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Immunology

Interleukin 4 is a growth factor for activated thymocytes: Possible role in T-cell ontogeny

(interleukin 2/cell-surface markers/lymphokine production)

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ABSTRACT **We have shown that recombinant or natural interleukin 4 (IL-4) (formerly called B-cell stimulatory factor 1) induces proliferation of activated adult or fetal thymocytes. In the case of adult thymocytes, IL-4 in combination with Con A or phorbol 12-myristate 13-acetate (PMA) stimulated the proliferation of peanut agglutinin (PNA)-negative (−) thymocytes, while PNA-positive (+) thymocytes showed only marginal responses. Further investigation revealed that day 14–17 fetal thymocytes, purified L3T4− LyT2− double-negative adult thymocytes, and single positive L3T4+ LyT2− or L3T4− LyT2+ thymocytes failed to respond to IL-4 or PMA alone but proliferated strongly with both IL-4 and PMA. In contrast, purified double-positive L3T4+ LyT2+ adult thymocytes showed only a marginal proliferative response to these stimuli. Responsiveness of thymic subpopulations to PMA and IL-4 could be inhibited with anti-IL-4 but not with anti-IL-2 monoclonal antibodies, indicating that they were IL-2 independent. Finally, we have observed that supernatants from calcium ionophore and PMA-stimulated adult double-negative L3T4− LyT2− thymocytes induce proliferation of double-negative adult thymocytes. This latter response is inhibited by anti-IL-4 monoclonal antibodies, suggesting that under appropriate stimulation conditions, these immature thymocytes are able to produce IL-4. These observations suggest a role for IL-4 in T-cell ontogeny.**

T-cell ontogeny is an important and complex issue that has received considerable attention in recent years. A central issue is the identification of the signals and growth factors that mediate proliferation and differentiation of immature T cells in the thymus (1, 2). Two main cell types in the thymic microenvironment are most likely to produce and/or deliver these signals: the thymic stromal cells and the immature thymocytes themselves. Workers in several laboratories have investigated the role of interleukin 2 (IL-2), a known growth factor for mature T cells, in early thymic development. Recent evidence indicates that IL-2 can be produced by thymocytes after appropriate stimulation (3, 4). However, the potential role of IL-2 in T-cell ontogeny remains controversial, and several reports have concluded that IL-2, by itself, is unlikely to be responsible for the high proliferative rate of thymocytes (2, 5). In the case of thymic stromal cells, some reports have identified soluble factors produced by these cells that are able to maintain the viability of immature thymocytes or induce proliferation and differentiation (6).

We have studied the role of the recently cloned lymphokine interleukin 4 (IL-4) (7, 8) (formerly called B-cell stimulatory factor 1*) in thymic development. Initially identified as a costimulator of B-cell proliferation (9), IL-4 has since been shown to stimulate mature T-cell proliferation (10, 11). Our data indicate that IL-4 is produced by activated immature thymocytes and is a potent costimulator of proliferation for some populations of both mature and immature thymocytes.

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MATERIALS AND METHODS

Mice. Three-week-old female BALB/c mice were used (The Jackson Laboratory). Timed BALB/c pregnant mice were also obtained from The Jackson Laboratory and were sacrificed at different time intervals to obtain fetal thymuses of the appropriate gestational age.

Antibodies. The monoclonal antibody 11B11, originally produced by Ohara and Paul (12) and specific for murine IL-4, was used as supernatant (SN) from antibody-producing cultures at a final concentration of 25%. This concentration totally neutralized the amounts of IL-4 used throughout this study. The monoclonal antibody S4B6, specific for murine IL-2, was a kind gift of Tim Mosmann (DNAX) and was used as ascites at a final dilution of 1:200. At this concentration, it completely neutralized 1000 IL-2 units as defined below.

Other Reagents. Con A was obtained from Sigma. The calcium ionophore A23187 and phorbol 12-myristate 13-acetate (PMA) were obtained from Calbiochem.

Culture Medium. The tissue culture medium used for these studies was RPMI 1640 medium (GIBCO) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT), penicillin, streptomycin, and 2-mercaptoethanol (5 μM). This medium is referred to as culture medium (CM).

