DIFFERENTIATION OF T CELLS INDUCED BY PREPARATIONS FROM THYMUS AND BY NONTHYMIC AGENTS

THE DETERMINED STATE OF THE PRECURSOR CELL*,‡

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"Determination" implies that although a given cell displays none of the recognizable phenotypic traits characteristic of a particular pathway of differentiation it may nonetheless be committed to that pathway; the cell's future genetic program is already decided, though not yet implemented. Implementation of this program ("differentiation") may normally involve the interaction of a specific molecule with a specific receptor on the determined cell, but alternately other agents interacting with the cell, or functioning generally as mediators of phenotypic expression, may initiate the process. An example of the latter is cyclic AMP (cAMP).

To understand the function of the thymus it is necessary to know whether this organ is responsible for genetic determination of the T lymphocyte or whether it only supplies the signal for differentiation. Among other considerations, this may decide whether restoration of immune function to thymus-deprived subjects, in the sense of providing them with functional T cells, requires that they be supplied with thymus itself or any product of the thymus.

Here we summarize evidence from our own work showing that it is differentiation and not genetic determination of T cells for which the thymus is responsible, a conclusion that is implicit in previous studies (1–3).

When precursor cells from spleen or bone marrow of normal or *nu/nu* thymusless mice, or from embryonic liver, are incubated with a product of mouse thymus, they differentiate into T cells, defined as cells expressing the antigens TL, Thy-1, G_{IX}, Ly-1, Ly-2/Ly-3, and Ly-5 (4,5; and subsequent unpublished data).

Table I shows that agents of nonthymic origin can induce T-cell differentia-

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TABLE I

Summary of Experiments Showing Generation of T Cells from Precursor Cells under the Influence of Thymic and Nonthymic Agents

Material tested for inducer activity		Criterion of T cell induction						
		Expression of TL or Thy-1 antigen		Appearance of helper effect in vitro‖				
		I: in vitro‡	II: in *nu/nu* mice§	PFC/culture				
Source	Description*	Induced cells		1st exp.	2nd exp.	3rd exp.	4th exp.	5th exp.
		%						
Mouse thymus	Fr. 2 (KK)	20–23	20	184	233	267	716	
Mouse spleen	Fr. 2 (KK)	<10	<10				116	
—	Medium	<10		0	12	42	134	24
Mouse thymus	Fr. 3 (AG)	40–45						
Calf thymus	Fr. 2 (KK)	20–25	20,40					
Calf thymus	Fr. 3 (AG)	40–45						
Calf thymus	5 (AG)	25–30						
Calf thymus	6 (AG)	18–22						
Calf spleen	Fr. 3 (AG)	40–45						
Calf muscle	Fr. 3 (AG)	40–45						
Rat thymus	Fr. 2 (KK)	20–25						
Chicken thymus	Fr. 2 (KK)	25–30						
Human thymus	Fr. 3 (AG)	25–30						
—	cAMP	20						
—	DB-cAMP	25						
—	Poly A:U	20–25	30				495	410
—	Endotoxin	15–19						
—	BSA, Medium	<10, <10	<10					
Calf thymus¶	Fr. 2 (KK) + Aminophylline	25						
	Fr. 2 (KK) + Insulin	2						
	Fr. 2 (KK) + Medium	10						

Because of space limitations, details of materials and methods can be indicated for the most part only by key references to where full descriptions can be found.

* Fr = fraction. (KK) = fraction prepared by Dr. K. Komuro according to Goldstein

tion from precursor cells in vitro, and that the induced T cells may acquire functional (helper) activity as well as T-cell antigens, regardless of whether differentiation was initiated by a thymic or a nonthymic product poly (A:U).

