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A Role for Tumor Necrosis Factor-α and Interferon-γ in the Regulation of Interleukin-4-Induced Human Thymocyte Proliferation *In Vitro*. Heightened Sensitivity in the Down Syndrome (Trisomy 21) Thymus

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ABSTRACT. The influence of recombinant interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF) on IL-4-induced proliferation of postnatal human thymocytes from eight children with Down syndrome (DS, trisomy 21) and 18 control children was evaluated. DS thymuses were studied because they are characterized by cortical depletion and abnormal thymocyte differentiation. IL-4, without mitogen, induced a dose-dependent proliferation of both DS and control thymocytes. The proliferation was comparable to that induced by IL-2 and far greater than the proliferation mediated by IL-1β in the absence of mitogen. The level of IL-4 responsiveness correlated with the proportion of cells expressing the γ,δ chains of the T cell receptor. Furthermore, thymocyte preparations greatly enriched for T cell receptorγ,δ-bearing cells were found to vigorously proliferate when treated with IL-4. Both IFN-γ and TNF inhibited IL-4-driven proliferation in a dose-dependent manner, but DS thymocytes were found to be significantly more sensitive to inhibition by both cytokines. Our studies suggest an important role for IL-4 in the proliferation of T cell receptorγ,δ^+ thymocytes and demonstrate regulatory functions for IFN-γ and TNF in human thymocyte proliferation. The increased sensitivity of DS thymocytes to IFN-γ and TNF may explain anatomical abnormalities in DS thymuses and suggests the involvement of genes encoded on human chromosome 21 in the responses to both IFN-γ and TNF. (*Pediatr Res* 32: 269–276, 1992)

Abbreviations

DS, Down syndrome
GAM, goat anti-mouse
IFN-γ, interferon-γ
TCR, T cell receptor
TNF, tumor necrosis factor-α
r, recombinant

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Individuals with DS (trisomy 21) have multiple immunologic and hematologic abnormalities (1–3). Their thymuses show altered patterns of thymocyte differentiation (4, 5) and have numerous anatomical abnormalities, including depletion of cortical thymocytes, loss of cortico-medullary demarcation and enlarged Hassall's corpuscles (1, 6, 7). Also, we recently found evidence for an inefficient release by the DS thymus of mature T cells into the periphery (8). It is unclear which chromosome 21-encoded gene or genes if overexpressed in trisomic cells may be responsible for the abnormalities of thymic anatomy and thymocyte maturation observed in DS. However, it is known that human chromosome 21 does encode some aspect of the response to IFN-γ (9, 10), making trisomy 21 cells more sensitive to the effects of this cytokine (11, 12). Taken together, these facts make the study of the effects of IFN-γ, and its interaction with other cytokines, on DS thymocyte proliferation and maturation particularly appropriate.

The importance of the thymus for the continuous production, maturation, and selection of T cells is well established. There is increasing evidence that cytokines play a role in the regulation of thymocyte maturation (13). IL-4, originally described as a B cell stimulatory factor (14), has been shown to promote *in vitro* proliferation of murine (15–21) and human (22–25) thymocytes. However, the cell type(s) that proliferates in response to IL-4 has not been clearly established. There is also evidence that IL-4 is a differentiation factor for immature T cell precursors in fetal murine (15) or postnatal human thymus (25, 26). Support for an important role of IL-4 in thymocyte proliferation and differentiation is strengthened by the observations that immature murine thymocytes express high affinity receptors for IL-4 (27) and can be stimulated to produce IL-4 *in vitro* (16, 28, 29). In addition, constitutive expression of IL-4 mRNA has been identified by *in situ* hybridization in fetal murine thymocytes (30) and in $CD3^-CD4^-CD8^-$ T cell precursors isolated from postnatal human thymus (26).

To elucidate the importance of IL-4 in human thymocyte proliferation, we have examined the effects of purified rIL-4 on postnatal human thymocytes from eight DS and 18 control children. We have found that IL-4 induces a dose-dependent proliferation of both DS and control thymocytes *in vitro* and does not require the addition of exogenous mitogen. Using a new method for purification of human TCRγ,δ-bearing thymocytes, we have also demonstrated that TCRγ,δ^+ human thymo-

cytes are an important target of the proliferation-inducing effects of IL-4.

IFN-γ (29, 31) and TNF (32, 33) are also produced by immature thymocytes and have been shown to influence thymocyte proliferation *in vitro* (29, 32–36). In particular, IFN-γ has been shown to inhibit IL-4-induced proliferation of phorbol ester-stimulated murine thymocytes (29). We have examined the influence of rIFN-γ and rTNF on IL-4-induced proliferation of DS and control thymocytes and demonstrated that both cytokines mediate a dose-dependent inhibition of IL-4-mediated proliferation. In addition, we observed that DS thymocytes are far more sensitive to the inhibitory effects of both IFN-γ and TNF.

Our observations define a possible role for IL-4 in the proliferation of TCRγ,δ-bearing thymocytes. They also demonstrate that IFN-γ and TNF have important regulatory roles in human thymocyte proliferation. Furthermore, the fact that DS thymocytes have an increased sensitivity to both IFN-γ and TNF suggests a mechanism for the lymphocyte depletion and abnormal thymocyte differentiation observed in DS thymuses. It also implicates the possible involvement of genes encoded on human chromosome 21 in the response not only to IFN-γ but also to TNF.

MATERIALS AND METHODS

Cytokines. Purified human rIL-4 (sp act 2×10^6 U/mg) was obtained from Dr. Alan Levine of Monsanto Co. (St. Louis, MO). A unit of IL-4 is defined as the amount of IL-4 required to cause half-maximal stimulation of ^{3}H-thymidine incorporation by phytohemagglutinin-stimulated human peripheral blood lymphocytes (22). Purified human rIFN-γ (sp act 2.5×10^7 U/mg) and rTNF (sp act 5.7×10^7 U/mg) were gifts from Dr. H. M. Shepard (Genentech, Inc., So. San Francisco, CA). Purified human rIL-2 (sp act 6.2×10^6 U/mg) was purchased from Collaborative Research, Inc. (Bedford, MA). Purified human rIL-1β (sp act 1×10^8 U/mg) was purchased from Genzyme Cytokine Research Products (Boston, MA).

