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Immunology

Induction of terminal deoxynucleotidyl transferase and Lyt antigens with thymosin: Identification of multiple subsets of prothymocytes in mouse bone marrow and spleen

(thymosin fraction 5 and α_1 peptide/immunofluorescence/thymocytopoiesis/congenitally athymic mice/DNA nucleotidylexotransferase)

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ABSTRACT **Thymosin (fraction 5 and synthetic α_1 peptide) induced prothymocytes in mouse bone marrow and spleen to express terminal deoxynucleotidyl transferase (TdT; DNA nucleotidylexotransferase; nucleosidetriphosphate:DNA deoxynucleotidylexotransferase, EC 2.7.7.31) or Lyt-1$^+$, 2$^+$, 3$^+$ alloantigens (or both) after brief incubation *in vitro*. Three antigenic phenotypes were generated: (*i*) TdT$^+$ Lyt$^+$, (*ii*) TdT$^-$ Lyt$^+$, and (*iii*) TdT$^+$ Lyt$^-$. The TdT$^+$ Lyt$^+$ phenotype was expressed by 80% of prothymocytes in bone marrow and 30% of prothymocytes in spleen from normal mice. The TdT$^-$ Lyt$^+$ phenotype was expressed by 81% of prothymocytes in bone marrow from athymic mice. More than 80% of TdT$^+$ bone marrow cells from normal and athymic mice expressed Lyt antigens after thymosin treatment. We interpret these observations as suggesting that (*i*) most TdT$^+$ hemopoietic cells in normal and athymic mice are thymocyte progenitors; (*ii*) two independent lineages of prothymocytes exist, one that expresses TdT and another that does not; (*iii*) commitment of prothymocytes to the TdT$^+$ cell pathway is partially regulated by a thymic feedback mechanism; and (*iv*) the bone marrow preferentially produces TdT$^+$ prothymocytes, whereas the spleen may serve as a repository for TdT$^-$ prothymocytes. A model of T-cell development is presented in which the thymus functions as a compound organ to process TdT$^+$ and TdT$^-$ thymocyte progenitors and to generate two lines of T cells.**

Terminal deoxynucleotidyl transferase (TdT; DNA nucleotidylexotransferase; nucleoside triphosphate:DNA deoxynucleotidyltransferase, EC 2.7.7.31) catalyzes deoxynucleoside triphosphate polymerization in the absence of any known template (1). Its distribution is restricted to immature members of the lymphocyte cell series and to their neoplastic counterparts (2–8).

Several recent studies using soluble thymus factors strongly suggest that many TdT$^+$ hemopoietic cells are thymocyte progenitors. Silverstone *et al.* (9, 10) showed that TdT specific activity decreased significantly in suspensions of mouse bone marrow cells that had been incubated *in vitro* with thymopoietin and then treated with anti-Thy-1 serum and complement. Pazmino *et al.* (11, 12) showed that TdT specific activity in bone marrow from thymectomized or congenitally athymic mice increased significantly after *in vivo* or *in vitro* treatment with thymosin. However, it was not possible in these studies to accurately determine the percentage of TdT$^+$ cells that were thymocyte progenitors or the percentage of thymocyte progenitors that were TdT$^+$. Such information would permit more-precise analyses of the early stages of T-cell development and of the possible role of thymic factors in this process.

We have used double immunofluorescence to simultaneously assay for TdT and Lyt antigens in or on hemopoietic cells from normal (*nu*/+) and athymic (*nu*/*nu*) mice. The results suggest that both of these markers can be induced by brief *in vitro* incubation with thymosin (fraction 5 and thymosin α_1).

MATERIALS AND METHODS

Animals. Three- to 6-week-old C57BL/6 female athymic (*nu*/*nu*) (Lyt-1.2$^+$, 2.2$^+$, 3.2$^+$) mice and their normal (*nu*/+) littermates were obtained from the colonies of the Naval Medical Research Institute and the Division of Animal Resources, National Institutes of Health, Bethesda, MD. Female C57BL/6-Ly1^a (Lyt-1.1$^+$, 2.2$^+$, 3.2$^+$) and C57BL/6-Ly2^{a}3^a (Lyt-1.2$^+$, 2.1$^+$, 3.1$^+$) strains of mice were obtained from the Jackson Laboratory.

