

DEVELOPMENT OF FETAL THYMOCYTES IN
ORGAN CULTURES
Effect of Interleukin 2

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When mature thymus-derived (T) lymphocytes from peripheral lymphoid organs are activated by antigen or mitogen, an increase in the expression of the surface receptor for interleukin 2 (IL-2-R) is observed that precedes the development of effector function (1-3). While IL-2 is known to be a specific growth factor for such mature T cells (4, 5), it is not known whether IL-2 is also involved as a regulatory molecule in the early maturation of thymocytes (6-9). It has recently been reported that the IL-2-R can be detected on cells in the fetal thymus at day 13 of gestation, and by day 14, >90% of fetal thymocytes are IL-2-R⁺ (6-8). Little is known of the origin of fetal thymocyte precursors or the events controlling their development.

Several questions arise from the finding of such a high proportion of Thy-1⁺, IL-2-R⁺ thymocytes in the day-14 fetus. First, is IL-2 involved in regulating the maturation of these cells? Second, are cells in the 14-day fetal thymus capable of synthesizing IL-2? There are several remarkable changes that occur in the fetal thymus. While the phenotype of most cells on day 14 of gestation is Thy-1⁺, IL-2-R⁺, by day 16 this has changed so that a majority of cells are Thy-1⁺, L3T4⁺, Ly-2⁺, IL-2-R⁻ (6, 7). In addition, the events that are associated with the development of antigen specificity and MHC restriction of effector function appear to occur as L3T4⁺, Ly-2⁺ cells emerge (10-16).

Organ culture of murine fetal thymus lobes at day 14 of gestation has been successfully used to follow the maturation of thymocyte precursors that have already seeded this tissue (11, 13, 17). The progression of Thy-1⁺, IL-2-R⁺, cells to Thy-1⁺, L3T4⁺, Ly-2⁺ cell populations can be followed over a period of days in vitro (13, 17, 18).

The organ culture system has potential for the purpose of analyzing the mechanisms that control thymocyte maturation. We describe here the influence of IL-2 on fetal thymocyte development. When IL-2 is added to organ cultures on 14-d fetal thymic lobes, an inhibition of thymocyte proliferation is observed and the development of Thy-1⁺, L3T4⁺, Ly-2⁺ cells is impaired. A population of IL-2-activated cytotoxic cells emerge. This report summarizes the kinetics of

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thymocyte maturation in fetal thymus organ cultures in the presence of IL-2, and leads to a discussion of possible pathways of thymocyte differentiation.

Materials and Methods

Mice. Male and female C57BL/10J (H-2^b), BALB/c (H-2^d), and BALB.B (H-2^b) mice were obtained from breeding colonies maintained in the School of Medicine, University of Auckland. Embryos were obtained from timed matings with the day of detection of a vaginal plug being designated as day 0.

Lymphokines. Recombinant human IL-2 was a gift from Immunex Corporation, Seattle, WA. Units of IL-2 activity were determined in a IL-2-dependent T cell growth assay where 1 U was defined as the amount required to stimulate 50% of the maximal cell response (19).

Organ Cultures. Intact thymic lobes were removed from day-12 to day-18 embryos and established in organ culture as described elsewhere (11, 13, 17). Briefly, five individual lobes were placed on the surface of 0.45- μ m Millipore filters supported by a 1-cm square of Gelfoam gelatin sponge (Upjohn Co., Kalamazoo, MI). Each sponge was placed in a 15-cm petri dish and soaked in 4 ml of culture medium. The culture medium was Iscove's modified Dulbecco's medium supplemented with 10% FCS and glutamine (0.2 mg/ml). Cultures were incubated in a humidified incubator containing 10% CO₂ in air.

Cell Suspensions. Fetal thymus lobes were placed in fresh culture medium and cells were released by dropping a cover slip over the lobe and gently pressing the cover slip with forceps. A cell suspension was prepared from individual lobes and the numbers of viable cells were estimated by their ability to exclude trypan blue dye. The viability of cell suspensions was always >80%. When a large number of thymocytes was required, five lobes were treated simultaneously, the cells pooled, layered over FCS to remove debris, and washed twice in culture medium.

Cell Surface Staining. Cell suspensions were prepared from fetal thymic lobes. To detect expression of the Thy-1, L3T4, Ly-2 antigens and IL-2-R, cells were stained with T24-31.7 (20), GK1.5 (21), 53-6.7 (22), and 7D4 (23) mAb, respectively. Cells were incubated at 4°C in tissue culture medium containing 0.01% sodium azide and the appropriate mAb for 15 min. The cells were washed twice and stained with a fluorescein-conjugated rabbit anti-rat Ig antibody for 15 min as detailed elsewhere (24). The cells were washed twice and then examined using a Nikon Optiphot epifluorescent microscope for the presence of membrane fluorescence.

