages are shown in Table 1.

In contrast to the striking proliferative response of splenocytes, thymocytes were relatively unresponsive to either mitogen at every larval stage tested (Fig. 4). The responses were usually, but not consistently, significant when cultured at high density (5×10^5 cells/ml) with stimulation indices ranging from 1·2 to 6·9 and 1·4 to 2·9 for PHA and Con A, respectively. Four representative experiments are shown in Table 2.

Neither increased nor decreased concentrations of Con A improved the thymocyte response, and the best responses were seen when thymocytes were cultured at relatively high density (5×10^5 cells/ml) (Table 3).

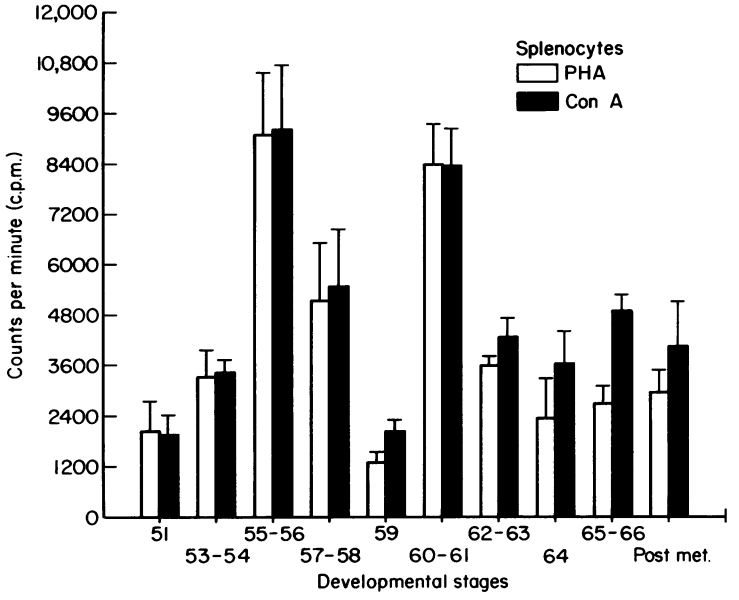

Fig. 3. Ontogeny of mitogen responses of splenocytes (1×10^5/ml) from various developmental stages to PHA and Con A. Data for each stage are plotted as mean stimulated c.p.m. (after normalization; see Materials and methods) plus SEM minus mean background c.p.m. (after normalization). Post Met = 2 months post-metamorphosis.

Table 1. Proliferation of larval splenic lymphocytes in response to PHA and Con A

Stage	C.p.m. cells only ($\bar{x} \pm$ SE)	C.p.m. cells + PHA* ($\bar{x} \pm$ SE)	C.p.m. cells + Con A† ($\bar{x} \pm$ SE)	Stimulation index	Significance‡
53–54	426 ± 70 (6)§	3259 ± 658 (6)		7·7	$P < 0{\cdot}001$
	426 ± 70 (6)		3217 ± 353 (6)	7·5	$P < 0{\cdot}001$
57–58	375 ± 91 (7)	6883 ± 383 (6)		18·4	$P < 0{\cdot}001$
	375 ± 91 (7)		5912 ± 458 (6)	15·8	$P < 0{\cdot}001$
60–61	242 ± 21 (6)	9138 ± 455 (6)		37·8	$P < 0{\cdot}001$
	242 ± 21 (6)		7363 ± 366 (5)	30·3	$P < 0{\cdot}001$
64	212 ± 24 (6)	1475 ± 155 (6)		7·0	$P < 0{\cdot}001$
	212 ± 24 (6)		1739 ± 114 (6)	8·2	$P < 0{\cdot}001$

* Concentration of PHA in culture was 2 μg/ml.

† Concentration of Con A in culture was 2 μg/ml.

‡ Average background and stimulated counts were compared by a two-tailed Student's *t*-test. Data in this table have not been normalized.

§ The number in parentheses after c.p.m. indicates the number of replicate wells plated.