Thymosin Preparations. The partially purified calf thymosin fraction 5 and the synthetic thymosin α_1 peptide (M_r 3108) were prepared by Hoffmann–La Roche as described (13–15). The known biological properties of these substances have been discussed elsewhere (16). A control spleen extract (spleen fraction 5) was prepared according to the protocol for thymosin fraction 5.

Antisera. Mouse alloantisera to Lyt-1.1, 1.2, 2.1, 2.2, 3.1, and 3.2 antigens were prepared according to the method of Shen *et al.* (17) and were absorbed with thymocytes from Lyt congeneic mice to ensure specificity and to remove autoantibodies and antiviral antibodies (18).

An F(ab')$_2$ fraction of rabbit antiserum to homogenous calf terminal deoxynucleotidyl transferase (anti-TdT) was rendered highly specific by elution from a terminal transferase immunoabsorbant column (3, 4, 19). Rhodamine-conjugated goat IgG against mouse IgG, and fluorescein-conjugated goat IgG against rabbit IgG, were obtained from Cappel Laboratories (Dowingtown, PA). The former antiserum was absorbed with mouse erythrocytes before use, and the latter was purified on a normal mouse serum affinity column (10 mg/ml coupled to CNBr-activated Sepharose 4B) to remove crossreacting antibodies to mouse Ig.

Medium. The medium used was RPMI-1640 supplemented with penicillin, streptomycin, L-glutamine, and 25 mM Hepes at pH 7.4.

Treatment of Cell Suspensions. Bone marrow and spleen cell suspensions were separated into four fractions by discontinuous bovine serum albumin density-gradient centrifugation as de-

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Abbreviations: TdT, terminal deoxynucleotidyl transferase; sIg, surface immunoglobulin; IF, immunofluorescence.

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scribed (18). Aliquots containing 2×10^6 cells were taken from each fraction and from the unfractionated control and incubated with either thymosin fraction 5 at 10 μg/ml, thymosin α_1 at 1 μg/ml, or spleen fraction 5 at 10 μg/ml for 3 hr at 37°C (18). (In preliminary experiments, these concentrations had been found to be optimal for inducing TdT and Lyt antigens.) After incubation, the cell samples were washed twice and divided into two portions, one of which was assayed for Lyt antigens by cytotoxicity (20) and the other of which was frozen in 7.5% dimethyl sulfoxide/10% fetal calf serum (21) for subsequent immunofluorescence analysis of Lyt, TdT, and Ig antigens.

Control suspensions of untreated cells were freshly prepared for IF. These were neither fractionated nor cryopreserved.

Imunofluorescence Assay for Lyt Antigens and TdT. Approximately 5×10^5 cells in 0.2 ml of Tyrode's buffer/10% fetal calf serum were incubated with combinations of antisera to Ly 1.2, 2.2, and 3.2 antigens (1:10 final dilution) for 20 min at 4°C, washed twice, and developed with a 1:10 dilution of rhodamine-conjugated goat IgG against mouse IgG. Smears of the cells were prepared on a Cytospin cytocentrifuge (Shandon Instruments, Sewickley, PA), fixed for 5 min in cold absolute methanol, incubated with anti-TdT at 100 μg of $F(ab')_2$ per ml, washed three times in phosphate-buffered saline, and developed with fluorescein-conjugated goat IgG against rabbit IgG (1:20) as described (4). Individual cells were analyzed for surface rhodamine and intracellular fluorescein fluorescence with a Zeiss Universal Microscope equipped with interchangeable narrow band filters.

Specificity controls included substituting anti-Lyt-1.1, 2.1, and 3.1 sera for anti-Lyt-1.2, 2.2, and 3.2 sera and substituting cells from the C57BL/6-Ly-1^a and Ly-2^a3^a congenic mice. Also, normal mouse and rabbit sera were substituted for the primary antisera, and the fluorescence-developing antisera were interchanged. The fact that none of the TdT^+ cells had detectable surface immunoglobulins either before or after thymosin treatment permitted the use of the rhodamine-conjugated goat IgG against mouse IgG as the developing antiserum for the detection of Lyt antigens on TdT^+ cells. Moreover, Lyt^+ cells (indirect immunofluorescence) fluoresced much more intensely than did surface immunoglobulin-positive cells (direct immunofluorescence), permitting the detection of Lyt^+ cells in the presence of immunoglobulin-positive cells. The validity of this assay is shown by the close correlation between the percentages of Lyt^+ cells identified by immunofluorescence and cytotoxicity assays (data not included; see refs. 16, 18, 20).