Cytotoxicity Assay. Cytotoxicity was assayed by a standard ⁵¹Cr-release assay with minor modifications (25). Target cells were P815 (H-2^d) mastocytoma cells, 14-d BALB/c (H-2^d), or BALB.B (H-2^b) fetal thymocytes. Briefly, fetal thymocyte effector cells were harvested from organ cultures, cell numbers determined, and various numbers added in triplicate to V-bottomed wells in 100- μ l volumes. Target cells were labelled with 150 μ Ci ⁵¹Cr for 45 min at 37°C, washed once, and allowed to stand at room temperature for 30 min. After the final wash, cell numbers were determined and 5 \times 10³ target cells were added to the thymocyte effector cells in the V-bottomed wells to give a total volume of 200 μ l. For assay of lectin-dependent cytotoxicity, PHA was added at a concentration of 1% vol/vol to the effector/target cell mixture. After incubation for 4 h at 37°C, 100 μ l of supernatant was removed from each well for radioactive counting. Spontaneous release of radioactivity was determined by incubating target cells in culture medium, and percent specific lysis was determined as 100 \times [(experimental cpm) - (spontaneous cpm)]/[(maximum uptake cpm) - (spontaneous cpm)]. Standard deviations of triplicate determinations were generally <3% of the specific lysis.

Histology. Thymic lobes from organ cultures were fixed in formalin while still in position on the Millipore membranes. Sections were cut from wax-embedded tissue and stained with hematoxylin and eosin (H and E) (26).

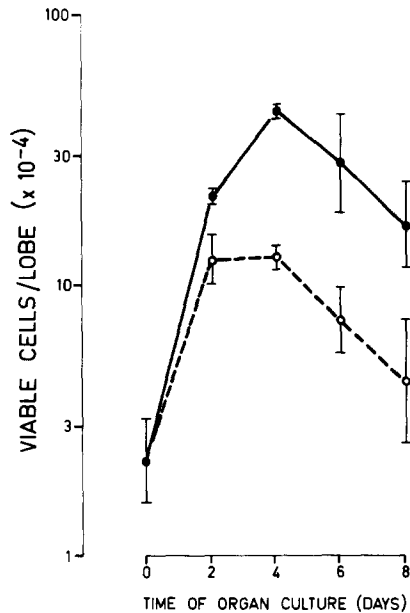


FIGURE 1. Effect of IL-2 on the growth of cells in fetal thymic organ cultures. 14-d C57BL/10J thymic lobes were cultured with or without IL-2 at 20 U/ml and the number of viable cells per lobe was estimated at the times indicated. Vertical bars indicate SD of five replicates. (●) controls, (○) + IL-2.

Results

Inhibition of Thymocyte Growth in Fetal Thymic Lobes Cultured with IL-2. Fetal thymic lobes were taken from 14-d C57BL/10J embryos and placed in organ culture for 8 d. The number of viable cells in the thymic lobes in organ culture increased 20-fold (2.5×10^4 – 5×10^5 cells) during the first 4 d, after which time growth ceased (Fig. 1). By contrast, when fetal thymic lobes placed in organ culture in medium supplemented with 20 U/ml of human recombinant IL-2, a decrease in the cell growth rate was observed in thymic lobes (Fig. 1). Although cell numbers increased over 2 d, the thymic lobes cultured with IL-2 for 6 d yielded fivefold fewer cells (1.5×10^4 – 7.5×10^5 cells) than observed in control lobes (Fig. 1). The viability of cells from thymic lobes in each of the organ culture systems was similar during the entire culture period and was consistently in the range of 80–90%. In each culture system it was also observed that an increase in the number of cells within thymic lobes ceased after 4 d.

The effect of the IL-2 concentration in culture medium on thymocyte growth in organ cultures was studied. Fetal thymic organ cultures were established from 14-d BALB/c embryos in medium supplemented with concentrations of IL-2 from 2 to 100 U/ml. The cell numbers harvested from thymic lobes were measured after 3 d (Fig. 2A). Concentrations of IL-2 up to 2 U/ml had no detectable effect on the numbers of cells harvested from the thymic lobes, however increasing the concentration of IL-2 to 20 U/ml in the culture medium resulted in a major decrease in cell numbers harvested from lobes. For comparison, the titration of the same IL-2 on a murine IL-2-dependent T cell line has been shown in Fig. 2B. Here a dose-dependent increase in radioactive thymidine uptake was routinely observed. Inhibitory effects of IL-2 have not been seen using a range of long-term cell lines as targets. Growth inhibition of fetal

