

## INDUCTION OF T LYMPHOCYTES FROM PRECURSOR CELLS IN VITRO BY A PRODUCT OF THE THYMUS\*

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We have reported that a product of mouse thymus, prepared according to the method of Goldstein et al. for extracting "thymosin" (1), induces precursor cells found in the spleen and bone marrow of adult mice to differentiate in vitro into T lymphocytes, defined as cells expressing the T lymphocyte antigens TL, Thy-1, and Ly, within about 2 h (2). (We have avoided calling the inducer thymosin in case these may be different products occurring in the same preparation.) The simplest interpretation is that the noninduced precursor cell has not passed through the thymus, but would or could in the normal course of events do so, and thereby become differentiated into a T lymphocyte. (Whether these precursor cells are as yet uncommitted to T lymphocyte differentiation or are already primed or genetically "determined" to respond to the T lymphocyte thymic inducer is unknown.) While many interesting points can be raised regarding this in vitro induction system, we shall consider here only this one question: Is the inducible cell truly a precursor that has not yet undergone any thymus-mediated process? To answer this we have tested the inducibility of cells from the spleen and bone marrow of *nu/nu* mice (which lack a thymus) and from the liver of embryonic mice.

### *Materials and Methods*

*Mice.*—All mice, including the congenic stocks A/Thy-1.1 (formerly called A/ $\theta$ -AKR) and A/TL<sup>-</sup>, were bred in our own colonies. The *nu/nu* mice come from a stock that has undergone four to five backcrosses to BALB/c; the *+/nu* mice were heterozygotes of this line; the *nu/nu* mice were bred and maintained under normal conditions without protection from environmental infection.

*Induction Procedure.*—The procedure of Goldstein et al. for thymosin (1) was used to prepare "thymic extract" from thymuses of TL-negative mice and "splenic extract" (for control); the final preparations had approximately equivalent protein concentrations (OD/280 nm 0.5 U/ml for thymus and 0.7 for spleen) and were used undiluted.

Cells from spleen, bone marrow, or embryonic liver (separated by teasing) were fractionated by centrifugation on gradients of bovine serum albumin (BSA) yielding four layers: A (10–23% BSA interface), B (23–26%), C (26–29%), and D (29–35%). Three aliquots of cells from each layer were incubated with (a) thymic extract, (b) splenic extract (control), and (c) without extract (control), for 2 h at 37°C. (Meanwhile, cytotoxicity tests with Thy-1 antiserum were

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carried out on a fourth aliquot, to ascertain the number of T lymphocytes present in untreated cells of each layer, data shown in Table II.) After the 2-h incubation period the cells were washed and tested for Thy-1 or TL antigens in cytotoxicity tests with antiserum (prepared in our congenic mouse strains) diluted 1/10 and 1/20 (the two dilutions always gave very similar results). The formula used in Table I gives the proportion of cells expressing Thy-1 or TL antigen in consequence of exposure to the extract. More complete technical details, and references to methods, will be found in our previous report (2).

## RESULTS AND DISCUSSION

The data are summarized in Tables I and II. As before (2), the inducible precursor cells were found in the A and B layers of spleen and bone marrow (Table I) and preexisting T lymphocytes in the C and D layers (Table II).

*Induction of Cells from nu/nu Mice.*—Table I shows that thymic extract in-

TABLE I  
*Differentiation of Thy-1<sup>+</sup> or TL<sup>+</sup> Cells Induced In Vitro by a Product of the Thymus. Positive Results with Cells from Adult Bone Marrow, Spleen and Bone Marrow of nu/nu Mice, and Fetal Liver*

Mouse strain	Cells from	Antiserum	Thy-1 <sup>+</sup> or TL <sup>+</sup> cells (%)* induced in layer			
			A	B	C	D
A/Thy-1.1	Bone marrow	Anti-Thy-1.1	28 (-1)‡	26 (7)	1 (3)	—
		Anti-Thy-1.2§	1 (0)	0 (3)	—	—
		Anti-TL	30 (8)	27 (4)	5 (4)	—
A/TL <sup>-</sup>		Anti-TL§	4 (5)	3 (2)	—	—
nu/nu	Spleen	Anti-Thy-1.2	31 (0)	34 (-4)	-4 (2)	—
		Anti-Thy-1.1§	-1 (0)	-2 (-1)	-2 (5)	—
	Bone marrow	Anti-Thy-1.2	35 (3)	16 (4)	-2 (-3)	-1 (-2)
		Anti-Thy-1.1§	-3 (0)	-4 (-2)	—	—
+/nu	Spleen	Anti-Thy-1.2	24 (3)	25 (6)	-2 (-2)	—
		Anti-Thy-1.1§	1 (2)	3 (3)	—	—
A	Fetal liver (18 days)	Anti-Thy-1.2	—	27 (3)	4 (-1)	5 (2)
		Anti-Thy-1.1§		-4 (1)	1 (2)	—
		Anti-TL		26 (-1)	13 (3)	4 (2)
	Fetal liver (14 days)	Anti-Thy-1.2		27 (4)	4 (4)	-1 (-2)
		Anti-Thy-1.1§		-5 (-1)	2 (2)	—
		Anti-TL		34 (4)	7 (0)	3 (1)

\* Calculation:  $100 \times \frac{a-b}{a}$ .

a = % cells viable in cytotoxicity test after preincubation without extract;

b = % cells viable in cytotoxicity test after preincubation with thymic extract or splenic (control) extract.