RESULTS

Induction of TdT by Thymosin. Both thymosin fraction 5 and thymosin α_1 caused ≈8-fold increases in TdT^+ spleen cells and ≈2-fold increases in TdT^+ bone marrow cells above levels found in spleen fraction 5-treated or untreated controls from both *nu/+* and *nu/nu* animals (Table 1). TdT induction was confined almost entirely to cells in fractions A and B from the density-gradient centrifugation (i.e., those obtained at lower densities).

Thymosin treatment of spleen and bone marrow cells altered the intracellular distribution of terminal transferase as well as the percentage of TdT^+ cells. In untreated and in spleen fraction 5-treated suspensions, TdT^+ cells displayed a diffuse nuclear pattern of TdT fluorescence (Fig. 1*A*). In contrast, TdT^+ cells in thymosin-treated suspensions showed a marked heterogeneity of fluorescence patterns, including cytoplasmic, mixed nuclear and cytoplasmic, and nuclear "ring" patterns (Fig. 1 *B–D*). These latter patterns are ordinarily restricted to cortical thymocytes (4, 5). A greater degree of heterogeneity was displayed by thymosin-treated cells from normal mice than from athymic mice; the mixed nuclear-cytoplasmic patterns predominated in the former animals, the diffuse nuclear pattern predominated in the latter.

Table 1. Induction of TdT in mouse spleen and bone marrow cells

	TdT^+ cells after incubation, %		
	Spleen fraction 5	Thymosin fraction 5	Thymosin α_1
	nu/+ C57BL/6 mice		
Spleen			
Fraction A (9)	0.4 ± 0.3	9.4 ± 2.6	8.1 ± 1.2
Fraction B (16)	0.5 ± 0.2	6.3 ± 0.8	6.8 ± 1.0
Fraction C (53)	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.2
Fraction D (22)	0.1 ± 0.1	0.0 ± 0.0	0.2 ± 0.2
Unfractionated	0.3 ± 0.2	2.3 ± 0.1	2.0 ± 0.3
Bone marrow			
Fraction A (17)	3.8 ± 1.3	11.5 ± 0.8	5.4 ± 1.6
Fraction B (48)	3.6 ± 0.2	9.5 ± 2.1	7.8 ± 2.6
Fraction C (15)	1.6 ± 0.9	4.4 ± 1.9	4.4 ± 2.3
Fraction D (19)	2.2 ± 1.2	2.4 ± 1.4	2.8 ± 1.6
Unfractionated	3.3 ± 0.4	8.0 ± 0.2	6.0 ± 2.2
	nu/nu C57BL/6 mice		
Spleen			
Fraction A (13)	0.1 ± 0.1	3.1 ± 0.5	2.8 ± 0.5
Fraction B (26)	0.1 ± 0.1	1.6 ± 0.8	1.1 ± 0.6
Fraction C (40)	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1
Fraction D (21)	0.0 ± 0.0	0.2 ± 0.1	0.2 ± 0.1
Unfractionated	0.1 ± 0.1	1.0 ± 0.4	0.8 ± 0.3
Bone marrow			
Fraction A (14)	1.5 ± 0.3	3.6 ± 0.8	2.4 ± 0.4
Fraction B (47)	1.1 ± 0.6	3.2 ± 0.2	2.0 ± 0.3
Fraction C (15)	1.2 ± 0.3	1.2 ± 0.2	1.1 ± 0.3
Fraction D (24)	1.5 ± 0.2	0.9 ± 0.2	2.2 ± 1.1
Unfractionated	1.3 ± 0.2	2.6 ± 0.3	1.8 ± 0.3

Cells were separated by density-gradient centrifugation. Values in parentheses are mean percentages of total nucleated cells recovered per fraction. Fraction A, least dense cells; fraction D, most dense cells. TdT^+ values were determined by indirect immunofluorescence. Values given are mean ± SEM for three experiments, and 500–1000 cells were counted per point per experiment.