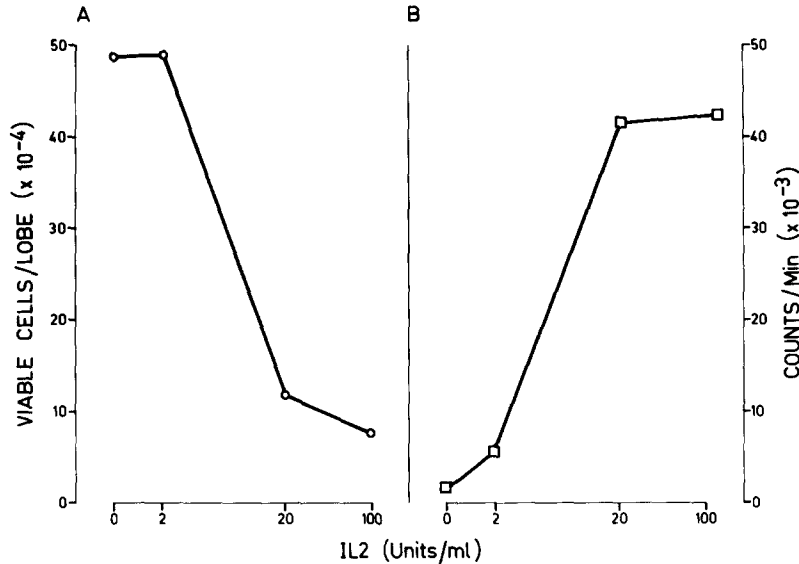


FIGURE 2. Effect of different doses of IL-2 on the growth of thymocytes in organ culture. Thymic lobes from 14-d embryos of BALB/c mice were set up in organ culture containing various amounts of IL-2. After 3 d the viable cell yield of each lobe was estimated (A). IL-2 was similarly titrated in cultures of the IL-2-dependent T cell line CTB, and the cell proliferation measured by the incorporation of [3 H]thymidine into DNA (B).

thymocytes was clearly evident at concentrations of IL-2 stimulatory for the proliferation of continuous T cell lines (Fig. 2).

Morphology of IL-2-treated Fetal Thymus Organ Cultures. The histological structure of the thymic lobes was examined following the period of organ culture. Thymic lobes from 14-d mouse embryos were placed in organ culture in the presence and absence of IL-2. After 6 d, lobes were isolated, embedded in wax, and stained with H and E. Thin sectioning of the thymic lobes prepared from organ cultures lacking IL-2 revealed thin-walled sacs filled with typical lymphoid cells (Fig. 3A). Thymic lobes that were prepared from organ cultures supplemented with 20 U/ml IL-2 contained a few small lymphoid cells interspersed with a layer of stromal cells (Fig. 3B).

Effect of IL-2 on the Phenotype of Fetal Thymocyte Populations. The expression of the Thy-1, L3T4, Ly-2 and IL-2-R surface markers was followed on thymocytes developing in organ culture. Several phenotypic changes were consistently observed (Fig. 4). Most thymocytes in fetal thymic lobes prepared from embryos after 14-d gestation were large, Thy-1⁺, IL-2-R⁺ cells. When these thymic lobes were placed in organ culture, small Thy-1⁺, L3T4⁺, Ly-2⁺ cells appeared within 3 d. After 6 d, Thy-1⁺, L3T4⁺, Ly-2⁺ thymocytes represented >90% of the cells harvested from the thymic lobes (Fig. 4A). The increase in Thy-1⁺, L3T4⁺, Ly-2⁺ cell numbers was 400-fold (10^3 – 4×10^5 cells/lobe) during the 6-d culture period. The staining for L3T4, Ly-2, and Thy-1 antigens was done separately. At all times in the organ cultures the numbers of L3T4⁺ cells correlated with the number of Ly-2⁺ cells (Fig. 4A and B). This observation is consistent with previous studies by Kingston et al. (17), where it was demonstrated that the

