Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 1862–1866, March 1986
Immunology

Early precursor thymocytes can produce interleukin 2 upon stimulation with calcium ionophore and phorbol ester

(intrathymic T-cell ontogeny/interleukin-2 gene activation/signal transduction)

JAMES P. LUGO, SANTOSH N. KRISHNAN, ROCHELLE DIAMOND SAILOR, AND ELLEN V. ROTHENBERG

Division of Biology, California Institute of Technology, Pasadena, CA 91125

Communicated by Eric H. Davidson, November 1, 1985

ABSTRACT **T-cell precursors were stimulated with a conventional T-cell mitogen or with the calcium ionophore A23187 in order to determine whether pre-T cells acquire the ability to produce interleukin 2 (IL-2) before they acquire the ability to respond to antigen or mitogenic lectins. Immature T cells were obtained by eliminating mouse thymocytes that expressed the Lyt2 and L3T4 cell surface proteins. The remaining Lyt2$^-$,L3T4$^-$ cells were stimulated for IL-2 production by using concanavalin A (Con A) or A23187, together with phorbol 12-myristate 13-acetate (PMA). We found that these "double-negative" thymocytes were unresponsive to Con A plus PMA but produced substantial amounts of IL-2 when stimulated with A23187 plus PMA. In contrast, both stimulation regimens induced more mature T-lymphocyte populations to produce IL-2. This implies that developing T cells acquire the ability to make IL-2 upon induction before they acquire the ability to be triggered by Con A. Day-15 fetal and cortical thymocytes were also tested for their ability to make IL-2. Both populations failed to synthesize this growth factor, even when stimulated with A23187 and PMA. For cortical thymocytes, this result, together with the finding that A23187 plus PMA fails to activate these cells, suggests that this population is immunologically inert rather than immature. On the other hand, the inability of day-15 fetal thymocytes to produce IL-2 indicates that these T-cell precursors are developmentally distinct from adult Lyt2$^-$,L3T4$^-$ thymocytes, which they phenotypically resemble.**

Intrathymic T-cell ontogeny is a complex process that is not well understood. During their development, immature T cells rearrange gene segments that code for the variable portion of the α, β, and γ chains of the T-cell antigen receptor (1–3), develop the capacity to transduce antigen binding into a mitogenic signal (4), and begin to express cell surface molecules that will allow them to interact with each other and with nonlymphoid components of the immune system (5). This maturation process involves the activation of genes that appear to be expressed constitutively by mature T cells and the activation of genes whose transcription is antigen-induced. Some constitutively expressed gene products are cell surface glycoproteins (for example, Lyt2, L3T4, and TL) that become permanent lineage markers (reviewed in ref. 6). Activation of inducible genes, such as those whose products help mediate immune responsiveness, is more difficult to assess, since it involves both the acquired ability of the gene to be transcribed in response to inducer and the ability of the cell to convert the external inductive stimulus into an internal one. Therefore, to determine the developmental stage at which these genes can first be transcribed, their status must be assessed as independently as possible of the membrane receptors involved with antigen recognition and of the intracellular proteins that transmit signals from these molecules to the nucleus.

One way to determine whether particular genes are inducible in immature T cells is to bypass the antigen receptor by mimicking the signals it would normally deliver. Within minutes of exposure to antigen or a lectin like Con A, T cells show two signs of stimulation: their concentration of intracellular free calcium ($[Ca^{2+}]_i$) increases, and protein kinase C is activated (7, 8). These changes appear to be involved in signal transduction, since mature T cells can also be stimulated by agents that effect these changes directly. When their $[Ca^{2+}]_i$ is raised by treatment with the calcium ionophore A23187, and when the tumor promoter phorbol 12-myristate 13-acetate (PMA) is used to activate their protein kinase C, mature T cells and cloned T-cell lines are induced to divide and to produce the T-cell growth factor interleukin 2 (IL-2) (9, 10). Because their activity is independent of the T-cell antigen receptor, these reagents may activate mature T cells and their thymic precursors equally well. For these reasons, we used stimulation with A23187 and PMA to determine whether Lyt2$^-$, L3T4$^-$ thymocytes, which include the most immature pre-T cells in the adult thymus (11, 12), are able to activate the inducible gene encoding IL-2. Here we report that these "double-negative" thymocytes produce IL-2 after stimulation with the ionophore plus PMA but fail to do so upon stimulation with Con A plus PMA. We also show that day-15 fetal thymocytes and dividing lymphoblasts of the major cortical lineage fail to produce IL-2 *in vitro* regardless of the stimulation conditions used. Therefore, the ability to secrete IL-2 is not a universal attribute of T-cell precursors.