Induction of Lyt Antigens by Thymosin. Thymosin (fraction 5 and α_1 peptide) has been shown previously to induce the expression of Lyt antigens on mouse spleen and bone marrow cells after brief incubation *in vitro* (16). Cells with newly expressed Lyt antigens were recovered predominantly in the lower-density cell fractions (A and B), whereas most preexisting Lyt^+ cells were found in the higher-density fractions (C and D). Essentially all of the newly appearing Lyt^+ cells had the Ly-$1^+2^+3^+$ phenotype.

These findings were corroborated in this study by using immunofluorescence to quantify Lyt^+ cells (Tables 2 and 3). It is notable that Lyt^- to Lyt^+ conversion rates did not vary substantially between normal and athymic mice or between spleen and bone marrow cells.

Induction of TdT and Lyt Antigens on the Same Cell. Individual cells were examined for TdT and Lyt markers by double immunofluorescence. No cell with a TdT^+ Lyt^+ phenotype was identified in untreated or spleen fraction 5-treated spleen or bone marrow cells. In constrast, 99% of TdT^+ spleen cells and 88% of TdT^+ bone marrow cells from normal mice expressed Lyt antigens after *in vitro* incubation with thymosin fraction 5 (Table 4 and Fig. 2). Similarly, 74% of TdT^+ spleen cells and 80% of TdT^+ bone marrow cells from athymic mice expressed

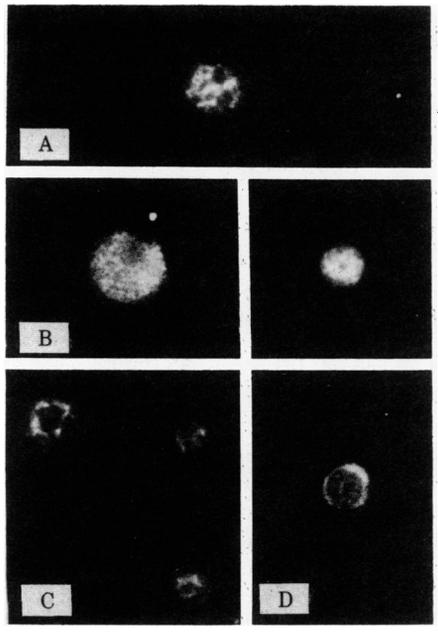

FIG. 1. TdT^+ cells. (*A*) From *nu/+* mouse spleen treated with spleen fraction 5. Note nuclear pattern of fluorescence. (*B–D*) From *nu/+* mouse spleen treated with thymosin α_1 peptide. (*B*) Large (*Left*) and small (*Right*) cell with diffuse nuclear TdT fluorescence. (*C*) "Ring" pattern of nuclear fluorescence. (*D*) Cytoplasmic pattern of TdT fluorescence. (×1250.)

Lyt antigens after thymosin treatment. Inasmuch as the same percentage of TdT^+ cells reacted with individual or combinations of antisera to Ly-1, Ly-2, and Ly-3 antigens, it would appear that their phenotype was Ly-$1^+2^+3^+$. By using the appropriate alloantisera, comparable results were obtained with thymosin-treated cells obtained from C57BL/6-Lyt 1^a (Ly-1.1, 2.2, 3.2) or C57BL/6-Lyt 2^a3^a (1.2, 2.1, 3.1) cogenic strains of mice.

Induction of TdT and Lyt Antigens on Separate Cells. Although most TdT^+ spleen and bone marrow cells expressed Lyt antigens after thymosin treatement (see Table 4), many thymosin-induced Lyt^+ cells did not express TdT (Table 5). Approximately 50% of newly induced Lyt^+ spleen cells from normal mice and 75% of those from athymic mice were TdT^-, and approximately 20% of newly induced Lyt^+ bone marrow cells from normal mice and 80% of those from athymic mice were TdT^-.

DISCUSSION

The ability of thymosin to induce TdT^+ bone marrow cells and spleen cells to express Lyt antigens is strong evidence that such cells are thymocyte progenitors. In this respect, our results are consistent with those of Silverstone *et al.* (9, 10), who used thymopoietin to induce Thy-1 antigen on TdT^+ cells. However, the results differ in two major aspects. First, thymosin, apparently unlike thymopoietin, can induce a subset of prothymocytes to express TdT. Second, thymosin can induce TdT^+ cells from *nu/nu* mouse bone marrow to express T-cell alloantigens. The simplest explanation for these discrepancies, aside from methodological differences, is that thymosin can stimulate TdT^+ prothymocytes earlier in their development than can thymopoietin. Thus, three thymosin-responsive stages in the differentiation of TdT^+ prothymocytes can be envisioned: (*i*) pre-TdT^+ cells, (*ii*) early TdT^+ cells, and (*iii*) late TdT^+ cells. The last of these is presumed to give rise to TdT^+ thymocytes.