Lymphokines. Con A-induced SN from the T-cell hybridoma D9C1.12.17 (13) were used. These SN were produced by incubating 1×10^6 cells with Con A (6 μg/ml) in RPMI 1640 medium supplemented with 1% fetal calf serum for 24 hr. These SN contain IL-2 and IL-4 but lack interferon-γ when screened by standard assays for these lymphokines (10, 14) (H. Hagiwara and A.Z., unpublished data). Recombinant murine IL-2 produced at our institution (15) and purified to homogeneity by Schering was diluted in CM (see below, *Proliferation Assays*) and added as indicated. Recombinant murine IL-4 was used as a single preparation of SN from IL-4 cDNA-transfected COS-7 monkey kidney cells containing 5120 units/ml. Mock-transfected COS-7 SN were used as negative controls and in all cases failed to produce a detectable response either alone or in combination with PMA. The bioassays used to quantitate these lymphokines using the lymphokine-dependent cell lines HT-2 and MC-9 have been described in detail (10, 14). A unit of lymphokine activity is defined as the reciprocal of the dilution producing a half-maximal response.

Abbreviations: IL-2, interleukin 2; IL-4, interleukin 4; PMA, phorbol 12-myristate 13-acetate; PNA, peanut agglutinin lectin; SN, supernatant(s); FACS, fluorescence-activated cell sorter.

*The name interleukin 4 was recently recommended for this lymphokine (formerly called B-cell stimulatory factor 1) by an ad hoc subcommittee of the International Union of Immunological Societies (IUIS)/World Health Organization (WHO).

Isolation of Peanut Agglutinin-Negative (PNA−) and PNA+ Thymocyte Subpopulations. After dissection, a single cell suspension was obtained by gently massaging the tissue between two microscope slides. The cells were assessed for viability, counted, and washed. The erythrocytes were lysed by hypotonic shock. In some experiments, thymocytes were fractionated on the basis of their binding to PNA (Sigma) as described (16). Briefly, cells were agglutinated with PNA (20 μg/ml) in balanced salt solution for 30 min at 37°C. The cells (two thymus cell equivalents per gradient) were overlayed on fetal bovine serum gradients (100%, 50%, 25%, and 10%) in a total vol of 10 ml and were allowed to sediment at room temperature at 1 × *g* for 30 min. After this time, clumped cells forming a pellet in the bottom of the tube were considered to be PNA+, and nonpelleted cells at the top 1.5 ml of the gradient were considered to be PNA−. In our experience, this procedure routinely yielded a population of PNA+ cells representing 10–20% of the initial thymocyte population. By fluorescence-activated cell sorter (FACS) analysis, PNA+ cells were greatly enriched for L3T4+ LyT2+ double-positive thymocytes (77%), but contained a small proportion of L3T4+ LyT2− (15%) and L3T4− LyT2+ (7%) cells. After separation, the cells were incubated for 10 min at 37°C in CM containing D-galactose (10 mg/ml) to neutralize the PNA, then washed twice in CM alone to remove the galactose.

Preparation of L3T4− LyT2− Double-Negative Adult Thymocytes. Several adult thymuses were dissected and a single cell suspension was prepared. Cells were then treated with the rat anti-L3T4 GK1.5 monoclonal antibody (kindly provided by Philippa Marrack; National Jewish Center for Immunology, Denver, CO) and mouse anti-LyT2.2 (Cedarlane Laboratories, Hornby, ON) monoclonal antibody at 4°C followed by treatment with low-toxicity rabbit complement (Cedarlane) at 37°C for 30 min. The cells were washed and then treated a second time with the same monoclonal antibodies and complement. This negative-selection procedure yielded a population of L3T4− LyT2− thymocytes representing ≈2% of the starting thymocyte population. Two-color analysis and analysis using fluorescein isothiocyanate (FITC)-conjugated rabbit anti-rat immunoglobulin (kindly provided by Robert Coffman; DNAX) or goat anti-mouse immunoglobulin (Becton Dickinson) indicated that these cells were >99% pure L3T4− LyT2−. In some experiments, L3T4− LyT2− double-negative, L3T4+ LyT2+ double-positive and single-positive L3T4+ LyT2− or L3T4− LyT2+ thymocytes were positively selected using two-color FACS.

FACS Analysis. Cells were stained with FITC-conjugated rat anti-mouse LyT-2.2 and phycoerythrin-conjugated rat anti-mouse L3T4 antibodies (Becton Dickinson) and were analyzed or sorted by double-color flow cytometry using a FACS IV (Becton Dickinson). Dead cells were eliminated by forward low-angle scatter.