MATERIALS AND METHODS

Animals. C57BL/6 (B6) mice were bred and maintained in the animal facility at the California Institute of Technology. Young adult mice were 4–6 weeks old. For timed pregnancies, breeding quartets were caged overnight and the females were checked for vaginal plugs the next day. The day of vaginal-plug detection was designated day 0.

Reagents. The calcium ionophore A23187 (Sigma) was stored as a 1 mg/ml stock in dimethyl sulfoxide at −20°C. The tumor promoter PMA (Sigma) was stored as a 10 μg/ml stock in 95% ethanol. Complement lysis medium consisted of a 1:30 final dilution of baby rabbit complement (Pel-Freez) and 100 μg of DNase I (Sigma) per ml in 0.25% bovine serum albumin/RPMI 1640 medium (BSA/RPMI). Bovine serum albumin stocks (30%, wt/vol) were purchased from Irvine Scientific.

Monoclonal Antibodies (mAbs) and Flow Microfluorometry. AD4.15 is a mouse IgM mAb specific for Lyt2.2 (13). Ascites from the corresponding hybridoma was produced in (B6 × BALB/c)F_1 females. GK1.5, a rat IgG mAb specific for the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: $[Ca^{2+}]_i$, intracellular calcium; IL-2, interleukin 2; mAb, monoclonal antibody; PnA, peanut agglutinin; PMA, phorbol 12-myristate 13-acetate.

L3T4 molecule (14), and Mar 18.5, a mouse IgG2a mAb specific for rat κ chains (15), were used as culture supernatants. The latter hybridoma was generously provided by K. Wall (University of Texas Health Science Center, San Antonio, TX). Monoclonal anti-Lyt1.2 (CG-16) and anti-Thy-1.2 (13-4) ascites were purchased from Accurate Chemical, (Westbury, NY) and New England Nuclear, respectively. The rat IgM mAb 7D4, which detects the mouse IL-2 receptor (16), was used as a culture supernatant. Flow microfluorometry was done as described (17).

Preparation of Thymocyte Subpopulations. Double-negative thymocytes were obtained by sequentially eliminating $Lyt2^+$ and $L3T4^+$ cells. Unfractionated thymocytes (5×10^7 cells per ml) were incubated for 45 min on ice in BSA/RPMI containing AD4.15 ascitic fluid (1:100 final dilution), washed, and then resuspended in complement lysis medium to $3–5 \times 10^7$ cells per ml. After 45 min of incubation at 37°C, viable cells (≈15% initial input) were separated from dead cells by flotation centrifugation (15 min; 1500 × *g*) through a 15/30% discontinuous bovine serum albumin gradient at 4°C. Sixty-five to seventy-five percent of the live cells were recovered, with viabilities >98%. These $Lyt2^-$ cells were then incubated for 45 min on ice in undiluted GK1.5 culture supernatant, then washed into Mar18.5 culture supernatant ($3–5 \times 10^7$ cells per ml) and placed on ice for 20 min, prior to treatment with complement. Eighty-five to ninety percent of the viable cells (≈21% initial input) were recovered after flotation centrifugation. Further treatment of these cells to remove Lyl^+ or IL-2 receptor-positive cells was done as described for the AD4.15 elimination step, using CG-16 ascites at 1:100 dilution and 7D4 as an undiluted culture supernatant. The fractionation of thymocytes according to both size and peanut agglutinin (PnA)-binding ability has been described (17, 18). PnA^+ blasts here represent the 50% of cells in the cycling population that bound most tightly to PnA-coated plates.

Production and Assay of IL-2. IL-2 induction cultures consisted of $3–5 \times 10^6$ cells per ml in RPMI 1640 supplemented with 10% fetal bovine serum, nonessential amino acids, 1 mM sodium pyruvate, 50 μM 2-mercaptoethanol, 2 mM L-glutamine, penicillin (100 units/ml), and streptomycin (100 μg/ml). Cells were stimulated with Con A or A23187, with or without PMA (17 nM), as indicated. Cultures were harvested after 20–24 hr of stimulation unless otherwise stated. IL-2 assays were performed as described, using the cloned T-cell line MTL 2.8.2 (18). IL-2 activity units are the reciprocal of the dilution that yielded 30% maximal incorporation of 5-[^{125}I]iododeoxyuridine.