Pazmino *et al.* (11) have shown that TdT activity in normal mouse bone marrow decreases progressively after thymectomy and that low TdT levels in thymectomized and in *nu/nu* mice can be restored to normal by adminstration of thymosin *in vivo* or *in vitro*. Our results suggest that most of this increase in enzyme activity is due to the induction of TdT^+ cells from TdT^- precursors.

Our results further suggest that the thymus influences both the commitment of early thymocyte progenitors to the TdT cell pathway and their subsequent differentiation. However, the thymus does not seem to influence the rate at which early thy-

Table 2. Percentage of Lyt^+ cells in C57BL/6 mouse spleen and bone marrow after incubation with spleen fraction 5

	Spleen		Bone marrow	
	nu/+	*nu/nu*	*nu/+*	*nu/nu*
Fraction A	4.7 ± 0.3	≤2.5	4.8 ± 1.0	≤2.0
Fraction B	3.7 ± 1.2	≤2.5	4.1 ± 0.9	≤2.0
Fraction C	38.7 ± 1.9	≤2.5	4.0 ± 1.0	≤2.0
Fraction D	48.0 ± 4.5	≤2.5	4.6 ± 0.5	≤2.0
Unfractionated	31.5 ± 3.5*	≤2.5	4.2 ± 1.1	≤2.0

Values were determined by immunofluorescence using a combination antisera to Ly 1.2, 2.2, and 3.2 antigens. Similar results were obtained with untreated cell suspensions. Fractions A–D were as given in Table 1. Results are expressed as mean ± SEM.
* Phenotypes: Ly-$1^+2^-3^-$ (41%); Ly-$1^-2^+3^+$ (10%); Ly-$1^+2^+3^+$ (49%).

Table 3. Induction of Lyt^+ cells in C57BL/6 mouse spleen and bone marrow

	Net increase in Lyt^+ cells, %			
	Spleen		Bone marrow	
	nu/+	*nu/nu*	*nu/+*	*nu/nu*
Incubation with thymosin fraction 5				
Fraction A	18.2 ± 2.2	10.0 ± 2.3	10.5 ± 2.9	24.2 ± 4.2
Fraction B	23.3 ± 4.1	19.7 ± 2.6	12.3 ± 1.7	8.8 ± 1.2
Fraction C	0.0	2.3 ± 0.3	2.0 ± 1.9	0.0
Fraction D	0.0	0.0	1.6 ± 0.9	0.0
Unfractionated	7.8	7.5	8.7	7.7
Incubation with thymosin α_1				
Fraction A	12.3 ± 0.9	12.8 ± 3.5	11.7 ± 1.2	24.4 ± 0.9
Fraction B	15.5 ± 0.9	13.4 ± 0.2	7.1 ± 0.6	7.3 ± 3.7
Fraction C	1.8 ± 0.4	1.0 ± 0.4	0.0	0.0
Fraction D	0.0	0.0	0.8 ± 0.5	0.0
Unfractionated	6.6†	5.7	5.2	7.0

Values were determined as in Table 2. Fractions A–D were as given in Table 1. Results represent the difference in number of Lyt^+ cells in aliquots of thymosin-treated and spleen fraction 5-treated cells (see Table 2) and are expressed as mean ± SEM of the percentage of total cells per fraction.
* Acquired phenotype in all groups: Ly-$1^+2^+3^+$ (>95%).

Table 4. Percentage of TdT^+ spleen and bone marrow cells expressing Lyt antigens after incubation with thymosin

Treatment	Cell fractions examined	Spleen		Bone marrow	
		nu/+	*nu/nu*	*nu/+*	*nu/nu*
None	Unfractionated	0	0	0	0
Spleen fraction 5	A–D	0	0	0	0
Thymosin fraction 5	A–D	99	74	88	80
Thymosin α_1	A–D	95	70	79	73

Values were determined by double immunofluorescence for TdT and Ly-1,2,3 antigens. Mean of 2 or 3 experiments. Each entry represents the analysis of 100 TdT^+ cells (untreated and spleen fraction 5 treated) or 400–600 TdT^+ cells (thymosin fraction 5 and α_1 treated).