Antibodies. MAb anti-Leu-4, anti-Leu-3a/b, anti-Leu-2a, and anti-TCR-1, reacting with CD3, CD4, CD8, and TCRα,β (37), respectively, were obtained from Becton Dickinson Immunocytochemistry Systems (San Jose, CA) and were used as FITC conjugates. Anti-TCR-δ1 (IgG1), reacting with the δ chain of the human TCR (38), was a gift of Dr. M. Brenner (Dana-Farber Cancer Institute, Boston, MA). Optimal concentrations of anti-TCRδ1 ascites fluid was used for purification of TCRγ,δ$^+$ thymocytes and, in most experiments, for the phenotypic analysis of thymocytes by indirect immunofluorescence. FITC-conjugated anti-TCRδ1, purchased from T Cell Sciences (Cambridge, MA), was used in indicated experiments. Neutralizing antibodies to human IL-2 and IFN-α were purchased from Collaborative Research, Inc. Neutralizing antibody to human IFN-β was a gift from Dr. Alfons Billiau, University of Leuven (Leuven, Belgium). Neutralizing antibody to human IFN-γ was generously provided by Drs. B. Perussia and G. Trinchieri of The Wistar Institute (Philadelphia, PA) and neutralizing antibody to human TNF was a gift from Dr. H. M. Shepard, Genentech, Inc.

cDNA probes. A 710-bp fragment of the pLW55 plasmid containing the human IL-2 cDNA (purchased from the American Type Culture Collection, Rockville, MD) was used to detect human IL-2 mRNA. A 900-bp fragment of the human IFN-γ cDNA plasmid p52 (provided by Dr. D. Goeddel, Genentech, Inc.) was used to detect human IFN-γ mRNA.

Human thymocyte preparations. Sterile samples of human thymus, routinely removed during corrective cardiac surgery, were obtained with the help of Dr. Kevin Turley and the members of the Department of Surgery, University of California, San Francisco. All thymus samples included in the study were from children less than 2 y of age. All DS children were diagnosed as trisomy 21 by phenotypic and cytogenetic analysis. Children with recent infections and children treated with medications known to affect immunologic development or function were excluded from the study. Thymic tissue was dissociated in PBS, and a single cell suspension was obtained by sequential passage through 18-, 20-, and 23-gauge needles. Viable mononuclear cells were isolated by gradient centrifugation with Ficoll-Hypaque (1.077 g/mL; Pharmacia, Piscataway, NJ) (39), washed three times with PBS, and depleted of stromal cells by adherence to plastic (37°C, 1 h). The resulting cell populations always contained > 98% thymocytes as identified by reactivity with anti-CD2 MAb (anti-Leu-5b, Becton Dickinson).

Purification of TCRγ,δ-bearing thymocytes. Nonadherent thymocytes (10×10^7 cells/mL) were reacted for 45 min at 4°C with optimal concentrations of anti-TCRδ1 (IgG1, 38) ascites fluid that had been extensively dialyzed against PBS and sterile filtered. Antibody-sensitized cells were washed twice with cold PBS containing 1% BSA and then reacted for 30 min at 4°C with an optimal concentration of biotinylated rat anti-mouse IgG1 MAb (CellPro, Inc., Bothell, WA). Cells were washed as above, and the biotin-bound cells were positively selected by loading the cell suspension (10×10^7 cells/mL in PBS-1% BSA, 3–6 mL per column) onto an avidin-gel column using the Ceprate LC Laboratory Cell Separation System provided by CellPro, Inc. Loading and elution of bound and unbound cells were performed according to the manufacturer's instructions and required less than 1 h to perform. The purification of bound and unbound cells was monitored immediately after purification by reactivity with optimal concentrations of affinity-purified FITC-conjugated goat F(ab')2 anti-mouse IgG (GAM-FITC, Organon Teknika, West Chester, PA). The bound cells were also cultured for 18 h at 37°C in RPMI-1640–10% FCS, washed extensively, and analyzed for anti-TCRδ1 reactivity to confirm purification. The purity of TCRγ,δ-bearing thymocytes was comparable by both methods used to assess purity. This purification method yielded an average of 2×10^6 viable cells with an average purity of at least 86% anti-TCRδ1$^+$ cells.

Measurement of thymocyte proliferation by ^{3}H-thymidine incorporation. Replicate wells of unseparated, CD4$^-$CD8$^-$-enriched, or anti-TCRδ1$^+$ or TcRδ1$^-$ thymocytes were cultured at 37°C in sterile, flat-bottom, 96-well tissue culture plates (1×10^5 cells/200 μL) in RPMI-1640–10% FCS containing the indicated concentrations of rIL-4, rIL-2, rIL-1β, rIFN-γ, or rTNF. Cytokines were always added at the initiation of culture. Wells were pulsed with 1 μCi/well ^{3}H-thymidine (Amersham Corp., Arlington Hills, IL; sp act 2 Ci/mmol) on d 5 of culture and harvested onto glass-microfiber filters after 18 h using a semiautomated cell harvester (Skatron, Inc., Sterling, VA). The amount of ^{3}H-thymidine incorporated was assessed by liquid scintillation counting of filters.