RESULTS

Double-Negative Thymocytes Produce IL-2. Thymic lymphocytes are divisible into four populations based on their Lyt2 and L3T4 phenotypes (11, 12). One of these populations, the $Lyt2^-$,$L3T4^-$ (double-negative) thymocytes, contains within it cells capable of repopulating irradiated thymus glands and generating all the other T-cell populations normally found in this organ (11). To examine IL-2 expression during early T-cell ontogeny, we isolated these precursors by eliminating all $Lyt2^+$ and $L3T4^+$ thymocytes by lysis with antibodies and complement. Flow cytometry was used to assess how well our elimination procedure worked. As shown in Fig. 1, we could not detect any $Lyt2^+$ or $L3T4^+$ cells over background staining. Most (85–90%) double-negative thymocytes were lymphoid, because they stained as brightly for Thy-1 as did unfractionated thymocytes (Fig. 1 *A* and *E*). Unlike thymocytes overall, however, a large fraction (50–60%) of double-negative thymocytes stained with mAb 7D4 (Fig. 1 *D* and *H*), suggesting that these cells express receptors for IL-2. This finding was consistent with reports from other groups (19–21).

As shown in Table 1, double-negative cells produced significant amounts of IL-2 in response to A23187 plus PMA but did not do so when stimulated with Con A plus PMA. The failure of Con A plus PMA to elicit IL-2 from these cells was not due to suboptimal stimulation conditions. Various concentrations of Con A, from 0.75 to 24 μg/ml, failed to induce these cells to make IL-2 (Fig. 2*D* and data not shown), nor did decreasing or increasing the time of exposure to Con A result in IL-2 synthesis (Fig. 3*A*). Moreover, the similar IL-2 production profiles of spleen cells, unfractionated thymocytes, and double-negative pre-T cells measured in response to various doses of A23187 suggested that the IL-2 producers in each of these cell populations have similar activation requirements (Fig. 2 *A*, *B*, and *D*). Finally, the inability of Con A to elicit IL-2 from double-negative thymocytes was not due to a lack of expression of Con A binding sites by the cells: double-negative cells and spleen cells were agglutinated equally well by Con A (data not shown). However, it is possible that double-negative thymocytes do not yet express

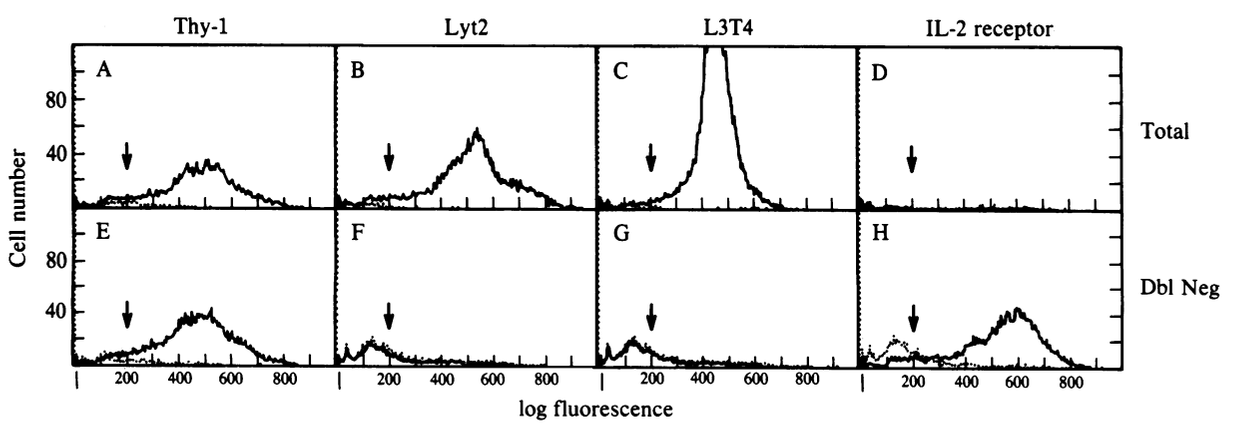

FIG. 1. Flow cytometric analysis of unfractionated and double-negative (Dbl Neg) thymocytes. Unfractionated (A–D) and double negative (E–H) thymocytes were incubated with saturating amounts of mAbs specific for the indicated cell surface proteins and then were stained with fluorescein isothiocyanate-conjugated goat anti-rat or anti-mouse IgG (—). In each case, the negative control was cells stained with the second-stage reagent alone (···). Each histogram represents the signals from 20,000 cells, gated to exclude aggregates and debris by the correlated intensity of forward light scatter (not shown). The arrow in each panel indicates the threshold of positive staining (≈5% background "positive" cells) with second antibody. By this criterion, 50% of double-negative thymocytes stained with anti-IL-2-receptor mAb, whereas only 2–3% stained with anti-L3T4 or anti-Lyt2 mAb.