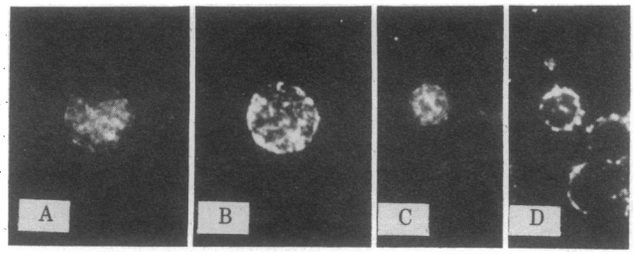

FIG. 2. Double immunofluorescence for TdT and Lyt antigens. Athymic (*nu/nu*) mouse spleen cells were treated with thymosin fraction 5. Large (*A*) and small (*C*) cell with nuclear pattern of TdT fluorescence (green). Corresponding cells with surface fluorescence (red) for Lyt (1.2 plus 3.2) antigens (*B* and *D*, respectively). The slight surface fluorescence in *A* and *C* is due to leaching of the rhodamine conjugate through the fluorescein conjugate filter. Two Lyt^+ TdT^- cells are also shown in *C* and *D*.

Table 5. Percentage of Lyt^+ spleen and bone marrow cells expressing terminal transferase after incubation with thymosin

Treatment	Cell fractions examined	Spleen		Bone marrow	
		nu/+	*nu/nu*	*nu/+*	*nu/nu*
None	Unfractionated	0	NS	0	NS
Spleen fraction 5	A plus B	0	NS	0	NS
Thymosin fraction 5	A plus B	33	12	80	19
Thymosin α_1	A plus B	31	11	87	16

Values were determined by double immunofluorescence for TdT and Ly-1,2,3 antigens. Mean of 2 or 3 experiments. Each entry represents the analysis of 500–750 Lyt^+ cells. NS, insufficient Lyt^+ cells for analysis.

mocyte progenitors are generated from presumptive lymphoid stem cells. Thus, *nu/nu* and *nu/+* mice had roughly equal percentages of thymocyte progenitors (≈8% of hemopoietic cells); yet, only 19% of thymocyte progenitors from athymic mouse bone marrow were TdT^+ after thymosin treatment as compared with 80% of thymocyte progenitors from normal mice. Moreover, most TdT^+ Lyt^+ cells from athymic mice had a "bone marrow" pattern of TdT fluorescence whereas those from normal mice had a "thymic" pattern.

An arrest in the generation of TdT^+ cells also occurs in spleen of normal postpubertal mice (5). This appears to be due to an age-related decrease in pre-TdT^+ prothymocytes (see Table 5). Hence, the spleen may serve as a repository for TdT^- prothymocytes in adult mice and bone marrow may serve as a repository for TdT^+ prothymocytes (22).

The ability of thymosin to induce prethymic cells to differentiate along two different pathways, TdT^+ and TdT^-, raises the intriguing possibility that separate progenitors exist for major thymocyte subsets—perhaps for cortical and medullary thymocytes. This hypothesis is consistant with the observations (*i*) that most cortical thymocytes in rats and mice are TdT^+ whereas medullary thymocytes are TdT^- (2–5), (*ii*) that cortical and medullary thymocytes have independent generative kinetics (23 and 24), (*iii*) that subsets of T cells may be released independently from thymus cortex and medulla (25–28). It is also consistent with the observation of independent homing of lymphoid stem cells to cortex and medulla of avian thymus (F. Jotereau, personal communication) and with the selective regeneration of TdT^- thymocytes in mice depleted of TdT^+ hemopoietic cells by low dose irradiation (29). We have recently identified two proliferating populations of low Thy-1^+, high H-2^+ thymocytes, one of which is TdT^+ and resides in the cortex and the other of which is TdT^- and resides in the medulla (unpublished results). It is tempting to speculate that these two subsets of thymocytes are the immediate descendants of TdT^+ and TdT^- thymocyte progenitors, respectively.