This formula gives the percent of newly induced Thy-1<sup>+</sup> or TL<sup>+</sup> cells, excluding those already present in the starting population.

‡ Numbers in parentheses are the control values for cells preincubated with splenic extract in place of thymic extract, the two extracts being prepared in the same way and having approximately the same protein concentrations.

§ Negative control.

|| Unsatisfactory for testing because of a high proportion of dead cells.

duced Thy-1-positive cells from spleen and bone marrow of *nu/nu* mice in numbers even higher than for normal mice (35% of *nu/nu* A layer bone marrow cells). Clearly there is no lack of inducible T lymphocyte precursors in mice that lack a thymus.

No T lymphocytes were demonstrable in untreated spleen or bone marrow of *nu/nu* mice (Table II). Reported demonstrations of Thy-1 antigen in *nu/nu* mice by serological absorption (3) do not necessarily indicate that *nu/nu* mice have some T lymphocytes, because Thy-1 antigen is expressed on epidermal cells and brain of *nu/nu* mice, and Thy-1 antigen is probably present on another cell type that may be present in hemopoietic tissues (unpublished work of Dr. M. Scheid in this laboratory). Absence of T lymphocytes from

TABLE II  
*Proportion of Cells Recovered in Each Layer, and Proportion of Preexisting Thy-1-Positive (Thy-1<sup>+</sup>)\* Cells within Each Layer†*

Mouse strain	Cells from	Cell layer							
		A		B		C		D	
		Yield‡	Thy-1 <sup>+</sup> cells	Yield	Thy-1 <sup>+</sup> cells	Yield	Thy-1 <sup>+</sup> cells	Yield	Thy-1 <sup>+</sup> cells
		%		%		%		%	
A/Thy-1.1	Bone marrow	5-12	<5	30-35	<5	13-20	<5	13-18	10-20
A/TL <sup>-</sup>		8-15	<5	34-38	<5	15-20	<5	10-15	15-20
<i>nu/nu</i>	Spleen	10-18	<5	25-32	<5	10-15	<5	5-10	<5
	Bone marrow	5-10	<5	35-40	<5	28-34	<5	2-5	<5
+/ <i>nu</i>	Spleen	6-10	<5	15-20	5-10	25-30	30-40	20-25	45-55
A	Fetal liver (18 days)	7-13	—	12-20	<5	10-20	<5	5-8	<5
	Fetal liver (14 days)	5-10	—	8-15	<5	10-15	<5	5-10	<5

\* Determined on cells of each layer immediately after their recovery from the BSA gradient. Calculation:  $100 \times \frac{a-b}{a}$ .

*a* = % cells viable in cytotoxicity test control with complement added but antiserum omitted;

*b* = % cells viable in cytotoxicity test with antiserum and complement: (anti-Thy-1.1 for A/Thy-1.1 mice and anti-Thy-1.2 for other mice in this table, all of which are Thy-1.2).

‡ These data come from the experiments summarized in Table I; each line corresponds to a line in Table I, negative controls and entries for TL antiserum omitted. The range of values quoted indicates results obtained in more than one test.

§ Expressed as percent of starting population.

*nu/nu* mice accounts for the approximately 50% reduction in the relative proportions of C and D layer spleen cells and the corresponding increase in the relative proportions of A and B layer spleen cells, in comparison with normal mice (Table II).

*Induction of Cells from Embryo Liver.*—As the thymus is thus seen to be unnecessary for the development of inducible potential T lymphocytes, it is not surprising that there was a high rate of induction of T lymphocytes from fetal liver cells (<34% for the B layer) at the 14-day stage of embryogenesis (Table I), the earliest time at which T lymphocytes can be detected in the thymus (4, 5). "T<sub>1</sub>" cells, probably the first T cells to appear in peripheral lymphoid tissue, have the ability to cooperate with B cells in humoral immune responses (6) but no T cell function has been demonstrable in mouse liver before birth (7), so it is hardly likely that the 14-day liver contains any thymus-processed cells (also see particularly references 8 and 9).

#### SUMMARY

A product of mouse thymus induces cells found in the spleen and bone marrow of *nu/nu* mice (which lack a thymus), and in 14-day embryonic mouse liver, to differentiate in vitro into T lymphocytes (defined as cells bearing TL and Thy-1 antigens). Thus the in vitro T lymphocyte induction mechanism acts on a cell that is antecedent to any thymus-mediated process.

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