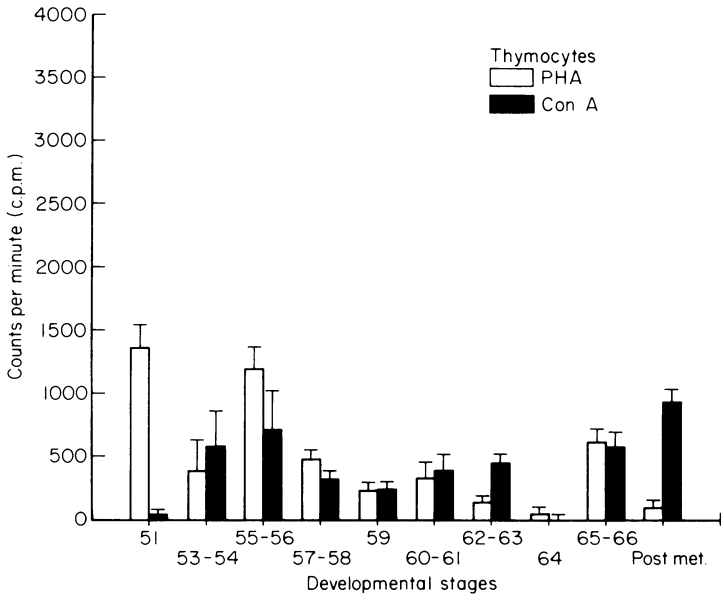

Fig. 4. Ontogeny of mitogen responses of thymocytes (5×10^5/ml) from various developmental stages to PHA and Con A. Data for each stage are plotted as mean stimulated c.p.m. (after normalization) plus SEM minus mean background c.p.m. (after normalization). Post Met = 2 months post-metamorphosis.

Table 2. Proliferation of larval thymocytes in response to PHA and Con A

Stage	C.p.m. cells only ($\bar{x} \pm$ SE)	C.p.m. cells + PHA* ($\bar{x} \pm$ SE)	C.p.m. cells + Con A† ($\bar{x} \pm$ SE)	Stimulation index	Significance‡
55–56	1161 ± 126 (8)§	2332 ± 266 (8)		2·0	$P < 0{\cdot}01$
	1161 ± 126 (8)		2108 ± 196 (7)	1·8	$P < 0{\cdot}01$
57–58	188 ± 18 (8)	564 ± 33 (9)		2·8	$P < 0{\cdot}01$
	188 ± 18 (8)		539 ± 46 (6)	2·9	$P < 0{\cdot}01$
60–61	212 ± 39 (6)	400 ± 80 (6)		1·8	$P < 0{\cdot}05$
	212 ± 39 (6)		415 ± 72 (6)	1·9	$P < 0{\cdot}01$
62–63	787 ± 168 (10)	832 ± 123 (10)		1·1	NS¶
	787 ± 168 (10)		1949 ± 160 (6)	2·4	$P < 0{\cdot}01$

* Concentration of PHA in culture was 2 μg/ml.
† Concentration of Con A in culture was 2 μg/ml.
‡ Average background and stimulated counts were compared by a two-tailed Student's *t*-test. Data in this table have not been normalized.
§ The numbers in parentheses following c.p.m. indicate the number of replicate wells plated.
¶ NS = not statistically significant.

Table 3. Neither increased nor decreased concentrations of Con A improved thymocyte proliferation; best responses were achieved with cells at relatively high cell density

Stage	Cell Density (cells/ml)	Con A conc. (μg/ml)	C.p.m. cells only ($\bar{x}\pm$SE)	C.p.m. cells+Con A ($\bar{x}\pm$SE)	Stimulation index	Significance*
55–56	1×10^5	0·8	125±20	285±73	2·2	$P<0.03$
		2·0	125±20	186±12	1·5	$P<0.01$
	2×10^5	0·8	213±24	316±71	1·5	NS†
		2·0	213±24	386±43	1·8	$P<0.01$
	5×10^5	0·8	839±98	934±137	1·1	NS
		2·0	839±98	1167±125	1·4	$P<0.03$
62–63	$2{\cdot}5\times10^5$	0·8	637±172	472±95	0·7	NS
		2·0	637±172	645±117	1·0	NS
	5×10^5	0·8	787±168	705±108	0·9	NS
		2·0	787±168	1949±160	2·4	$P<0.01$
		4·0	787±168	1016±70	1·4	NS
64–65	1×10^5	0·8	108±10	95±7	0·9	NS
		2·0	108±10	214±25	1·9	$P<0.01$
	2×10^5	0·8	164±12	234±33	1·4	$P<0.03$
		2·0	164±12	505±44	3·1	$P<0.01$
	5×10^5	0·8	472±87	360±53	0·8	NS
		2·0	472±87	1170±139	2·5	$P<0.01$

* Average background and stimulated counts were compared by a two-tailed Student's *t*-test. Data have not been normalized.

† NS = not statistically significant.

Table 4. Increased fetal calf serum (FCS) does not improve proliferative responses of larval thymocytes to 2 μg/ml PHA

Stage	Cell density (cells/ml)	FCS conc. (%)	C.p.m. cells only ($\bar{x}\pm$SE)	C.p.m. cells+mitogen ($\bar{x}\pm$SE)	Stimulation index	Significance*
55–60	5×10^5	1	502±51	965±94	1·9	$P<0.01$
		5	453±86	387±73	0·8	NS†
		10	370±82	481±51	1·3	NS
60	5×10^5	1	950±177	1531±52	1·6	$P<0.01$
		5	650±19	836±60	1·3	$P<0.05$

* Average background and stimulated counts were compared by a two-tailed Student's *t*-test. Data have not been normalized.