Analysis of surface antigen expression by immunofluorescence and flow cytometry. After isolation or culture, cells were washed three times with PBS and reacted with MAb for 30 min at 4°C (1×10^6 cells/100 μL). Optimal concentrations of MAb were used according to the manufacturer's instructions or results of titration. After exposure to fluorochrome-conjugated specific MAb, cells were washed three times with cold PBS containing 0.1% gelatin (Sigma Chemical Co., St. Louis, MO) and 0.1% NaN_3 (Sigma) and fixed with 2% paraformaldehyde (Sigma) in PBS for 10 min at 25°C. For staining with unconjugated anti-TCRδ1, cells were washed three times as above, reacted with optimal concentrations GAM-FITC for 30 min at 4°C, washed, and fixed as above. Cells stained with fluorochrome-conjugated nonspecific mouse IgG (Becton Dickinson) or GAM-FITC alone were used as negative controls. Ten thousand to 20 000 viable cells were analyzed on a FACScan flow cytometer (Becton Dickinson). Size thresholds and gates were used to exclude dead cells and cellular debris from analysis.

Extraction of total cellular RNA and Northern analysis. After culture with the indicated cytokines, $10–20 \times 10^6$ cells were washed three times with PBS and total RNA was prepared using

RNAzol (Cinna/Biotecx, Friendswood, TX) as previously described (40). Two to 5 μg of total RNA was electrophoresed on 1% agarose gels, and the gels were blotted and hybridized with ^{32}P-labeled cDNA inserts as previously described (40).

Statistical tests. A two-tailed *t* test was used for the statistical analysis of data comparing the results from DS and controls.

RESULTS

IL-4 effects on thymocyte proliferation. To determine whether IL-4 could induce proliferation of human postnatal thymocytes without the addition of mitogen, nonadherent mononuclear thymocytes (>98% CD2$^+$) were cultured in various concentrations of IL-4 for 5 d and proliferation was assessed by ^{3}H-thymidine incorporation. Kinetic analysis showed d 5 to be the time of peak ^{3}H-thymidine incorporation, followed at d 6 by the appearance of numerous large, blast-like cells and an increase in viable cell number. IL-4 induced a dose-dependent proliferative response that was significant at concentrations as low as 1 ng/mL and optimal for both DS and controls at 10 ng/mL (Fig. 1).

The proliferative response to IL-4, in the absence of mitogen, was comparable to that induced by well-established thymocyte growth factors. Figure 1 shows the results of a representative experiment in which the dose-dependent proliferation of control thymocytes to IL-4, IL-2, and IL-1β was compared. IL-4, in the absence of mitogen, induced proliferation that was comparable to that induced by IL-2 without mitogen. IL-4 was generally more potent at lower protein concentrations (10 ng/mL) than was IL-2. In the absence of mitogen, IL-1β, at the concentrations tested, did not induce proliferation of nonadherent mononuclear cells from human thymus. The proliferation-inducing effects of all three cytokines were enhanced in the presence of 25 μg/mL phytohemagglutinin (data not shown).

Proliferation of DS and control thymocytes in response to IL-4. The proliferative response of 18 control and eight DS thymocyte preparations to an optimal concentration of 10 ng/mL of IL-4 is shown in Figure 2. Both DS and control thymocytes proliferate in response to IL-4. The DS thymocytes, as a group, showed slightly lower levels of response to IL-4 than the controls (with median cpm × 10^{-3} of 4.32 and 5.74, respectively), but this difference was not found to be statistically significant.

Correlation of IL-4 response with proportion of TCRγ,δ$^+$ cells. There was considerable variability in the proliferative response to IL-4 among both control and DS thymocyte preparations tested (Fig. 2). Figure 3 demonstrates the range of responses of the 18 control thymocyte preparations to 10 ng/mL of IL-4, with the shaded area representing the mean ± SEM (14.63 ± 3.97 cpm × 10^{-3}) for the entire group. The mean response for cells cultured in the absence of IL-4 was 0.63 ± 0.09 cpm × 10^{-3}. Although younger subjects tended to show higher proliferative responses to IL-4, regression analysis did not reveal any statistically significant correlation between IL-4 responsiveness and age.

To determine the cell type(s) that is responsive to IL-4-induced proliferation and to ascertain the reason for the variability in IL-4 responsiveness among thymocyte preparations, we examined the correlation of the proliferative response to IL-4 (10 ng/mL) to the proportions of various thymocyte subpopulations, as de-

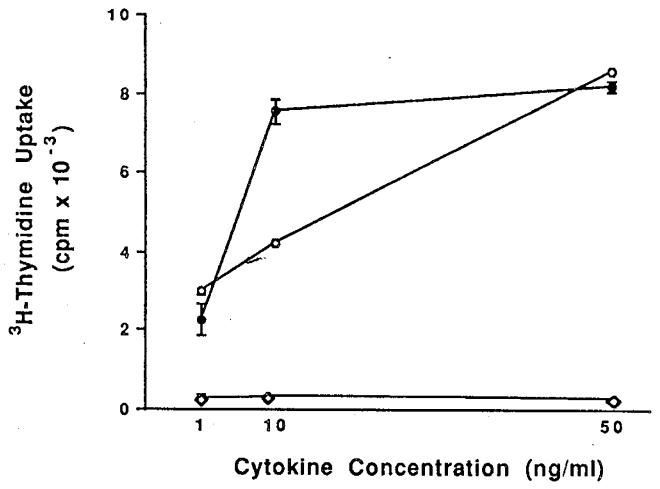

Fig. 1. Proliferative response of thymocytes to IL-4, IL-2, and IL-1β. A representative experiment, of three performed, in which nonadherent thymocytes were cultured in medium containing the indicated concentrations of IL-4 (●), IL-2 (○), or IL-1β (◇). Proliferation was measured by ^{3}H-thymidine uptake. The data points indicate the mean cpm and SEM of quadruplicate wells (1 × 10^5 cells/well). The mean cpm and SEM for thymocytes cultured in medium alone was 258 ± 57.