Table 1. IL-2 production by various spleen and thymocyte populations

Stimulation	IL-2 activity, units/ml				
	Spleen		Thymocytes‡		
	Total*	T-depleted†	Unfrac	Dbl Neg	D15
Con A	35 ± 15	<1	<1	<1	<1
Con A + PMA	218 ± 18	1	18 ± 2	<1	<1
A23187	<1	<1	<1	<1	<1
A23187 + PMA	702 ± 50	5	80 ± 3	103 ± 43	<1

Spleen cells and unfractionated thymoctyes were stimulated at 5 × 10^6 cells per ml; double-negative (Dbl Neg) and day-15 (D15) fetal thymocytes, at 3 × 10^6 cells/ml. Results are not normalized for cell number. Final concentrations for the various activating agents: Con A, 6 μg/ml; A23187, 0.48 μM; PMA, 17 nM.
*Mean ± SEM of two experiments.
†T cells were removed by a one-step complement procedure using the mAbs 13-4, GK1.5, and AD4.15. Results from one experiment.
‡Mean ± SEM of three experiments.

those Con A-binding molecules that can transduce lectin binding into an inductive stimulus.

Since ≈50% of the double-negative cells express IL-2 receptors (Fig. 1*H*), adsorption of IL-2 to receptor-bearing cells could have interfered with our measurements of IL-2 production, especially if Con A were to augment IL-2 receptor expression. To investigate this possibility, IL2-receptor-bearing cells were eliminated from double-negative thymocyte preparations, and the remaining cells were tested for IL-2 synthesis. The data presented in Table 2 show that double-negative cells that lack IL-2 receptors can produce IL-2, but only in response to ionophore and phorbol ester. Since these cells do not appear to be enriched for IL-2 producers with respect to double-negative thymocytes over-

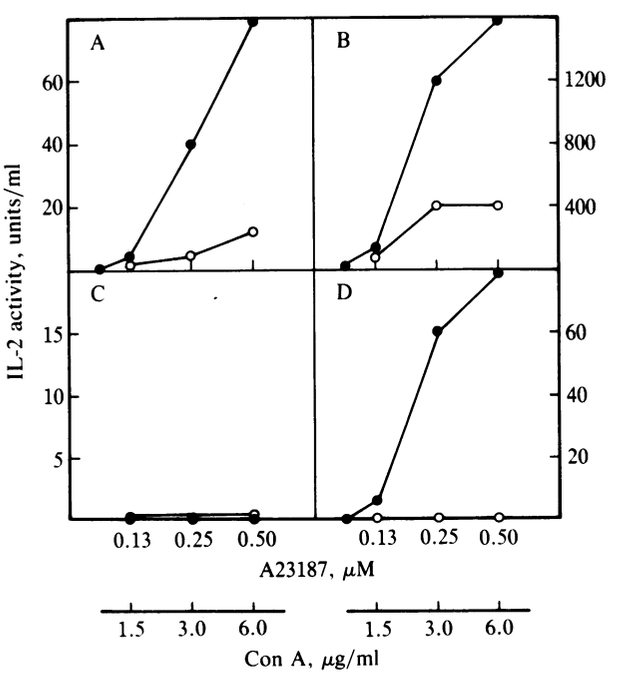

FIG. 2. IL-2 production by various T-cell populations in response to A23187 and Con A. Unfractionated thymocytes (*A*) and spleen cells (*B*) were cultured at 5 × 10^6 cells per ml in IL-2-induction medium containing the indicated amounts of A23187 (●) or Con A (○) plus 17 nM PMA. After a 24-hr incubation period, culture supernatants were harvested and assayed for IL-2 as described in *Materials and Methods*. Day-15 fetal (*C*) and adult double-negative (*D*) thymocytes were stimulated at 3 × 10^6 cells per ml.