Proliferation Assays. Unfractionated or fractionated thymocytes (1 × 10^5) were cultured in 100 μl of CM in 96-well plates. Two different methods were used to evaluate proliferation. Experiments were pulsed with [^{3}H]thymidine (1 μCi per well; 1 Ci = 37 GBq) after 48 hr of incubation and were harvested 16 hr later. Results shown are the mean cpm of duplicate samples. Alternatively, proliferation was assessed using the MTT-tetrazolium metabolic rate assay described by Mosmann (17). This assay is a reflection of the viability as well as the metabolic activity of the cells assayed. Therefore, it is positive when cells replicate or when they undergo metabolic activation.

Production of Supernatants from Double-Negative Thymocytes. Adult double-negative thymocytes were prepared as described above using negative selection (cytotoxic antibodies and complement). Approximately 10^5 cells per ml were incubated with 0.4 μM calcium ionophore A23187 (Behring, Somerville, NJ) and PMA (10 ng/ml) for 24 hr. At this time, the supernatants were collected, filtered, and stored at −70°C until use in the proliferation assays.

RESULTS

PNA− Thymocytes Proliferate in Response to Con A-Induced D9C1.12.17 SN. To explore the role of T-cell-derived lymphokines in T-cell proliferation, we initially investigated the response of thymocyte subpopulations to Con A-induced

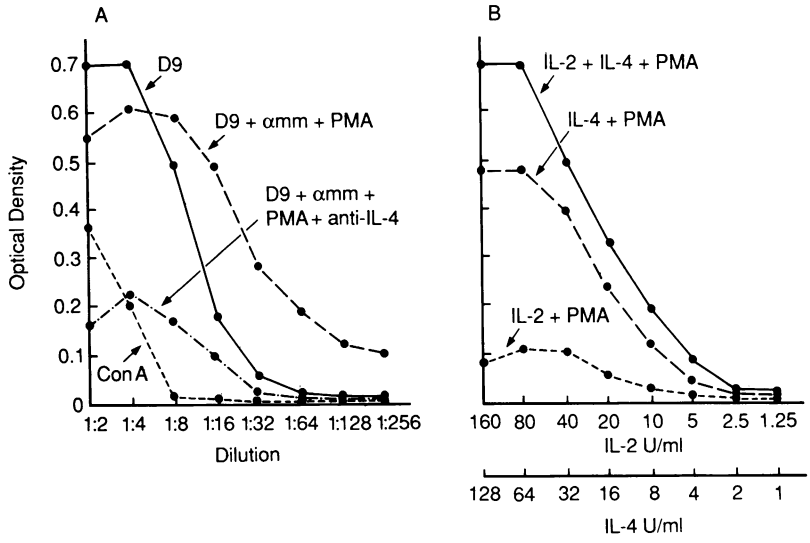

FIG. 1. Proliferation of PNA− thymocytes in response to IL-2 or IL-4 and Con A or PMA. The proliferative response was measured using the MTT colorimetric assay (see text). (*A*) Response to Con A-stimulated SN from the T-cell hybridoma D9C1.12.17 (D9), response to CM containing Con A (10 μg/ml), response to Con A-SN from D9C1.12.17 with methyl α-D-mannoside (αmm) (to neutralize the residual Con A present in the SN) and PMA (D9 + methyl α-D-mannoside + PMA), response to Con A SN from D9C1.12.17 SN with PMA and anti-IL-4 monoclonal antibody (used as culture SN at a final concentration of 25%) (D9 + methyl α-D-mannoside + PMA + anti-IL-4). No detectable response was observed to Con A-SN from D9C1.12.17 and methyl α-D-mannoside or to PMA alone (50–0.1 ng/ml). (*B*) Response to IL-2, IL-4, and PMA (IL-2 + IL-4 + PMA), response to IL-4 and PMA (IL-4 + PMA), response to IL-2 and PMA (IL-2 + PMA). No detectable response was observed to IL-4 or IL-2 alone or to mock-transfected COS-7 SN (titrated at the corresponding concentrations of IL-4 SN). PMA was used at a constant concentration of 10 ng/ml and methyl α-D-mannoside was used at 10 mg/ml.