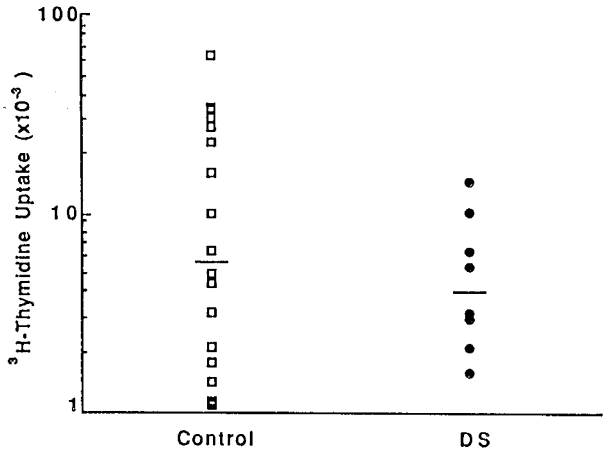

Fig. 2. Proliferative response of control and DS thymocytes to IL-4. Nonadherent thymocytes were cultured for 5 d in medium containing 10 ng/mL of IL-4. Proliferation was measured by ^{3}H-thymidine uptake. The data points indicate the mean cpm of quadruplicate wells of thymocytes (1 × 10^5 cells/well) from eight children with DS (mean age 6.8 mo) and 18 controls (mean age 4.1 mo). The mean cpm and SEM for thymocytes cultured in medium alone was 620 ± 100 and 350 ± 50 for control and DS cells, respectively.

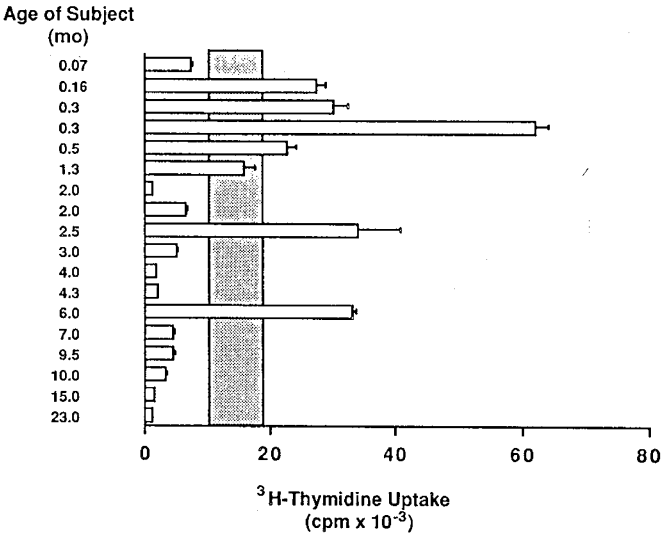

Fig. 3. Relationship between age and IL-4 responsiveness for control thymocytes. Nonadherent thymocytes from 18 control children were cultured for 5 d in medium containing 10 ng/mL IL-4. Proliferation was measured by ^{3}H-thymidine uptake. The data points indicate the mean cpm ± SEM of quadruplicate wells of thymocytes (1 × 10^5 cells/well). The *shaded area* represents the mean ± SEM of the proliferative response to IL-4 for the 18 experiments (14.63 ± 3.97 cpm × 10^{-3}). The mean response for cells cultured in the absence of IL-4 was 0.63 ± 0.09 cpm × 10^{-3}. The ages of the subjects examined are shown in the left column.

termined by dual-color immunofluorescence and flow cytometry. There was no significant correlation between IL-4 responsiveness and the proportion of TCRα,β^+ (dim or bright), $CD3^+$ (dim or bright), $CD4^-CD8^-$, $CD4^+CD8^+$, $CD4^+CD8^-$, or $CD4^-CD8^+$ thymocytes. However, Figure 4, which depicts the results of regression analysis correlating the proliferative response to IL-4 to the proportion of TCRγ,δ cells in total thymocyte populations from five control children, demonstrates that IL-4-induced proliferation is proportional to TCRγ,δ cell content ($R = 0.89$).

Development of new method for isolation of purified TCRγ,δ^+ thymocytes. To demonstrate directly that TCRγ,δ^+ thymocytes proliferate in response to IL-4, we sought to purify TCRγ,δ cells from human thymus. We first attempted purification by fluorescence-activated cell sorting. However, fluorescence-activated cell sorting separation took as long as 30 h to separate the requisite number of cells from total thymocyte populations and resulted in poor viability. Therefore, we developed a new procedure for positive selection of TCRγ,δ cells. Using an avidin-gel column system provided by CellPro, Inc., we took advantage of the fact that the pan TCRγ,δ MAb, anti-TCRδ1 (38), is a mouse IgG1 MAb and would react with the biotinylated rat anti-mouse IgG1 MAb developed for use with the avidin-gel column system.

Figure 5 shows histograms of the immunofluorescence analysis of a representative experiment in which TCRγ,δ thymocytes were purified from a control thymus using the avidin-gel column system. Only 1–5% of human postnatal thymocytes have been shown to express TCRγ,δ (Fig. 4) (41–43). The top panel shows reactivity of the total thymocyte population with anti-TCRδ1 MAb. In this experiment, 4% of the total thymocytes reacted with anti-TCRδ1 before purification. Immediately after purification, the bound cells that were eluted from the column were phenotyped by reactivity with GAM-FITC. In this experiment, 84% of the freshly purified cells reacted with GAM-FITC, indicating the presence of anti-TCRδ1 MAb on the cell surface (data not shown). The bound cells were also incubated in medium for 18 h at 37°C to regain reactivity with anti-TCRδ1, and the results are shown in the bottom panel. In this experiment, which is representative of five performed, 87% of the purified cells reacted with anti-TCRδ1 after the 18-h culture and the level of fluorescence was well above that observed for purified cells reacted with nonspecific mouse IgG alone (depicted by the broken line). In all five experiments, the results obtained by phenotypic analysis with GAM-FITC immediately after purification and those obtained after 18 h incubation and reactivity with anti-TCRδ1 were comparable. These estimates of purity may be conservative, considering the difficulty in performing immunofluorescence analysis on cells that have previously been reacted with antibody. The advantage of this procedure is that it requires only 2 h to perform and yields a highly purified population of TCRγ,δ cells with a viability of greater than 95%.