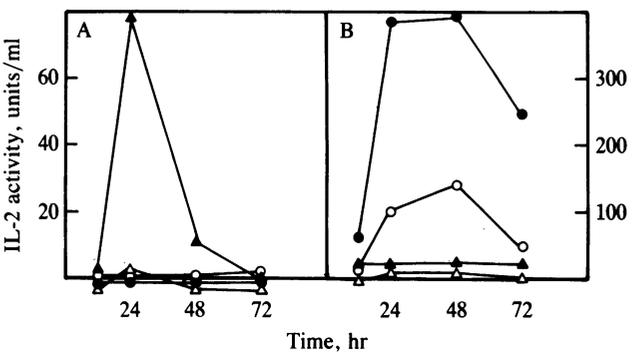

FIG. 3. Kinetic analysis of IL-2 synthesis by various thymocyte populations. Cells were stimulated with A23187 (0.48 μM, solid symbols) or Con A (6 μg/ml, open symbols) along with 17 nM PMA for the indicated periods of time. Supernatants were then harvested and assayed for IL-2. (*A*) Day-15 fetal (○, ●) and adult double-negative (△, ▲) thymocytes. (*B*) PnA^- (○, ●) and PnA^+ (△, ▲) thymic lymphoblasts.

all, it is likely that IL-2 producers may also be found in the IL-2-receptor-bearing class of double-negative cells.

In contrast to double-negative cells, unfractionated thymic lymphocytes and spleen cells produced readily detectable amounts of IL-2 when stimulated by Con A or A23187 plus PMA (Table 1 and Fig. 2 *A* and *B*). The lower titers of IL-2 secreted by thymocytes reflect the lower frequency of IL-2 producers in the thymus (refs. 4, 18, and 22; also see below). For each of these cell populations, A23187 plus PMA generally elicited 3- to 4-fold more IL-2 than did Con A plus PMA. This ratio appeared characteristic of mature T cells and at no point exceeded 8-fold (see Table 3). The basis for this effect is not known; however, it may in part be due to A23187 mediating a greater and perhaps longer-lasting increase in $[Ca^{2+}]_i$ than the transient increase elicited by Con A (23). Neither A23187 nor PMA alone effectively induced IL-2 biosynthesis in any population tested here (Table 1 and data not shown). Con A alone, however, induced spleen cells to make small amounts of IL-2, most likely because contaminating splenic accessory cells provide a "second signal" that facilitates IL-2 production (24).

A23187 and PMA do not induce IL-2 biosynthesis from all lymphocytes. T-cell-depleted splenic lymphocytes failed to make IL-2 under any stimulation conditions (Table 1). Further, of several T-cell hybridomas and cloned T-cell lines tested for IL-2 synthesis, only those previously shown to produce IL-2 in response to the appropriate antigen or Con A made IL-2 after treatment with A23187 plus PMA (data not shown). Thus, adult double-negative thymocytes represent a

Table 2. IL-2-receptor-negative and "Lyl-low" double-negative thymocytes produce IL-2

Treatment*	IL-2 activity, units/ml			
	Con A		A23187	
	−PMA	+PMA	−PMA	+PMA
None	<1	<1	<1	110 ± 15
Complement	<1	<1	<1	140 ± 20
Anti-IL-2 receptor plus complement	<1	<1	<1	70 ± 10
Anti-Lyl plus complement	<1	<1	<1	60 ± 20

Stimulations were carried out at 3 × 10^6 viable cells per ml, using the tissue culture conditions described for Table 1. Results represent mean ± SEM of three experiments.
*Fifty percent of the cells were killed with monoclonal anti-IL-2 receptor antibody (7D4) plus complement and 30% with monoclonal anti-Lyl (CG-16) plus complement.

developing pre-T-cell population that contains committed IL-2 producers that are not yet Con A-responsive.

Fetal Double-Negative Thymocytes Do Not Produce IL-2. Recent studies have shown that day-15 fetal and adult double-negative thymocytes resemble each other in several ways. Both populations are enriched for precursors that can give rise to all the other T-cell populations in the thymus (11, 25, 26). In addition, both types of cells lack detectable expression of Lyt2 or L3T4 and contain mRNA for the γ and β chains of the T-cell antigen receptor but few or no α-chain transcripts (2, 27–29). Further, 50–60% of the cells in each of these populations express IL-2 receptors (refs. 19–21 and unpublished data). These similarities prompted us to test day-15 fetal thymocytes for IL-2 production. As shown in Table 1 and Fig. 2*C*, these cells do not produce IL-2 under any of our *in vitro* stimulation conditions, including stimulation for up to 3 days with A23187 and PMA (Fig. 3*A*), even though the fetal thymocytes remain viable and lymphoblastoid throughout the culture period. The inability to make IL-2 distinguishes these cells not only from adult double-negative cells overall, but even from the adult subpopulation enriched for precursors that resemble fetal thymocytes by expressing low levels of Ly1 (11). As shown in Table 2, adult double-negative populations depleted of "Ly1-high" cells still produced readily detectable amounts of IL-2. These results demonstrate that day-15 fetal and "Ly1-low" adult double-negative thymocytes are not identical sets of cells.