It could be argued that the appearance of TdT^- Lyt^+ cells after thymosin treatment reflects incomplete induction of TdT or that the TdT^- Lyt^+ cells may have transiently expressed TdT during the 3-hr incubation period. Neither argument seems probable. Both the concentration of thymosin and the incubation time were optimal for the induction of TdT and Lyt antigens. Under these conditions, TdT specific activity and percent Lyt^+ cells (14, 16) increase linearly during the first 3–4 hr of incubation and then remain constant for at least 20 hr. It must be noted that the reported inability of thymosin α_1 to induce TdT *in vitro* (12) was based on a dose of α_1 peptide that was 1/12.5 that used here. Moreover, the high dose inhibition of TdT

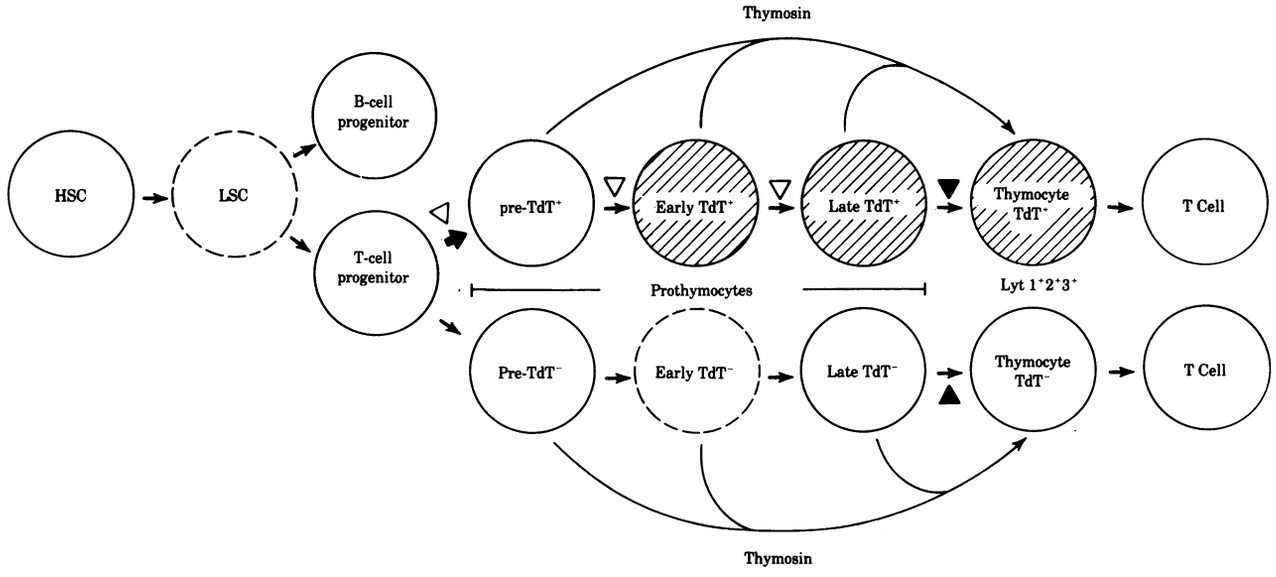

FIG. 3. Dual prothymocyte model of T-cell development in *nu/+* mouse bone marrow. In athymic mice, the equilibrium would be shifted toward the production of TdT^- prothymocytes. HSC, hemopoietic stem cells; LSC, lymphopoietic stem cells. ▽, Long-range thymuc influence; ▲, short-range thymic influence.

induction noted previously with thymosin fraction 5 (11) was not seen in this study. Instead, maximal induction of TdT^+ cells occurred at concentrations of 0.01–100.0 μg/ml.

In summary, this study suggests the existence of two thymosin-responsive lineages of prothymocytes in hemopoietic tissues, one TdT^+ and one TdT^-. The nature and relationship of the cell subsets in each lineage remain to be determined. A hypothetical scheme of prothymocyte development that is consistent with available evidence is shown in Fig. 3. In this scheme, the TdT^+ and TdT^- lineages of prothymocytes are postulated to arise from a common TdT^- ancestor. The proportion of prothymocytes committed to the TdT^+ or TdT^- lineage is influenced by a combination of long-range (thymic) and short-range (microenvironmental) interactions. Mature ("late") prothymocytes migrate to the thymus, where they undergo further differentiation. The anticipated result of this developmental process is the production of two independent lineages of thymocytes and, ultimately, of T cells.

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