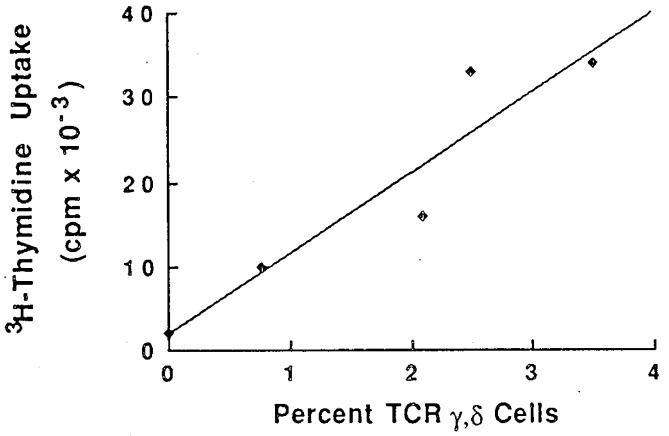

Fig. 4. Regression analysis of IL-4 proliferative response *vs* percentage of TCRγ,δ cells. Total nonadherent thymocyte preparations from five children were cultured for 5 d with 10 ng/mL IL-4. Proliferation was measured by ^{3}H-thymidine uptake. The mean cpm of quadruplicate wells (1×10^5 cells/well) are plotted on the vertical axis and the proportions of TCRγ,δ cells, as assessed by reactivity with anti-TCRδ1, are plotted on the horizontal axis. The formula for the regression line is: $y = 2.04 \pm 9.56x$, $R = 0.89$.

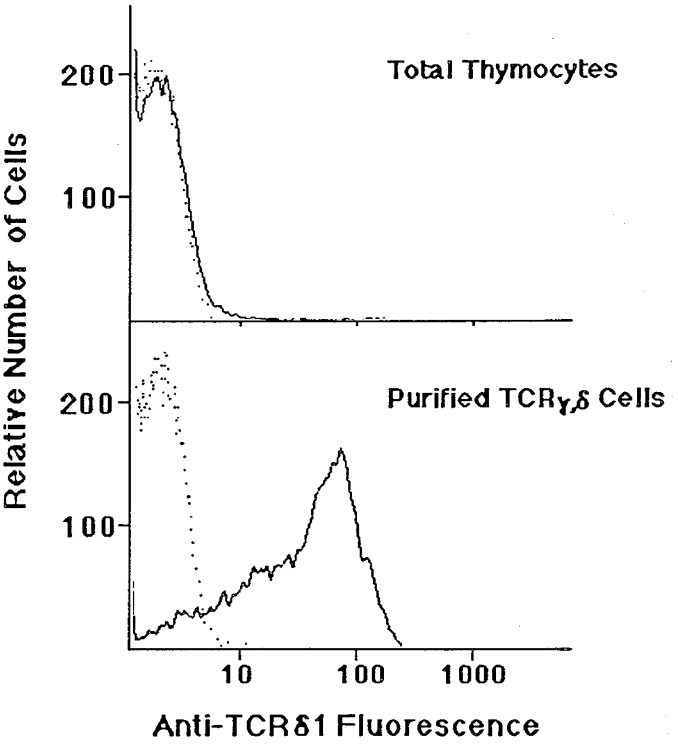

Fig. 5. Purification of TCRγ,δ^+ thymocytes by avidin-gel columns. A representative experiment, of five performed, in which total nonadherent thymocytes were purified using the avidin-gel column system. Cells eluted from the column were incubated in medium for 18 h at 37°C, washed extensively, and analyzed for reactivity with FITC-conjugated anti-TCRδ1 by immunofluorescence and flow cytometry. The horizontal axis represents the log fluorescence intensity of anti-TCRδ1 staining, and the vertical axis represents the relative number of cells. The *top panel* shows the level of anti-TCRδ1 staining of the total (unseparated) thymocyte population. The *bottom panel* shows anti-TCRδ1 staining of the purified TCRγ,δ cells. The level of fluorescence by cells reacted with nonspecific FITC-conjugated mouse IgG is indicated by the dashed lines. Four percent of the total thymocytes and 87% of the purified TCRγ,δ cells stained with anti-TCRδ1 beyond the level of fluorescence detected with nonspecific mouse IgG.

Proliferation of purified TCRγ,δ cells in response to IL-4. Figure 6 shows the results from three separate control thymuses in which we examined the IL-4-induced proliferation of purified TCRγ,δ cells as compared to total (unseparated) thymocytes and thymocytes that did not bind to the columns and were partially depleted of TCRγ,δ cells. In all three experiments, purified TCRγ,δ cells showed a greatly enhanced proliferative response to IL-4 as compared with the response of unseparated thymocytes. Of import was our additional observation that the proliferation of purified TCRγ,δ cells cultured without IL-4, but in medium alone, was not significantly higher than that of total thymocytes cultured in medium alone, demonstrating that the process of purification itself did not induce proliferation.