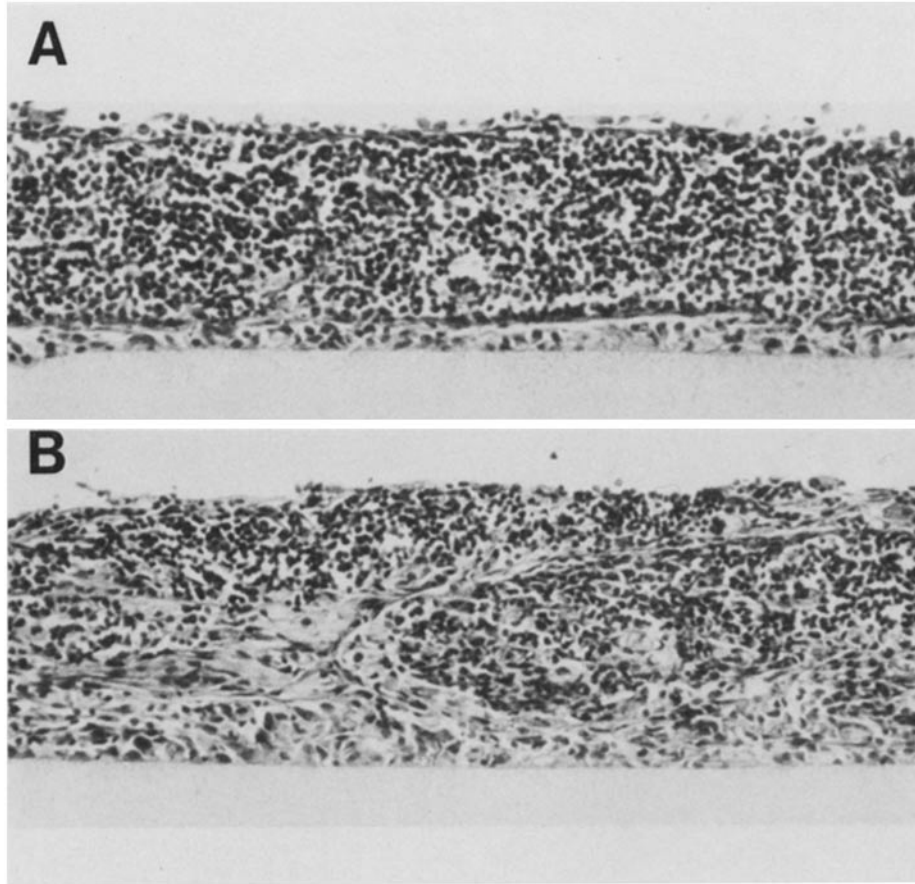


FIGURE 3. Morphology of fetal thymus in organ culture. Thymic lobes from 14-d C57BL/10J embryos were cultured for 4 d with or without IL-2 (20 U/ml), and wax-embedded sections were stained with hematoxylin and eosin. (A) control lobe, (B) IL-2-treated lobe.

majority of fetal thymocytes were L3T4⁺, Ly-2⁺. The proportion of Thy-1 cells was always >90% and was not included in the data of Fig. 4.

The addition of IL-2 to organ cultures of these 14-d fetal thymic lobes inhibited the appearance of Thy-1⁺, L3T4⁺, and Thy-1⁺, Ly-2⁺ thymocytes. After 6 d in culture, the same proportion of cells were L3T4⁺ as were Ly-2⁺. In normal organ cultures, this proportion was 90% compared to <15% in organ cultures with IL-2 (Fig. 4C).

It was of interest also to compare the fate of IL-2-R⁺ cells in these different organ cultures. In normal fetal thymus lobes prepared from 14-d embryos, 90% of the cell population (2.5×10^4 cells) was IL-2-R⁺, but when placed in organ culture over 6 d IL-2-R⁺ cells decreased to 5% ($2.5-3 \times 10^4$ cells) of the total cells (Fig. 4A). Thus, the actual number of IL-2-R⁺ cells in these fetal thymic lobes did not change significantly in organ culture, remaining at $\sim 2.5 \times 10^4$ cells/lobe (Fig. 4B). This suggests there were at least two maturational events occurring in these cell populations, those giving rise to L3T4⁺, Ly-2⁺ cells, and those giving rise to IL-2-R⁺ cells. Similar fetal thymic lobes placed in organ

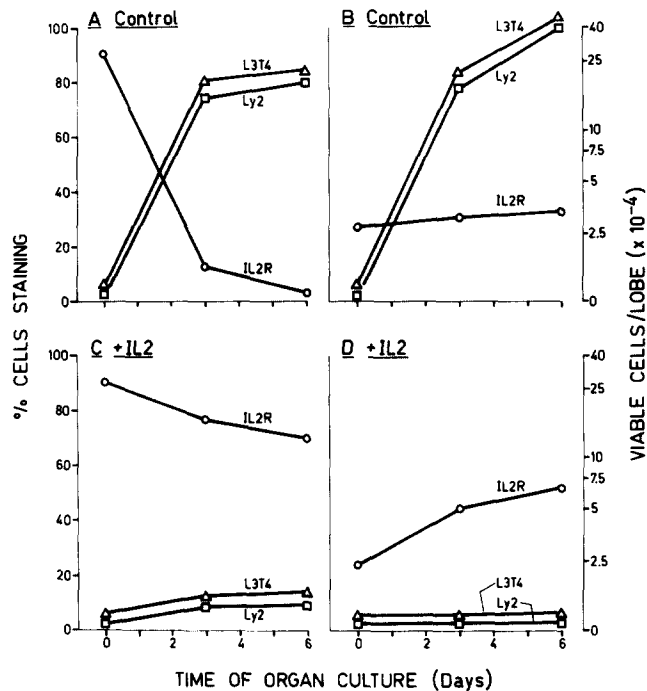


FIGURE 4. Phenotypic analysis of thymocytes from thymus lobes cultured in IL-2. 14-d C57BL/10J fetal thymic lobes were cultured for 6 d with or without IL-2. The cells staining with anti-L3T4 (Δ) anti-Ly2 (\square) and anti-IL-2-R (\circ) antibody were quantitated after 3 and 6 d. Data are expressed as percentage of cells staining with each antibody (see Fig. 4, A and C) and as the total number of cells per lobe staining (Fig. 4, B and D). Fig. 4, A and B are data from control cultures, and C and D are from cultures containing IL-2.