To determine further whether intracellular cAMP may be implicated in T-cell

et al. (6) with minor modification (4). (AG) = fraction sent by Dr. A. L. Goldstein for testing at Sloan-Kettering Center. Fractions numbered according to their degree of purification (6). cAMP = adenosine 3′,5′-cyclic monophosphoric acid (Sigma Chemical Co., St. Louis, Mo.) 1 mM. DB-cAMP = N_6,O_2-dibuturyl-adenosine 3′,5′-cyclic monophosphoric acid (Sigma) 1 mM (also active at 0.1 mM). Poly A:U = polyadenylic-uridylic acid (provided by Dr. F. Lacour, Paris; sample SK 30-184B) tested in five serial log 10 dilutions from 1 to 0.0001 μg/ml; induction activity in vitro optimal between 0.1 and 0.001 μg/ml; inactive at higher concentrations. Endotoxin = *Escherichia coli* lipopolysaccharide (LPS) (Bayer, Germany) 20 μg/ml. BSA = bovine serum albumin (Path-o-cyte-5, lot 20, Pentex Biochemical, Kankakee, Ill.) 5%. Aminophylline = [(theophylline)$_2$·ethylenediamine·$2H_2O$] crystalline (Sigma) 1 mM. Insulin = 40 U regular Iletin (Eli Lilly and Co., Indianapolis, Ind.) 80 mU/ml.

‡ Figures in this column represent percent of starting cell population induced to express TL and Thy-1 antigens in vitro (for details see Komuro and Boyse, reference 4). The starting population was the "B" layer of mouse spleen cells fractionated on BSA density gradients as before (4) but at the interface 20–23% with the source of BSA (lot 20, Pentex) now in use. This fraction contains virtually no T (Thy-1$^+$) cells; TL$^+$ T cells are normally confined exclusively to the thymus. The percent cells induced is calculated from the results of the cytotoxicity test with TL or Thy-1 antiserum according to the formula $100 \times (a - b)/a$; a = viable cells percent (no fraction or agent included); b = viable cells percent fraction or agent included) (4). Results for TL and Thy-1 antigens were always concordant. A figure of 10 or higher is taken as significant, although with present standardization of technique a negative control reading higher than 5 is exceptional.

The only inducer-positive tissue so far identified in the mouse is thymus (4). Previous and present negative results with mouse spleen Fr. 2 (KK), contrasting with the positive results reported here for calf spleen Fr. 3 (AG), can have numerous explanations based on differences in preparation and concentration, but the salient point is that Fr. 3 from a tissue other than thymus can have inducer activity; thus the activity of Fr. 3 of thymus is not necessarily attributable to a specific thymic factor. Fetal bovine serum has shown occasional low inducer activity at a concentration of 5%, as J. F. Bach has observed with the rosette test (personal communication). A positive result was obtained with DB-cAMP; also cAMP was active, but with a higher minimal inductive dose, which accords with reports that although the permeability of most cell membranes to cAMP is small, some cells do admit cAMP to at least some extent (reviewed in reference 7).

§ Thymus-less *nu/nu* mice were injected intraperitoneally with mouse thymus Fr. 2 (KK) or mouse spleen Fr. 2 (KK) 1.0 ml daily for 6 days; or with calf thymus Fr. 2 (KK) 0.5 ml daily for 3 wk; or with poly A:U 500 μg once; and their lymph node cells tested for Thy-1 (θ) expression 3 days later.

‖ Induction of the helper property was assayed according to the culture technique of Mishell and Dutton (8) and expressed as plaque-forming cells (PFC) per culture on day 4 (exps. 1–4) or day 3 (exp. 5). Either (*a*) the fraction or agent to be tested was added directly to cultures of unfractionated *nu/nu* spleen cells, or (*b*) unfractionated *nu/nu* spleen cells processed for induction of TL and Thy-1 antigens as described for column I of this table were washed and then cultured.