IFN-γ and TNF inhibition of IL-4-induced thymocyte proliferation. IFN-γ has been shown to inhibit the IL-4-induced proliferation of murine thymocytes (29). To determine if human thymocyte proliferation is also regulated by IFN-γ, we tested the effects of varying concentrations of IFN-γ on IL-4-induced proliferation. The effects of TNF were also evaluated. Figure 7

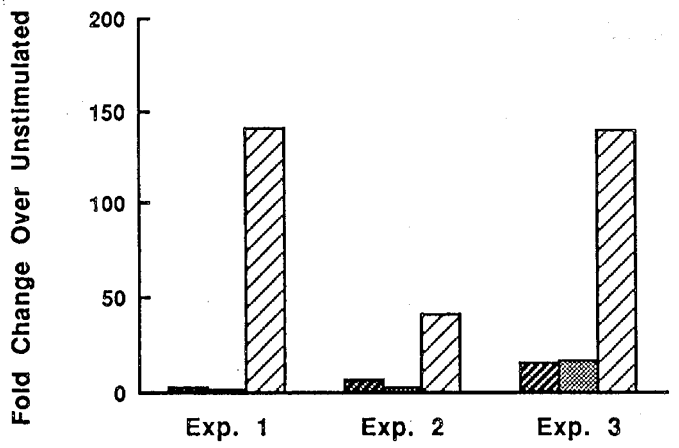

Fig. 6. IL-4-induced proliferation by purified TCRγ,δ cells. TCRγ,δ-bearing thymocytes were purified using avidin-sepharose columns. In three experiments, total thymocytes (▨), TCRγ,δ-depleted thymocytes (▩), or purified TCRγ,δ cells (▨) were cultured for 5 d in medium with or without IL-4 (10 ng/mL). Proliferation was measured by ^{3}H-thymidine uptake. The *bars* represent the fold change in the mean cpm of cells cultured with IL-4 as compared to cells cultured in medium alone. The mean cpm for total thymocytes, TCRγ,δ-depleted thymocytes, and purified TCRγ,δ cells cultured in medium alone were 230, 71, and 77, respectively, for experiment 1; 187, 188, and 606 for experiment 2; and 308, 206, and 703 for experiment 3.

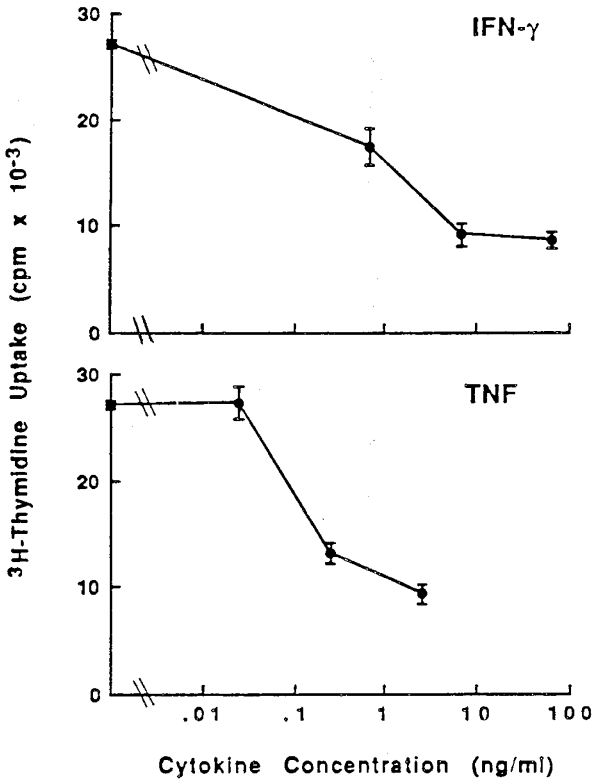

Fig. 7. Dose-dependent inhibition by IFN-γ or TNF of IL-4-induced proliferation. A representative experiment, of four performed, in which nonadherent thymocytes were cultured for 5 d in medium containing IL-4 (10 ng/mL). The indicated concentrations of IFN-γ (*top panel*) or TNF (*bottom panel*) were added at the initiation of culture. Proliferation was measured by ^{3}H-thymidine uptake. The data points indicate the mean cpm and SEM of quadruplicate wells of thymocytes (1×10^5 cells/well).

depicts a representative experiment demonstrating the inhibition of IL-4-induced proliferation of unseparated control thymocytes by IFN-γ (top panel) or TNF (bottom panel). Both cytokines elicited a dose-dependent inhibition of IL-4-induced thymocyte proliferation that was optimal at concentrations of 1–10 ng/mL. Synergistic inhibition of IL-4-induced proliferation by the combination of suboptimal concentrations of IFN-γ and TNF was not observed. Proliferation of purified TCRγ,δ cells was also inhibited by 30% by IFN-γ (6.7 ng/mL) and 21% by TNF (2.5 ng/mL) in one experiment, comparable to the inhibition observed for total, unseparated thymocytes (Fig. 8). IFN-γ or TNF alone, in the absence of IL-4, did not affect spontaneous thymocyte proliferation of total thymocytes or purified TCRγ,δ cells.

Greater sensitivity of DS thymocytes to inhibitory effects of IFN-γ and TNF. We have shown that DS fibroblasts are more sensitive to IFN-γ (11, 12). Therefore, we examined whether DS thymocytes are also more sensitive to inhibition of IL-4-induced proliferation by IFN-γ. In addition, because of our finding that TNF inhibits IL-4-induced proliferation, we also examined the effects of TNF on IL-4-mediated proliferation by DS thymocytes. Proliferation was induced in DS thymocyte preparations and controls by the optimal concentration of 10 ng/mL IL-4. Optimal inhibitory concentrations of IFN-γ (6.7 ng/mL) and TNF (2.5 ng/mL) were added at the initiation of culture. As expected, DS thymocytes were more sensitive to inhibition by IFN-γ, with a mean of 45% inhibition compared to 27% for controls ($p < 0.01$) (Fig. 8, left panel). To our surprise, we found that DS thymocytes were also more strongly inhibited by TNF, with a mean of 56% inhibition compared to 25% for controls ($p < 0.02$) (Fig. 8, right panel). Although the mean response of the DS group to IL-4 alone was lower than that of the controls (Fig. 8, legend), this is unlikely to explain the increased sensitivity to IFN-γ or TNF, because there was no correlation between responsiveness to inhibition by IFN-γ or TNF and the level of IL-4-induced proliferation of a given thymocyte preparation.