culture with IL-2, showed that, while 90% of the starting cell population was IL-2-R⁺ (2.5×10^4 cells) the total number of IL-2-R⁺ cells increased to 8×10^4 cells/lobe after 6 d of culture (Fig. 4C). Thus, the number of IL-2-R⁺ cells increased in these organ cultures containing IL-2 approximately threefold per lobe. These data indicated that the presence of IL-2 was markedly slowing the normal maturation of thymocytes expressing L3T4 or Ly-2, but stimulating a detectable increase in the numbers of IL-2-R⁺ cells. It has not yet been possible to determine whether the remaining L3T4⁺ and Ly-2⁺ cells represented the immature L3T4⁺, Ly-2⁺ population, or the late-developing L3T4⁺ Ly-2⁻ and L3T4⁻, Ly-2⁺ populations.

Age of Fetal Thymus and the Effect of IL-2. The data summarized in Fig. 5 represent the effects of IL-2 in organ cultures containing fetal thymic lobes prepared from embryos of increasing age, from day 13 to day 18 of gestation. Thymus lobes taken from the day-13 fetus have the lowest number of L3T4⁺ or Ly-2⁺ thymocytes (5×10^2 cells). The rapid increase in cell numbers during their first 3 d in organ culture was attributed to the accumulation of L3T4⁺, Ly-2⁺ cells, which was inhibited completely in organ cultures containing IL-2. As thymic lobes were taken at days 13–18 of gestation, the number of L3T4⁺, Ly-2⁺ cells found increased progressively from 10^3 to 5.5×10^5 cells/lobe. When cultured for 3 d in the absence of IL-2, the numbers of L3T4⁺, Ly-2⁺ cells increased in lobes taken from day-14, -15, and -16 embryos, but day-17 and -18 fetal thymic lobes appeared to contain the maximum number of these cells (5×10^5 cells/lobe), thus no increase in cell numbers was observed in culture. The effect of IL-2 on the development of L3T4⁺, Ly-2⁺ fetal thymocytes was directly proportional to the age of embryos from which thymic tissue was prepared. The presence of IL-2 markedly inhibited development of L3T4⁺, Ly-2⁺ thymocytes in organ cultures

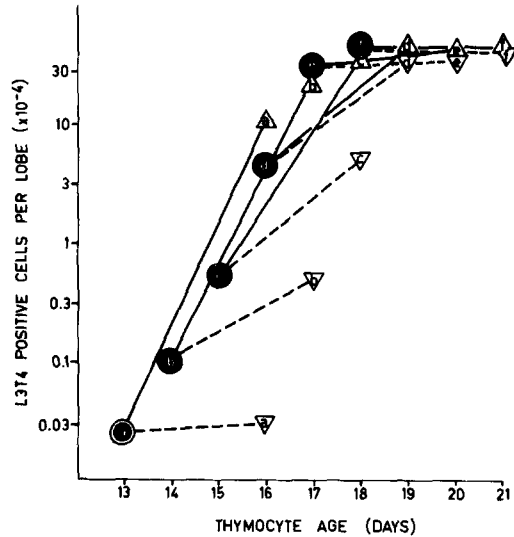


FIGURE 5. Effect of IL-2 on organ culture of different-aged thymic lobes. Thymic lobes from different-aged C57BL/10J embryos (a, 13; b, 14; c, 15; d, 16; e, 17; f, 18) were organ cultured for 3 d in the presence (dashed line) or absence (solid line) of IL-2. The numbers of L3T4⁺, Ly-2⁺ thymocytes per lobe were estimated in IL-2-treated and control cultures. The data only presents L3T4⁺ staining results, as this correlated strictly with Ly-2⁺ cells.