Only PnA$^-$ Thymic Lymphoblasts Produce IL-2 in Response to A23187 and PMA. T-cell precursors differentiate along at least two developmental pathways. One lineage leads to the appearance of small, PnA-binding (PnA$^+$) cortical thymocytes. These cells die intrathymically and exhibit little or no responsiveness to either Con A or alloantigens (22, 30). The other lineage is characterized by the formation of immunocompetent T lymphocytes that bind PnA weakly (PnA$^-$). To determine whether lymphoblasts committed to the cortical lineage (large PnA$^+$ cells) or their nondividing offspring (small PnA$^+$ cells) make IL-2 in response to A23187 and PMA, thymocytes were fractionated according to their size and their PnA-binding ability and then were stimulated with these activators. The results are shown in Table 3. In agreement with previous results (18), PnA$^-$ thymic lymphoblasts produced IL-2 whether stimulated with Con A or A23187 and PMA. However, neither lymphoblasts nor postmitotic cells of the PnA$^+$ class could be induced to make IL-2. The small amount of IL-2 that dividing PnA$^+$ cells produced in some experiments is most likely due to PnA$^-$ cell contamination. Varying the incubation periods or concentrations of A23187 and Con A did not lead to higher IL-2 outputs from PnA$^+$ cells, when compared with their PnA$^-$ counterparts (Fig. 3*B* and Fig. 4 *A* and *B*). Thus, cortical thymocytes, including proliferating cortical blasts, do not appear to be inducible for IL-2 expression. This characteristic sets these cells apart both from PnA$^-$ "medullary" cells and from

Table 3. IL-2 production by fractionated thymocytes

	IL-2 activity, units/ml			
	Con A		A23187	
Fraction	−PMA	+PMA	−PMA	+PMA
Small cells				
PnA$^-$	<1	10 ± 3	<1	75 ± 9
PnA$^+$	<1	<1	<1	<1
Large cells				
PnA$^-$	12 ± 10	140 ± 20	<1	520 ± 120
PnA$^+$	<1	6 ± 5	<1	30 ± 28

Stimulations were carried out at 5 × 10^6 cells per ml, using the tissue culture conditions described for Table 1. Results represent the mean ± SEM of two (small cells) or three (large cells) experiments.

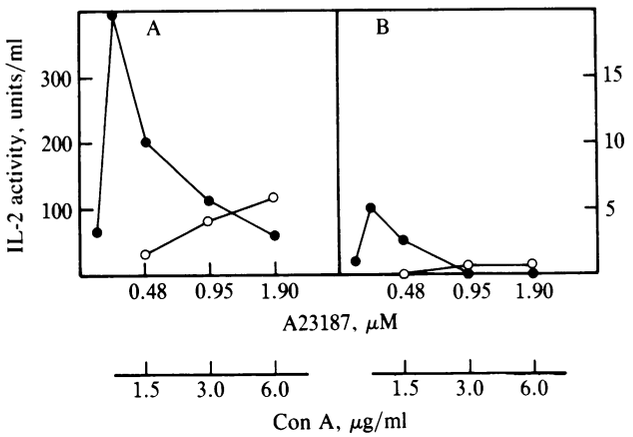

FIG. 4. IL-2 production by PnA$^-$ and PnA$^+$ thymic lymphoblasts in response to various concentrations of A23187 and Con A. PnA$^-$ (*A*) and PnA$^+$ (*B*) large thymocytes were prepared by centrifugal elutriation followed by PnA fractionation using PnA-coated dishes (17, 18). Stimulations were done at 5 × 10^6 cells per ml in IL-2-induction medium containing the indicated amounts of A23187 (●) or Con A (○) plus 17 nM PMA. After a 24-hr incubation period, culture supernatants were harvested and assayed for IL-2.

double-negative thymocytes, some of which can differentiate in culture into cortical cells (25, 26). Therefore, cortical (PnA$^+$) cells overall seem not so much to be "immature" as to be the mature products of a developmental process that leads to death instead of clonal expansion. In accord with this possibility, we have found (unpublished data) that PnA$^+$ blast cells also fail to proliferate in response to IL-2 when treated with A23187 and PMA, in contrast to double-negative thymocytes or mature T cells.