DISCUSSION

We have demonstrated that postnatal human thymocytes from both control and DS children proliferate in a dose-dependent manner to IL-4 and that this proliferation is unlike the IL-4-mediated proliferation of murine thymocytes, which requires a second signal such as that supplied by mitogen or phorbol ester (15–21). This difference between the response of human and

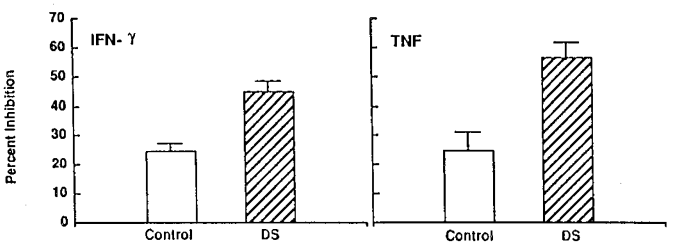

Fig. 8. Comparative responses of DS and control thymocytes to inhibition of IL-4-induced proliferation by IFN-γ or TNF. Nonadherent thymocytes from DS and control children were cultured for 5 d in medium containing IL-4 (10 ng/mL). In the *left panel*, an optimal inhibitory concentration of IFN-γ, 100 U/mL (6.7 ng/mL), was used. In the *right panel*, 100 U/mL (2.5 ng/mL) of TNF was used. Proliferation was measured by ^{3}H-thymidine uptake. The *data bars* indicate the mean and SEM of the percentage of inhibition for controls ($n = 18$ in left panel and 10 in right panel) or for children with DS ($n = 8$ in left panel and 6 in right panel). The percentage of inhibition for each experiment was calculated as follows: $100 \times [1 - (\text{mean cpm of quadruplicate wells containing IL-4 + IFN-}\gamma \text{ or TNF/mean cpm of quadruplicate wells containing IL-4 alone})]$. The mean cpm ($\times 10^{-3}$) of cultures containing IL-4 alone were 5.8 for DS and 14.6 for controls in the left panel and 6.7 for DS and 13.9 for controls in the right panel.

murine thymocytes may be explained by differences in either the number or affinity of IL-4 receptors on thymocytes. Immature murine thymocytes have been shown to express few high-affinity IL-4 receptors, the number of which is greatly enhanced by activation with phorbol ester (27). It is possible that postnatal human thymocytes constitutively express higher numbers of IL-4 receptors, alleviating the need for previous activation. In support of a species difference in IL-4 responsiveness, the induction by IL-4 of IgE synthesis in human peripheral blood mononuclear cells is not dependent on the addition of mitogen (44), whereas IL-4-mediated induction of IgE in murine cells requires previous activation with mitogen (45).

In the absence of mitogen, IL-4-stimulated human thymocyte proliferation occurs to an extent that is comparable to that induced by IL-2 and far greater than that induced by IL-1β. The IL-4-induced proliferation is independent of IL-2 because antibody to human IL-2, which effectively inhibited IL-2-induced proliferation, had no effect on the thymocyte proliferation stimulated by IL-4. Furthermore, IL-2 mRNA or protein was not detected in cultures of IL-4-stimulated cells (data not shown). IL-1 without the addition of mitogen did not induce significant proliferation of human postnatal thymocytes. Class II MHC$^+$ accessory cells have been shown to be necessary for IL-1-induced thymocyte proliferation (35), probably due to the production of IL-6 (46, 47). This suggests that our cultures, which were performed on nonadherent thymocyte preparations, may contain relatively few such accessory cells. However, it remains possible that low levels of thymic stromal cells were present in our cultures and that the activation of these cells, before or during culture, resulted in the production of numerous cytokines that may influence thymocyte proliferation (48, 49). Using the very sensitive technique of *in situ* hybridization, we have recently documented that both normal and DS thymuses contain cells that constitutively express IFN-γ and TNF mRNA and that DS thymuses contain 2-fold and 3.9-fold elevated numbers of IFN-γ- and TNF mRNA-expressing cells, respectively, than age-matched control thymuses (50). These results suggest that the thymocytes used in our experiments are likely to have been exposed to these cytokines before culture. In support for the endogenous production of TNF in our IL-4 cultures, we observed in two experiments that thymocytes cultured with IL-4 in the presence of neutralizing antibody to TNF proliferate to a greater extent than thymocytes cultured in IL-4 alone. Our finding of elevated levels of IFN-γ and TNF in DS thymuses, as well as the increased sensitivity of DS thymocytes to these cytokines, suggests an explanation for the slightly lower proliferative response of DS thymocytes to IL-4.

The considerable differences in the level of response to IL-4 among both the DS and the control thymocyte preparations examined is of interest. Although thymocytes from younger children were generally more responsive to IL-4, there was no obvious correlation between age and IL-4 responsiveness for either group. However, we did observe good correlation between the proportion of TCRγ,δ-bearing cells in thymocyte populations and IL-4 responsiveness, suggesting that the variability in the proliferative response to IL-4 may be due to differences in the proportion of TCRγ,δ cells. It is possible that the slightly lower median response of DS thymocytes to IL-4 may also be due to a lower proportion of TCRγ,δ cells in DS thymuses. However, thus far, we have observed no statistically significant difference in the proportion of TCRγ,δ cells in DS ($n = 3$) and control ($n = 7$) thymuses examined (1.7 and 1.4%, respectively).