Table 1. PNA− thymocytes proliferate to IL-4 and PMA

Cells	Incubated with	cpm*
PNA−	PMA†	781
PNA−	IL-4‡	588
PNA−	IL-4 + PMA	15,790
PNA−	Mock§ + PMA	717
PNA−	IL-4 + PMA + anti-IL-4¶	683
PNA−	IL-4 + PMA + anti-IL-2‖	18,218
PNA+	PMA	446
PNA+	IL-4	344
PNA+	IL-4 + PMA	2,156
PNA+	Mock§ + PMA	436
PNA+	IL-4 + PMA + anti-IL-4	482
PNA+	IL-4 + PMA + anti-IL-2	3,068

*Means of triplicate samples.
†Used at 10 ng/ml.
‡IL-4 at 256 units/ml.
§Mock-transfected COS-7 SN.
¶Culture SN at 25%.
‖Ascites fluid at 1:200 dilution.

SN from the T-cell hybridoma D9C1.12.17. This cell line is known to be a good producer of several lymphokines, including IL-2 and IL-4 (13). PNA− thymocytes proliferated in response to D9C1.12.17 SN (D9; Fig. 1*A*). This proliferation was totally inhibited by methyl α-D-mannoside, indicating that the Con A present in the SN was necessary. Indeed, Con A alone induced significant proliferation of PNA− thymocytes (Con A; Fig. 1*A*). The costimulatory effect of Con A could be replaced by PMA (D9 + methyl α-D-mannoside + PMA; Fig. 1*A*). In contrast to Con A, PMA alone did not induce proliferation of PNA− thymocytes at concentrations ranging from 0.1 to 50 ng/ml. This response could be partially inhibited by both anti-IL-4 antibodies (D9 + methyl α-D-mannoside + PMA + anti-IL-4; Fig. 1*A*) and anti-IL-2 antibodies (not shown) and totally inhibited by the combination of anti-IL-4 and anti-IL-2 antibodies. The role of IL-4 and IL-2 in these responses was confirmed using recombinant lymphokines on PNA− thymocytes as shown in Fig. 1*B*. Both IL-4 and/or IL-2 induced responses in combination with PMA. The response observed with IL-2, IL-4, and PMA (IL-2 + IL-4 + PMA; Fig. 1*B*) resulted in a response comparable to the one produced by D9C1.12.17 SN (D9 + methyl α-D-mannoside + PMA; Fig. 1*A*). Neither lymphokine alone nor in combination induced a significant response in the absence of PMA. The combination of IL-4 and PMA (IL-4 + PMA; Fig. 1*B*) induced more proliferation than IL-2 and PMA (IL-2 + PMA; Fig. 1*B*). Similar results were obtained using either the MTT colorimetric assay or [^{3}H]thymidine incorporation (data not shown).

PNA− but not PNA+ Thymocytes Proliferate in Response to IL-4 and PMA. The data presented in Fig. 1 *A* and *B* were obtained using PNA− thymocytes since preliminary experiments indicated that this population was highly responsive. We next studied which thymocyte populations were the targets for the action of IL-4. Thymocytes from 3-week-old mice were separated into PNA− and PNA+ and tested for their response to IL-4 and PMA. PNA+ thymocytes were <1% double negative, whereas PNA− thymocytes were 6–8% double negative, suggesting that the double-negative population is responsive (see below). As shown in Table 1, PNA− thymocytes proliferated in response to IL-4 and PMA whereas PNA+ showed only a marginal response. As PNA+ thymocytes are greatly enriched for L3T4+ LyT2+ double-positive thymocytes (see *Materials and Methods*), these data suggested that double-positive thymocytes are unresponsive to IL-4 and PMA. Consistent with previous data (Fig. 1), the response of PNA− thymocytes to IL-4 and PMA could be inhibited by anti-IL-4 but not by anti-IL-2 antibodies (Table 1).