¶ In this single experiment with aminophylline and insulin the level of induction by Fr. 2 (KK) alone (control) was unusually low (10%); we regard this experiment as no more than a favorable indication as to how the mechanism of induction may be better defined (see text).

differentiation, aminophylline, an inhibitor of phosphodiesterases (9), and insulin, reported to be an inhibitor of adenylate cyclase (10), were tested for their ability to enhance and inhibit induction, respectively. A single experiment tending in that direction is shown in Table I, suggesting possibly that thymosin may function via adenylate cyclase and that cAMP production may play an essential intracellular role in signalling the determined procursor cell to differentiate. In the same context, we note that poly A:U is reported to raise adenylate cyclase activity in mouse spleen (11).

COMMENT

(*a*) The T-cell precursor, found in spleen and bone marrow of *nu/nu* mice (5) as well as of normal mice (4), and in embryonic liver (5), is already determined, i.e., is already genetically programmed for the T-lymphocyte differentiation pathway, although as yet it has none of the known phenotypic traits that distinguish the T lymphocyte. The thymus is not concerned in this, but supplies the starting signal for differentiation. This being so, a number of agents, of which cAMP has the clearest rationale, can substitute for the physiological thymic inducer (and see reference 12).

(*b*) There is no compelling reason at present to postulate any effector function for the thymus other than to issue a signal for differentiation, for a precursor cell induced to differentiate into a cell bearing the T-cell surface components (antigens specified by genes in linkage groups II, IX, XI, XII, and others) also becomes competent to cooperate with B cells in the production of antibody to sheep red blood cells (SRBC) in vitro (Table I and reference 3).

(*c*) Agents such as poly A:U that appear to substitute for T cells in cooperative tests with B cells are in reality more probably inducing differentiation of T cells from precursor cells present in the T cell-depleted populations used to supply B cells. Restoration of immune faculties to thymectomized irradiated mice by such an agent (13) presumably has the same basis, i.e., induction of T cells from precursor cells (note the induction of Thy-1^+ cells in *nu/nu* mice given poly A:U, Table I). Before classifying any antigen as "T-cell independent" it is necessary to know whether it has the property of inducing T-cell differentiation.

(*d*) It will be essential to establish what correlations there may be among the three assays for T-cell induction, which depend on the criteria: (*i*) presence of T-cell antigens, (*ii*) rosette formation, and (*iii*) helper function. An important distinction is that the first of these tests measures the total number of T cells induced, whereas the latter two tests measure only the small fraction of cells acquiring the capacity to react specifically with SRBC. Complete correlation of the three assays can be expected only if the proportion of SRBC-reactive T cells induced is constant under all conditions. On such grounds explanations can be sought for the apparent discrepancy that rosette-forming cells decline in numbers more rapidly after adult thymectomy (14) than do Thy-1^+ cells (and more rapidly than the overall immunocompetence of the thymectomized mouse).

(*e*) Accepting that T-cell differentiation can be induced both specifically by thymosin, an exclusively thymic factor, and also nonspecifically by other agents, how can thymosin best be distinguished from these other agents in an assay in vitro? One possibility, supported by a few encouraging tests we have made, is to develop a similar assay for induction of B cells from B-cell precursors. The two assays side-by-side would exclude agents capable of inducing both types of precursors to differentiate. Further scrutiny could then be limited to agents or fractions with the specific capacity to induce differentiation of T-cell precursors exclusively.

(*f*) The fact that T-cell differentiation can be initiated by cAMP and poly A:U suggests ways of ascertaining whether agents that are not exclusive products of thymus can induce a full range of antigen receptors on T cells, and whether normal immunocompetence can be fully restored to thymus-deprived animals by such agents. This might bear on whether the thymus plays a part in generating diversity of antigen receptors on T cells, or has any sentinel role in limiting the range of T-cell antigen receptors to those that are physiologically permissible. But whether initiation of T-cell differentiation, indicated by the appearance of T-cell antigens in the in vitro assay, inevitably entails irreversible complete maturation of the precursor cell regardless of whether the inducer is thymosin or some other agent has yet to be decided.

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