† NS = not statistically significant.

Increased concentrations of fetal calf serum (FCS) in culture also did not improve the proliferative response of thymocytes to PHA (Table 4).

To determine whether thymocyte unresponsiveness to mitogens might be due to limited numbers of functional accessory cells, we provided accessory cells by adding autologous larval splenocytes to thymocytes cultured with mitogen. Co-culture of 1×10^5 larval thymocytes with 2×10^4 larval splenocytes plus Con A or PHA did not result in increased thymocyte proliferation in two of three experiments. That is, with or without mitogen, [^{3}H]TdR uptake by the co-cultured cells was approximately the sum of the uptake by splenocytes and thymocytes cultured separately in an experiment with stages 59 or 61 larvae. In contrast, proliferation by co-cultured thymocytes and splenocytes from stage 60 larvae with mitogens was significantly greater than the sum of proliferation by each

Table 5. Proliferation of larval thymocytes in response to PHA or Con A with or without the addition of autologous larvel splenocytes

Stage	Cell population	Mitogen	C.p.m. cells only ($\bar{x}\pm$SE)	C.p.m. cells+mitogen ($\bar{x}\pm$SE)	Stimulation index	Significance*
59	Thymocytes only†	PHA‡	299±21	642±8	2·1	$P<0.01$
	Splenocytes only§	PHA	106±12	1390±110	13·0	$P<0.01$
	Thymocytes+splenocytes¶	PHA	388±10	1505±191**	3·9	$P<0.01$
	Thymocytes only	Con A††	299±21	524±41	1·8	$P<0.01$
	Splenocytes only	Con A	106±12	1796±130	16·9	$P<0.01$
	Thymocytes+splenocytes	Con A	388±10	2215±156**	5·7	$P<0.01$
60	Thymocytes only	PHA	950±177	1531±52	1·6	$P<0.01$
	Splenocytes only	PHA	297±62	11,018±907	37·0	$P<0.01$
	Thymocytes+splenocytes	PHA	2100±925	18,129±2167‡‡	8·6	$P<0.01$
	Thymocytes only	Con A	950±177	1837±177	1·9	$P<0.01$
	Splenocytes only	Con A	297±62	9847±651	33·1	$P<0.01$
	Thymocytes+splenocytes	Con A	2100±925	25,269±1687‡‡	12·5	$P<0.01$
61	Thymocytes only	PHA	458±28	535±43	1·2	NS
	Splenocytes only	PHA	149±10	3092±173	20·7	$P<0.01$
	Thymocytes+splenocytes	PHA	592±46	4192+308**	7·1	$P<0.01$
	Thymocytes only	Con A	458±28	546±28	1·2	$P<0.05$
	Splenocytes only	Con A	149±10	2809±163	18·9	$P<0.01$
	Thymocytes+splenocytes	Con A	592±46	4126±95f	6·8	$P<0.01$

* Average background and stimulated counts were compared by a two-tailed Student's *t*-test. Data in this table have not been normalized.

† Thymocytes were cultured at a density of 5×10^5 cells/ml.

‡ Concentration of PHA in culture = 2 μg/ml.

§ Splenocytes were cultured at a density of 1×10^5 cells/ml.

¶ In mixed cultures, thymocytes and splenocytes were cultured at a density of 5×10^5 cells/ml and 1×10^5 cells/ml, respectively.

** Response is apparently additive. No evidence for synergy.

†† Concentration of Con A in culture = 2 μg/ml.

‡‡ Response is greater than additive; there appears to be synergism in the response of thymocytes with splenocytes.

cell population alone (Table 5). We are currently investigating this apparent synergy of stage 60 larval splenocytes and thymocytes.

The profile of changes in the number of thymocytes and splenocytes during larval development and metamorphosis (Fig. 5) shows cell numbers increasing to a maximum at stages 60–61 followed by a decline to their lowest numbers at stage 64, and a recovery at stages 65–66 (completion of metamorphosis). The decrease in lymphocyte responses to mitogens by cells from each organ, therefore, preceded the reduction of cell numbers in each organ.