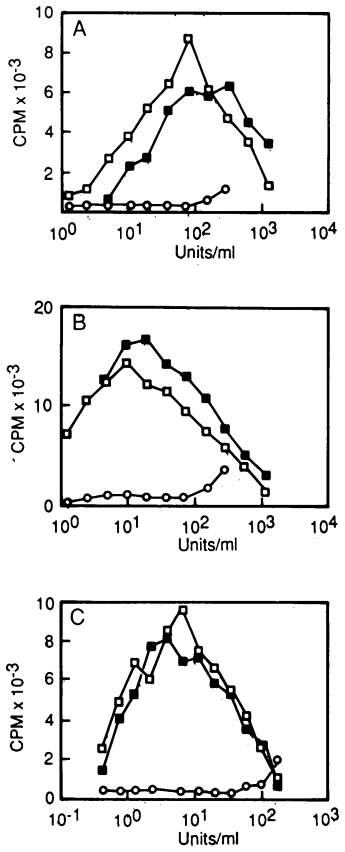

FIG. 2. (*A*) Response of fetal thymocytes to IL-4 and PMA (□) or to IL-4 and PMA in the presence of anti-IL-2 antibody used as ascites 1:200 constant concentration (■) or to IL-2 and PMA (○). (*A*) Day-14 fetal thymocytes. (*B*) Day-15 fetal thymocytes. (*C*) Day-17 fetal thymocytes. PMA was used at a constant concentration of 10 ng/ml and did not induce a detectable response when used alone. In all cases, addition of anti-IL-4 monoclonal antibody (used as culture SN at a final concentration of 25%) completely inhibited the IL-4-driven responses (<500 cpm). Each point represents mean of duplicate samples.

Fetal Thymocytes (Days 14–17), Adult Double-Negative L3T4− LyT2−, and Single-Positive L3T4+ or LyT2+ Thymocytes Proliferate in Response to IL-4 and PMA. The PNA− thymocytes found to respond in the above experiments contained both phenotypes of mature single-positive cells (i.e. L3T4+ or LyT2+) as well as double-negative thymocytes (see *Materials and Methods*). In contrast, the unresponsive PNA+ subset was greatly enriched for immature double-positive L3T4+ LyT2+ thymocytes. To clarify whether any immature thymocytes were capable of responding to IL-4 and PMA, we investigated the response of both fetal and purified adult double-negative L3T4− LyT2− thymocytes. Fig. 2 shows the response of fetal thymocytes collected at different gestational ages. A strong response to IL-4 and PMA was observed in fetal thymocytes as early as 14 days of gestational age and continued through gestational development. This response was blocked by anti-IL-4 but not by anti-IL-2 antibodies (Fig. 2). The inhibitory effect of high IL-4 concentrations in these experiments may reflect the high dose inhibition of IL-4 previously observed in B-cell proliferation (18) or, alternatively, it may be due to by-products of the transfected COS-7 cells. As with adult PNA− thymocytes, the proliferative responses obtained using fetal thymocytes were significantly greater with IL-4 and PMA than with IL-2 and PMA (Fig. 2). This observation suggests that IL-4 may be a more potent growth factor for fetal thymocytes

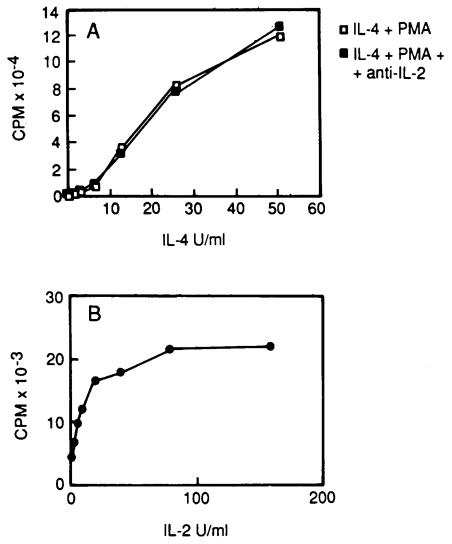

FIG. 3. Response of adult double-negative (L3T4− LyT2−) adult thymocytes. The cells were obtained by treatment with anti-L3T4 and anti-LyT-2.2 monoclonal antibodies and complement. (*A*) □, Response to IL-4 and PMA, ■, response to IL-4 and PMA in the presence of anti-IL-2 (see Fig. 2 legend). The response to IL-4 and PMA was completely inhibited by the addition of anti-IL-4 antibodies (<400 cpm) (used as culture SN at a final concentration of 25%). (*B*) Response to IL-2 and PMA. PMA was used at a constant concentration of 10 ng/ml and did not induce a detectable response when used alone or in combination with mock-transfected COS-7 SN (<400 cpm). Each point represents mean of duplicate samples.

than is IL-2. Separate experiments have indicated that day-14 fetal thymocytes could be expanded in IL-4 and PMA for at least 5 days without significant loss of viability.