DISCUSSION

The results presented here demonstrate that production of IL-2 is inducible in immature thymocytes that are not yet Con A-responsive; i.e., that responses associated with mature T cells can be elicited from phenotypically immature precursor cells. The double-negative population studied here includes few if any cells that express the mature T-cell receptor for antigen, since this population is strikingly deficient in mRNA for the α chain of the antigen-binding receptor (2, 27). Any antigen-binding structures that exist on these cells could be comprised of γ and β chains, rather than the $\alpha\beta$ heterodimers that are documented to bind antigen on mature T cells (31, 32). With mature T cells, Con A may act as a proxy for antigen by binding to the T-cell antigen receptor complex. Engagement of this complex triggers an increase in $[Ca^{2+}]_i$, which is a major controlling factor for IL-2 secretion (33). The lack of α chains in these cells and the probable lack of glycosylation of γ chains (34, 35) may account for the inability of Con A to stimulate double-negative thymocytes. Nevertheless, cells in the double-negative population are clearly competent to secrete IL-2, provided that the increase in $[Ca^{2+}]_i$ which activates the IL-2 gene is induced by a calcium ionophore. Thus, stimulation with Con A fails to reveal the degree to which these early precursors may already be programmed for function.

Inducibility of the IL-2 gene appears to mark an intermediate stage, perhaps restricted to a specific lineage, in the differentiation of immature thymocytes. Day-15 fetal thymocytes resemble adult double-negative cells in many ways, lacking Lyt2 and L3T4, but expressing Thy-1, IL-2 receptors, and mRNA for the γ and β chains of the T-cell receptor for antigen (20, 27–29). However, they do not make IL-2, even when stimulated with A23187 and PMA. Yet, by day 16, an IL-2 response is clearly detectable (data not

shown). These bulk-population data suggest that IL-2 inducibility begins after rearrangement of the γ- and β-chain genes, but before the α-chain gene is expressed. Thus, the adult double-negative cells may include an additional subpopulation, more mature than day-15-type double-negative cells. To identify the precise stage at which the IL-2 gene becomes inducible, however, it will be necessary to make a critical assessment of the heterogeneity in both the adult and fetal double-negative populations. It is not yet certain whether the IL-2-secreting cells represent the majority of adult double-negative thymocytes or an atypical minority.

Although the lack of IL-2 production by fetal thymocytes could be attributed to immaturity, we have found evidence that thymocytes of the major cortical lineage may also be blocked from carrying out this response. Both cortical lymphoblasts and postmitotic cells fail to make IL-2 even when stimulated with A23187 and PMA, suggesting a lesion in some intracellular component of this inductive response pathway. This explains the failure of cortical (PnA^+) cells to be activated by Con A (18, 30) in spite of their expression of apparently "mature" T-cell receptors for antigen (36). These cells may develop from a lineage of double-negative cells that never acquire the ability to make IL-2 or that lose this ability with further differentiation.

We thank Katherine Wall for Mar18.5 antibody, Patrick Koen for invaluable help with the flow cytometry, and Eric Davidson and Lee Hood for thoughtful criticism of the manuscript. The work was supported by Public Health Service Grants AI19752 and CA39605 to E.R. and CA32911 to the Caltech Cancer Center. J.P.L. is a Fellow of the Jane Coffin Childs Memorial Fund for Medical Research. S.N.K. was supported in part by a Summer Undergraduate Research Fund at Caltech.