DISCUSSION

The proliferative response of *Xenopus* splenocytes to PHA and Con A is evident as soon as the spleen becomes lymphoid (stage 51), increases to a maximum at the last pre-metamorphic larval stages (55–56), declines during the early stages of metamorphosis (stages 57–59) and recovers in the latter stages of the metamorphic crisis (stages 60–66) to post-metamorphic levels. A similar pattern can be seen in the very weak responses of larval thymocytes, although the numbers of PHA- and Con-A-responsive cells in the thymus are very low throughout larval development. The decline in mitogen responses at stages 57–59 precedes the loss of lymphocytes from spleen and thumus during metamorphosis. Thus, the recovery of mitogen responsiveness is occurring at the same time that the overall numbers of thymocytes and splenocytes are decreasing (stages 60–64). This may suggest a thyroxine-dependent and/or metamorphosis-related

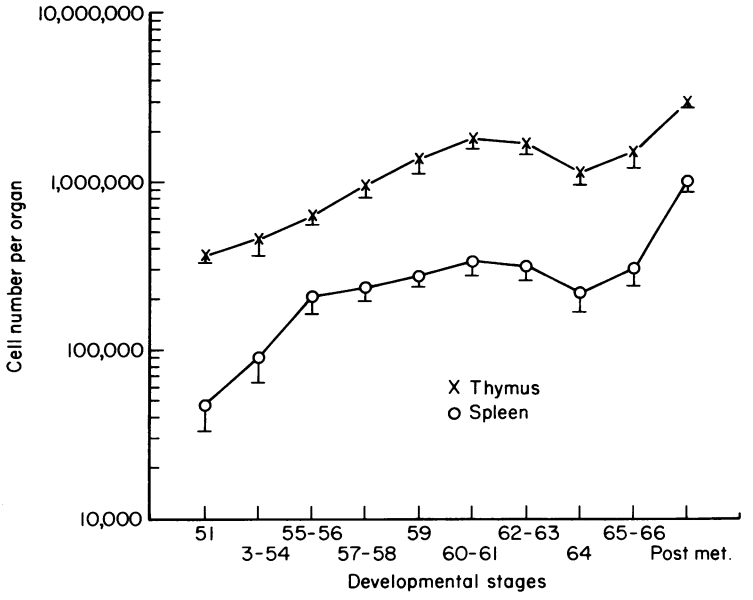

Fig. 5. Changes in the numbers of recoverable cells from bilateral thymuses (×——×) and spleen (O——O) at various developmental stages. Data are plotted as mean cell number per organ minus SEM.

loss of cells from each organ and a replacement of those cells by a population of cells with a different antigen or mitogen response profile.

The minimal larval thymocyte responses that we observed are similar to those reported by Williams *et al.* (1983). They observed that thymocyte responses to Con A (1 μg/ml) did not exceed a 2-fold increase until about 5–6 months of age, and the responses increased steadily until the frogs were about 1 year of age. PHA (20 μg/ml) responses of thymocytes did not reach appreciable levels until about 4 months of age (~2 months post-metamorphosis).

It is interesting to compare the ontogeny of mitogen responsiveness in developing frogs and mice. The mouse thymus anlage is first observed at 11–13 days of gestation and becomes lymphoid by days 14–15. Already by day 16, thymocytes are capable of proliferation in response to PHA and Con A (Mosier, 1974). Over the next few days, the PHA response declines and the Con-A response increases gradually to adult levels at about 2 weeks after birth. Although fetal spleen contains T cells from about day 16 of gestation (Spear *et al.*, 1973), fetal mouse splenocytes are relatively unresponsive to PHA (Mosier & Cohen, 1975) and good responses to PHA and Con A do not emerge until several weeks after birth, increasing to adult levels at about 6 weeks of age (Adler *et al.*, 1970; Harve & Manziello, 1972; Papiernik, 1976; Mosier, 1977).

In contrast to the developmental progression in the mouse, the frog shows very early functional lymphocyte activity in the spleen manifested by mitogen responsiveness (Fig. 3) and responsiveness to allogeneic cells in mixed leucocyte culture (Du Pasquier *et al.*, 1979; Cohen *et al.*, 1983) at a time when the thymus is only weakly responsive to mitogens (Fig. 4) and inconsistently responsive to allogeneic cells in MLC (Du Pasquier & Weiss, 1973; Rollins-Smith & Cohen, unpublished). Perhaps an explanation lies in the differences between the protected fetal existence of the developing mammal and the free-living life of a young frog. The larval frog is likely to contact environmental pathogens during its development. Thus, it needs a functional peripheral immune system. The larval immune system, however, must be suppressed or eliminated at metamorphosis to enable the frog to become tolerant of a whole new set of self-antigens that define the adult character of its body. Thus, in larval life, it needs a functional peripheral immune system and a reservoir of immature and inactive lymphocytes to replace those lost at metamorphosis. This may explain why larval thymocytes are relatively unresponsive to mitogenic stimuli.

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