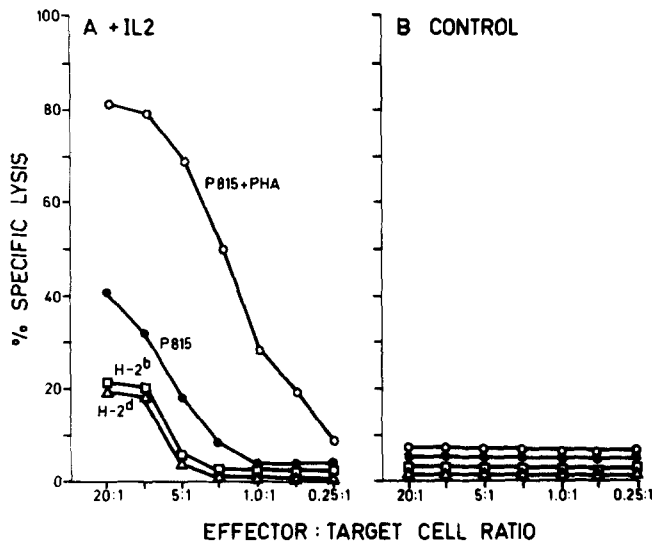


FIGURE 6. Induction of cytotoxic cells in cultured thymic lobes by IL-2. Cells from IL-2-treated (A) and control (B) BALB/c fetal thymic lobes were assayed against Cr-labelled BALB/c (H-2^d, Δ) and BALB/c (H-2^b, \square) fetal thymocytes and the tumour target P815 (H-2^d, \bullet). Cytotoxic activity against P815 was also assayed in the presence of 1% vol/vol PHA (O).

prepared from day-13, -14, and -15 thymic lobes, but had very little effect on the development of L3T4⁺, Ly-2⁺ thymocytes in organ cultures of day-16, -17, and -18 thymic lobes (Fig. 5). Once L3T4⁺, Ly-2⁺ cells appeared, IL-2 did not cause the disappearance of these cells.

Development of Cytotoxic Cells in Organ Cultures. Because Thy-1⁺, IL-2-R⁺ cells that have cytotoxic activity have been described (27), the development of cytotoxic activity in organ cultures of 14-d BALB/c fetal thymic lobes was examined (Fig. 6). No detectable cytotoxicity was detected in the fetal thymus at day 14 of gestation (Fig. 7), or using thymocytes harvested after 6 d from organ cultures of these 14-d fetal thymic lobes (Fig. 6B). A variety of ⁵¹Cr-labelled target cells were used: syngeneic and allogeneic fetal thymocytes, and the syngeneic tumor

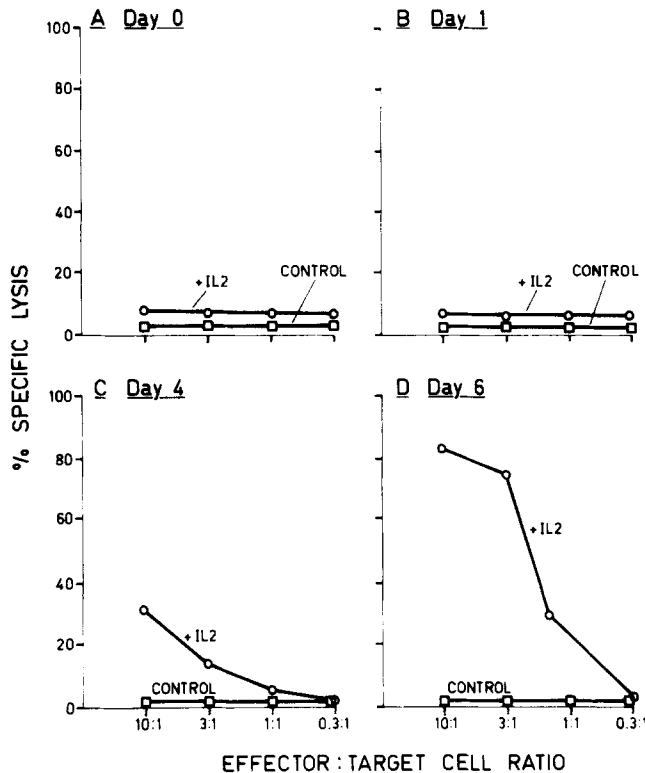


FIGURE 7. Development of cytotoxic activity in thymocytes from thymic lobes cultured in IL-2. 14-d fetal thymic lobes were cultured for various times in medium containing 20 U/ml IL-2. Thymocytes were harvested from lobes cultured for 0 (A), 1 (B), 4 (C), and 6 d (D), and the level of cytotoxic activity was assayed in a 4-h ^{51}Cr -release assay. The target cells were P815 in the presence of 1% vol/vol PHA.

cell line, P815. Thymocytes harvested after 6 d from organ cultures of these 14-d fetal thymic lobes with 20 U/ml IL-2 lysed syngeneic and allogeneic thymocyte targets and the syngeneic tumor target P815 (Fig. 6A). The addition of PHA to the cytotoxic assay using P815 target cells increased lysis significantly (Fig. 6A).