1. Born, W., Yagüe, J., Palmer, E., Kappler, J. & Marrack, P. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 2925–2929.
2. Samelson, L. E., Lindsten, T., Fowlkes, B. J., van den Elsen, P., Terhorst, C., Davis, M. M., Germain, R. N. & Schwartz, R. H. (1985) *Nature (London)* **315,** 765–768.
3. Trowbridge, I. S., Lesley, J., Trotter, J. & Hyman, R. (1985) *Nature (London)* **315,** 666–669.
4. Ceredig, Rh., Dialynas, D. P., Fitch, F. W. & MacDonald, H. R. (1983) *J. Exp. Med.* **158,** 1654–1671.
5. Reichert, R. A., Gallatin, W. M., Butcher, E. C. & Weissman, I. L. (1984) *Cell* **38,** 89–99.
6. Rothenberg, E. & Lugo, J. P. (1985) *Dev. Biol.* **112,** 1–17.
7. Imboden, J. B. & Stobo, J. D. (1985) *J. Exp. Med.* **161,** 446–456.
8. Weiss, A., Imboden, J., Shoback, D. & Stobo, J. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4169–4173.
9. Truneh, A., Albert, F., Golstein, P. & Schmitt-Verhulst, A.-M. (1985) *Nature (London)* **313,** 318–320.
10. Albert, F., Hua, C., Truneh, A., Pierres, M. & Schmitt-Verhulst, A.-M. (1985) *J. Immunol.* **134,** 3649–3655.
11. Mathieson, B. J. & Fowlkes, B. J. (1984) *Immunol. Rev.* **82,** 141–173.
12. Scollay, R. & Shortman, K. (1985) *J. Immunol.* **134,** 3632–3642.
13. Gottlieb, P. D., Marshak-Rothstein, A., Auditore-Hargreaves, K., Berkoben, D. B., August, D. A., Rosche, R. M. & Benedetto, J. D. (1980) *Immunogenetics* **10,** 545–555.
14. Dialynas, D. P., Quan, Z. S., Wall, K. A., Pierres, A., Quintas, J., Loken, M. R., Pierres, M. & Fitch, F. W. (1983) *J. Immunol.* **131,** 2445–2451.
15. Lanier, L. L., Gutman, G. A., Lewis, D. E., Griswold, S. T. & Warner, N. L. (1982) *Hybridoma* **1,** 125–131.
16. Ortega, R. G., Robb, R. J., Shevach, E. M. & Malek, T. R. (1984) *J. Immunol.* **133,** 1970–1976.
17. Lugo, J. P., Krishnan, S. N., Sailor, R. D., Koen, P., Malek, T. & Rothenberg, E. (1985) *J. Exp. Med.* **161,** 1048–1062.
18. Caplan, B. & Rothenberg, E. (1984) *J. Immunol.* **133,** 1983–1991.
19. Raulet, D. H. (1985) *Nature (London)* **314,** 101–103.
20. Ceredig, Rh., Lowenthal, J. W., Nabholz, M. & MacDonald, H. R. (1985) *Nature (London)* **314,** 98–100.
21. von Boehmer, H., Cristani, A., Kisielow, P. & Haas, W. (1985) *Nature (London)* **314,** 539–540.
22. Ceredig, Rh., Glasebrook, A. L. & MacDonald, H. R. (1982) *J. Exp. Med.* **155,** 358–379.
23. Tsien, R. Y., Pozzan, T. & Rink, T. J. (1982) *Nature (London)* **295,** 68–70.
24. Gillis, S. & Mizel, S. B. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 1133–1137.
25. Ceredig, Rh., Sekaly, R. P. & MacDonald, H. R. (1983) *Nature (London)* **303,** 248–250.
26. Fowlkes, B. J., Edison, L., Mathieson, B. & Chused, T. M. (1984) in *Regulation of the Immune System: UCLA Symposia on Molecular and Cellular Biology*, New Series, eds. Sercarz, E., Cantor, H. & Chess, L. (Liss, New York), Vol. 18, pp. 275–293.
27. Raulet, D. H., Garman, R. D., Saito, H. & Tonegawa, S. (1985) *Nature (London)* **314,** 103–107.
28. Snodgrass, H. R., Dembíc, Z., Steinmetz, M. & von Boehmer, H. (1985) *Nature (London)* **315,** 232–233.
29. Snodgrass, H. R., Kisielow, P., Kiefer, M., Steinmetz, M. & von Boehmer, H. (1985) *Nature (London)* **313,** 592–595.
30. Chen, W.-F., Scollay, R. & Shortman, K. (1982) *J. Immunol.* **129,** 18–24.
31. Kappler, J., Kubo, R., Haskins, K., Hannum, C., Marrack, P., Pigeon, M., McIntyre, B., Allison, J. & Trowbridge, I. (1983) *Cell* **35,** 295–302.
32. Meuer, S. C., Fitzgerald, K. A., Hussey, R. E., Hodgdon, J. C., Schlossman, S. F. & Reinherz, E. L. (1983) *J. Exp. Med.* **157,** 705–719.
33. Mills, G. B., Cheung, R. K., Grinstein, S. & Gelfand, E. W. (1985) *J. Immunol.* **134,** 1640–1643.
34. Saito, H., Kranz, D. M., Takagaki, Y., Hayday, A., Eisen, H. N. & Tonegawa, S. (1984) *Nature (London)* **309,** 757–762.
35. Hayday, A. C., Saito, H., Gillis, S. D., Kranz, D. M., Tanigawa, G., Eisen, H. N. & Tonegawa, S. (1985) *Cell* **40,** 259–269.
36. Roehm, N., Herron, L., Cambier, J., DiGuisto, D., Haskins, K., Kappler, J. & Marrack, P. (1984) *Cell* **38,** 577–584.