Similarly, purified double-negative L3T4− LyT2− thymocytes from 3- to 4-week-old mice were examined for responsiveness to IL-4 and PMA. Double-negative thymocytes were obtained either by negative selection using cytotoxic antibodies and complement (Fig. 3) or by positive selection using two-color FACS (Table 2). In both cases, double-negative thymocytes proliferated in response to IL-4 and PMA but no significant response was observed to either stimulant alone (Fig. 3*A*; Table 2). This response was inhibited by anti-IL-4 but not by anti-IL-2 antibodies (Fig. 3*A*). As with fetal thymocytes, the response of adult double-negative thymocytes to IL-4 and PMA (Fig. 3*A*) was greater than that induced by IL-2 and PMA (Fig. 3*B*).

Table 2. Double-negative thymocytes proliferate to IL-4 and PMA

Cells*	Incubated with	cpm†
L3T4− LyT2−	PMA‡	410
L3T4− LyT2−	IL 4§	693
L3T4− LyT2−	IL-4 + PMA	17,985
L3T4+ LyT2+	PMA	347
L3T4+ LyT2+	IL-4	344
L3T4+ LyT2+	IL-4 + PMA	1,890
L3T4+ LyT2−	PMA	200
L3T4+ LyT2−	IL-4	1,058
L3T4+ LyT2−	IL-4 + PMA	21,967
L3T4− LyT2+	PMA	97
L3T4− LyT2+	IL-4	474
L3T4− LyT2+	IL-4 + PMA	172,427

*FACS-sorted cells (5 × 10^4 cells per well) were used in this experiment.
†Means of duplicate samples. In all populations, induction with mock-transfected SN + PMA produced <500 cpm.
‡At 10 ng/ml.
§At 512 units/ml.

Table 3. Adult double-negative thymocytes produce IL-4 with A23187 and PMA

Double-negative thymocytes incubated with	cpm*
CM	337
IL-4† + PMA	26,833
IL-4 + PMA + anti-IL-4‡	404
Double-negative SN§ + PMA	6,770
Double-negative SN + PMA + isotype control¶	8,342
Double-negative SN + PMA + anti-IL-4	1,114
Double-negative SN + PMA + anti-IL-2	4,086
Double-negative SN + PMA + anti-IL-2 + anti-IL-4	670

*Means of duplicate samples.
†At 512 units/ml.
‡Antibodies used as described in Table 1.
§SN from double-negative thymocytes activated with A23187 and PMA.
¶Isotype control monoclonal antibody for anti-IL-4 used at equivalent concentration.

Sorted single positive L3T4+ LyT2− or L3T4− LyT2+ thymocytes were also tested for their response to IL-4 and PMA. As shown in Table 2, both of these populations responded to IL-4 and PMA. LyT2+ cells proliferated more than L3T4+ cells.

To further explore the apparent unresponsiveness of double-positive thymocytes to IL-4 and PMA (Table 1), these cells were purified by positive selection using two-color FACS. Consistent with the data obtained using PNA+ cells (Table 1), sorted double-positive thymocytes proliferated poorly to IL-4 and PMA (Table 2).

SN from Adult Double-Negative Thymocytes Activated with Calcium Ionophore and PMA Induce Proliferation of Double-Negative Thymocytes. Since the results presented above indicated that IL-4 is an important growth stimulus for immature thymocytes, we investigated whether immature thymocytes could produce IL-4. Adult double-negative thymocytes were induced with the calcium ionophore A23187 and PMA, and after 24 hr their SN was harvested and tested for its ability to induce proliferation in freshly prepared adult double-negative thymocytes. In our experience, most of the A23187 and PMA are absorbed and neutralized by the cells during the initial incubation. The calcium ionophore A23187 in particular has a very narrow range of concentrations where it exhibits mitogenic activity (0.3–0.5 μM), so it was unlikely that any mitogenic effects of these SN would be due to residual A23187 and, as shown in Table 2, double-negative thymocytes do not replicate to PMA alone. A representative experiment is shown in Table 3. Double-negative thymocytes proliferated to SN from adult double-negative thymocytes stimulated with A23187 and PMA. Furthermore, this response was inhibited by anti-IL-4 antibodies. The remaining response was eliminated by a combination of anti-IL-4 and anti-IL-2 antibodies. These results indicate that double-negative adult thymocytes are capable of producing IL-4.