Kinetics of Appearance of Cytotoxic Cells. Day-14 BALB/c fetal thymic lobes were placed in organ culture with 20 U/ml IL-2. At days 0, 1, 4, and 6, thymocytes were harvested and assayed for cytotoxicity using P815 tumor cells as targets in the presence of PHA. No cytotoxic activity was detected in 14-d fetal thymic lobes (Fig. 7A), or after organ culture of these lobes for 24 h (Fig. 7B). After 4 d in culture, cytotoxic activity could be detected in thymocytes harvested from lobes (Fig. 7C), and this increased significantly by day 6 (Fig. 7D). The kinetics of appearance of IL-2-induced cytotoxicity in fetal thymic organ cultures revealed that the cells found in the 14-d fetal thymus were not cytotoxic. This time period may reflect the proliferation of these IL-2-activated killer cells. It was not clear whether these cells developed from the Thy-1^+ , IL-2-R^+ population or from the smaller L3T4^+ , Ly-2^+ population present at the start of culture.

Discussion

The development of thymocytes was followed in organ cultures of thymic lobes taken from the mouse fetus. Most thymocytes found in the fetal thymus at day 14 of gestation were Thy-1^+ and IL-2-R^+ , and had no known effector

function. Organ culture of these 14-d fetal thymic lobes resulted in the proliferation and maturation of cells in the lobes that were predominantly L3T4⁺, Ly-2⁺, IL-2-R⁻. The interesting finding described in this paper was that the addition of IL-2 to organ cultures of 14-d fetal thymus, at concentrations known to support the growth of mature thymocytes, inhibited normal maturation and growth of L3T4⁺, Ly-2⁺ cells. Also, a cytotoxic population of cells was found to develop in organ cultures containing IL-2.

In normal 14-d fetal thymus, the total number of fetal thymocytes was 1–3 × 10⁴ cells/lobe (Fig. 1) and ~90% were Thy-1⁺ IL-2-R⁺. When placed in organ culture, the total number of thymocytes increased 20–40-fold in 4 d (Fig. 1), while the number of L3T4⁺, Ly-2⁺ cells increased 200-fold in the same period (Fig. 5). Two questions that are important in considering the effect of IL-2 on thymocyte ontogeny arise from the data. The first concerns whether the Thy-1⁺ IL-2-R⁺ cells contained precursors of the L3T4⁺, Ly-2⁺ cells or whether the L3T4⁺, Ly-2⁺ cells were derived from a small pool of IL-2-R⁻ cells. The second question concerns the nature of the regulatory events that governed the proliferation and maturation of fetal thymocytes. As Thy-1⁺, IL-2-R⁺ cells were such a large component of the thymocytes in a 14-d fetal lobe, it was of interest to see whether IL-2 would affect the appearance of L3T4⁺, Ly-2⁺ cells, presumably as a consequence of interacting with IL-2-R⁺ cells.

Our results indicated that adding IL-2 to 14-d fetal thymocytes resulted in a fivefold increase in cell number, which is predominantly a reflection of the increase in the number of IL-2-R⁺ cells. The emergence of the L3T4⁺, Ly-2⁺ population was inhibited (Fig. 4). The inhibitory effect of IL-2 on the normal pattern of development progressively diminished as the age of the donor fetus increased. The thymic lobes from mouse embryos at days 14–18 of gestation showed a steady increase in the size of the L3T4⁺, Ly-2⁺ cell population. The smaller the population, the greater the inhibition of the L3T4⁺ pool by IL-2 in 3 d of culture (Fig. 5). This implies that the recruitment of precursor cells into the L3T4⁺, Ly-2⁺ pool, rather than the proliferation of the mature L3T4⁺, Ly-2⁺ cells, was inhibited by IL-2.

In considering the mechanisms that underlie the inhibitory influence of IL-2 on the maturation of L3T4⁺, Ly-2⁺ cells, it may be important that cytotoxic cells were detected in cultured thymic lobes that lysed syngeneic and allogeneic fetal thymocytes and the syngeneic tumor P815 (Fig. 6). The cytotoxic cells are detectable after some days in culture (Fig. 7), and only in the presence of IL-2. Some cytotoxic cells have been implicated in differentiation processes (28), and in the thymus it may only be in the presence of IL-2 that they are amplified to a detectable level. The IL-2-R⁺ cells may mature to cytotoxic cells directly although, in other studies, IL-2-R⁺ cells give rise to L3T4⁺, Ly-2⁺ cells (17, 18). Thus, if the cytotoxic activity is part of a normal physiological process in the thymus, there are several ways such cells might function. Rather than destroy thymus cells randomly, it seems likely that the cytotoxicity would be directed towards a subpopulation. Because there is an increase in IL-2-R⁺ cells, it is unlikely that these are the major target; a more likely target might be those fetal thymic cells that regulate the development of L3T4⁺, Ly-2⁺ cells. Such possibil-

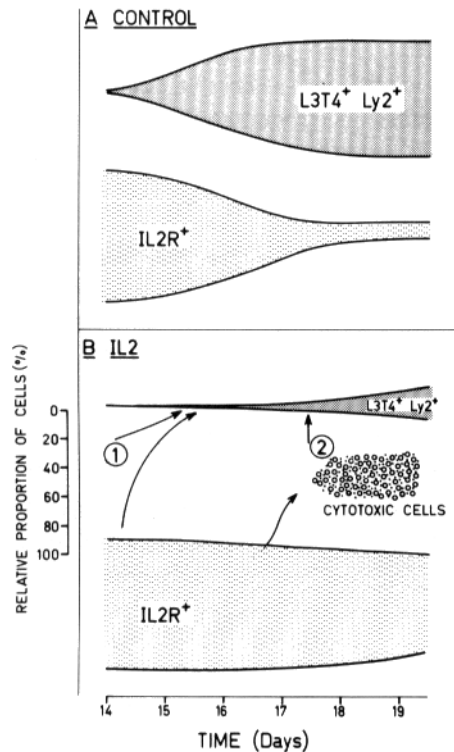


FIGURE 8. Summary of major changes during fetal thymocyte development.

ities can be examined further by the selective use of thymocyte subpopulations as target cells to assay cytotoxic cells from the fetal thymus.

A marked increase in the amount of cytotoxicity was observed when PHA was added to the assay for cytotoxic cells in the thymus cultured with IL-2 (Fig. 6). This is presumably a reflection of the diverse polyclonal nature of the cytotoxic cells, as the inclusion of PHA appears to result in an overriding of the normal effector cell recognition system. Although the precursor frequency of lymphokine-activated killer cells has been measured (29), the development and physiological role of cells that adopt a cytotoxic mode in the presence of IL-2 has yet to be clarified.

The changes in the populations of cells of the cultured thymus that we have described are summarized in Fig. 8. The percentage of cells with different phenotype are presented over a number of days, bearing in mind that the total number of cells was increased at different rates in the presence or absence of IL-2. The effects appear to be more marked at early stages, and it is suggested that the IL-2-induced effect is on the regulation of the L3T4⁺, Ly-2⁺ lineage (one in Fig. 8B). The presence of cytotoxic cells may fill, either directly or indirectly, such a regulatory role, particularly as they could act at a time that is earlier than when they were detectable in these studies (two in Fig. 8B).

These data lead to the important questions of what is the growth stimulus for L3T4⁺, Ly-2⁺ cells, and what regulates the development of Thy-1⁺, IL-2-R⁺ cells in a 13–14-d fetal thymus. It is not clear why the IL-2-R is expressed on early thymocytes. A number of possibilities might be considered. If the IL-2-R gene is

expressed coordinately with other genes that are used to regulate thymocyte growth, it may not be used during ontogeny. Alternatively, IL-2-R expression on fetal thymocytes may be regulated in a manner similar to that of mature T cells, as a result of cell activation. In the 14-d fetal thymus, the products of genes encoding the α and β chains of the functional antigen receptor do not appear to be expressed (10). In the absence of an antigen receptor, cell activation may be through another receptor structure that is expressed and functional. The physiological function of such a receptor remains unknown, although a homing receptor may have such a role (30). The finding that the L3T4⁺, Ly-2⁺ population fails to develop normally in the presence of IL-2 may reflect a deviation from the normal pathway of thymocyte development. According to such a notion, the diversion of IL-2-R⁺ cells into a cytotoxic population may be a minor maturation pathway, the significance of which has yet to be determined. The use of cell sorting to isolate cells followed by repopulation studies in organ cultures (17) will be used to address these questions. The alteration of the microenvironment by including growth factors in fetal organ cultures may reveal a series of alternative differentiation pathways that are normally masked by more dominant pathways.

Summary

Most fetal thymocytes from 14-d mouse embryos are Thy-1⁺, L3T4⁻, Ly-2⁻, and express the receptor for interleukin 2 (IL-2). The development of thymocytes has been followed in fetal thymus organ cultures. When fetal thymus from 14-d embryos were cultured for a 6-d period, thymocytes increased in number 20–40-fold, and 95% became Thy-1⁺, L3T4⁺, Ly-2⁺. The addition of IL-2 to organ cultures of 14-d fetal thymus inhibited, in a dose-dependent manner, cell proliferation and the appearance of Thy-1⁺, L3T4⁺, Ly-2⁺ thymocytes. The addition of IL-2 also resulted in the appearance of a population of cells that were cytotoxic for syngeneic and allogeneic fetal thymocytes and syngeneic tumour targets. While the events that lead to the expression of the IL-2 receptor on 14-d fetal thymocytes are unknown, IL-2 in fetal thymus organ cultures inhibits the normal maturation of fetal thymocytes and raises the question of whether the cytotoxic cells that appear reflect selection through an alternative pathway of development.

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