DISCUSSION

In this study, we demonstrate that several immature thymocyte populations are able to proliferate in response to IL-4 and a second costimulatory signal. These observations strongly suggest a role for IL-4 in T-cell ontogeny.

To understand the role of IL-4 in thymic development, it was important to determine which thymocyte subpopulations responded to this lymphokine. The following subsets were found to be responsive to the combination of IL-4 and PMA: PNA− thymocytes, double-negative L3T4− LyT2− and single-positive L3T4+ LyT2− or L3T4− LyT2+ adult thy-

mocytes, and thymocytes obtained from fetal mice from days 14–17 of gestation. In contrast, PNA+ thymocytes and purified L3T4+ LyT2+ double-positive thymocytes showed little or no response to IL-4 and PMA. These data indicate that some (double negative) but not all (double positive) immature thymocytes are responsive to IL-4 in the presence of a costimulator. Interestingly, responsiveness to IL-4 appears at a time in ontogeny that precedes the expression of the antigen-specific T-cell receptor (19). This indicates that antigenic stimulation is not a requirement for IL-4 receptor expression. Although our data define thymocyte subpopulations that respond to IL-4, it is not yet possible to determine whether other thymic cell type(s) are susceptible to IL-4 induction. Recent data from several laboratories have indicated that in addition to the well-documented effects of IL-4 on B cells (9, 13, 20), it is capable of stimulating a variety of hemopoietic and lymphoid cell types (10, 11, 21). It is therefore possible that IL-4 is acting on thymocytes directly, on thymic stromal elements, or on both. Thus, IL-4 may act at several different levels of T-cell ontogeny.

An important finding to emerge from our studies is that thymocytes require a costimulator (i.e., Con A or PMA) to respond to IL-4. While the role of this costimulator remains obscure, it is important to define its physiological equivalent in thymic development. An attractive candidate may be the stimulus provided by some kind of thymic stromal cell.

Finally, our data suggest a role for IL-4 as a growth factor for immature thymocytes. Previous reports (2, 4, 22, 23) have documented that thymocytes are able to produce and respond to IL-2. However, a large proportion of thymocytes fail to express IL-2 receptors of high affinity (5), suggesting the involvement of another growth factor distinct from IL-2. As shown in Figs. 1*B* and 3 *A* and *B*, adult PNA− as well as purified double-negative L3T4− LyT2− thymocytes showed greater responses to IL-4 and PMA than to IL-2 and PMA. The IL-4-mediated responses appear to be IL-2 independent since anti-IL-2 antibodies failed to show significant inhibition. Furthermore, we have observed that only IL-4 and PMA can maintain the proliferation and viability of these populations (as well as day-14 fetal thymocytes) for up to 5 days. Taken together, these observations suggest that IL-4 may be an important growth factor for immature thymocytes. However, to establish a physiological role for IL-4 in thymic development, it is also important to demonstrate its production by immature thymocytes. Previous studies have documented that immature thymocytes (adult double negative or fetal day 15) are able to produce IL-2 when induced with calcium ionophores (ionomycin or A23187) and PMA (3, 4). These stimuli are actually mitogenic for these thymocytes, although their mechanism of action is not clear. Thus, we used this stimulation protocol to investigate whether immature thymocytes could produce IL-4. As shown in Table 3, SN from A23187 and PMA-stimulated double-negative thymocytes induced proliferation of freshly prepared adult double-negative thymocytes. Furthermore, anti-IL-4 and to a lesser extent anti-IL-2 antibodies inhibited the ability of conditioned SN from calcium ionophore/PMA-stimulated double-negative thymocytes to induce proliferation of double-negative thymocytes. These results indicate that double-negative thymocytes are able to produce IL-4 when activated. Thus, our data indicate the most immature population of thymocytes in the adult thymus, the L3T4− LyT2− double-negative thymocytes, are able to both produce and respond to IL-4. Taken together, our studies strongly suggest that IL-4 is an important growth factor for immature thymocytes, and as such it may play an important role in T-cell ontogeny.

Note Added in Proof. Since this manuscript was submitted for publication, a paper has appeared documenting the effects of IL-4 on fetal thymocytes (24). We have also now observed that fetal thymocytes (day 14 or 15) are able to produce IL-4 in experiments similar to the one shown in Table 3.

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