Using a new and rapid avidin-gel column method for the purification of human TCRγ,δ-bearing thymocytes, we have demonstrated that positively selected TCRγ,δ cells proliferate in response to IL-4. Human TCRγ,δ cells are CD3$^+$ and predominantly CD4$^-$CD8$^-$ (41–43), although expression of CD4 (43, 51) and CD8 (41–43) on TCRγ,δ cells has been observed. Our demonstration that human TCRγ,δ-bearing thymocytes proliferate in response to IL-4 is consistent with reports in the murine system that IL-4-responsive thymocytes are generally CD4$^-$CD8$^-$ (15, 16, 19, 21, 27). It is also consistent with reports suggesting that IL-4-responsive human thymocytes (24) or bone marrow cells (52) are CD3$^+$. By directly purifying TCRγ,δ cells using positive selection, we have eliminated many of the problems presented by working with heterogenous populations of CD4$^-$CD8$^-$ thymocytes. We cannot rule out the possibility that our purified TCRγ,δ cells have been stimulated by cross-linking of the TCR. However, our data correlating IL-4 responsiveness with the percentage of TCRγ,δ cells in unseparated thymocyte populations suggests that resting TCRγ,δ cells also proliferate in response to IL-4. Our studies define IL-4 as an important proliferation factor for TCR$\gamma\delta$-bearing human thymocytes. Barcena *et al.* (25, 26) have shown that purified CD3$^-$CD4$^-$CD8$^-$ human thymocytes proliferate in response to IL-4 and that IL-4 can induce these cells to differentiate into TCRγ,δ cells *in vitro*. Therefore, IL-4 has effects not only on TCRγ,δ cells but on their precursors as well and plays a role in the maturation of human TCRγ,δ cells.

It is clear from our results, and those of others, that IL-4 has potent effects on developing thymocytes. *In vivo* data in IL-4 transgenic mice (53, 54) or murine fetal thymic organ culture (55, 56) suggest that overexpression of IL-4 results in a depletion of CD4$^+$CD8$^+$ thymocytes (53–56) and an inhibition in the production of TCRα,β-bearing cells (55, 56). Two explanations for the depletion of TCRα,β cells in these thymuses are possible. Given the known antagonism of the effects of IL-2 by IL-4 in other systems (57–59), the first possibility is that overexpression of IL-4 prevents the production or activity of IL-2, which has been proposed to play a critical role in differentiation of TCRα,β cells (19, 25, 26, 60–63). In light of our observation that IL-4 induces proliferation of TCRγ,δ-bearing thymocytes, the other possibility is that overexpression of IL-4 results in an increase in the proportion of TCRγ,δ cells and that TCRγ,δ cells (64), or cytokines produced by them (65), may influence TCRα,β cell maturation.

Although analysis of the effects of purified rIL-4 on human thymocytes *in vitro* is critical in defining a role for IL-4 in thymocyte proliferation and differentiation, other signals, including numerous other cytokines, are present in the thymus *in vivo* and may influence the effects induced by IL-4. For example, IL-4-mediated effects on the growth of murine fetal thymic organ cultures (56) and on mitogen-stimulated murine thymocytes *in vitro* (29, 66) were reversed by the addition of IFN-γ to the cultures. We also found antagonistic effects between IFN-γ and IL-4 in our human system, in that IFN-γ inhibited IL-4-induced proliferation of human thymocytes in a dose-dependent manner, with DS thymocytes being more strongly inhibited by IFN-γ than the controls. The 1.7-fold increased inhibition by IFN-γ of DS thymocytes compared to control thymocytes further illustrates the increased sensitivity of trisomy 21 cells to the effects of IFN-γ (11, 12) and conforms to expected gene-dosage effects due to an extra copy of a chromosome 21-encoded gene(s).

We observed that TNF also inhibits IL-4-mediated proliferation of human thymocytes. This finding was unexpected considering that TNF has been shown to have comitogenic effects on murine (33–36) or human (32) thymocyte proliferation when added together with IL-1 (34–36), IL-2 (32–34), or IL-4 (34). It is possible that the differences between human and murine thymocytes are due to differences in the expression of TNF receptors. Two distinct receptors for human (67–70) and murine (71) TNF have recently been identified and cloned. The 75-kd form of the murine TNF receptor, termed TNF-R2, has been shown to be responsible for the initiation of signals for murine thymocyte proliferation and to be the species-specific form of the TNF receptor (72). It is not known whether the comparable form of the human TNF receptor mediates the same effects as has been observed in the murine system.

It is unclear why DS cells are 2.2-fold more sensitive to inhibition by TNF. It is possible that DS cells express relative

differences in the abundance of one or both of the TNF receptors. However, neither receptor is encoded on human chromosome 21 (73). We also explored the possibility that TNF-mediated inhibition could be due to induction of IFN-α, -β, or -γ, inasmuch as DS cells are more sensitive to all three interferons (11), but we were unable to detect mRNA for IFN-γ in thymocytes cultured with IL-4 and TNF for 2 or 5 d. Furthermore, neutralizing antibodies to human IFN-α, -β, or -γ did not block TNF-mediated inhibition of IL-4-driven proliferation. In a reciprocal fashion, an MAb to TNF did not block IFN-γ-mediated inhibition, whereas it completely neutralized the inhibition caused by TNF (data not shown). Our current theory is that heightened sensitivity to TNF by DS thymocytes occurs because chromosome 21 encodes an additional gene or genes that confer sensitivity to TNF, or that the as yet undefined chromosome 21-encoded IFN-γ response element (74–76) may also play a role in the response to TNF.

An ordered and highly regulated production of cytokines in the thymus is most likely necessary for normal thymocyte maturation (13). Careful analysis of the production of cytokines by purified, freshly isolated human TCRγ,δ cells obtained by our new procedure will aid in elucidating the role of TCRγ,δ cells and in understanding the signals required for normal thymocyte maturation. In addition, our findings of increased sensitivity of DS cells to the inhibitory effects of IFN-γ and TNF suggest a mechanism in which a perturbation in the response to one or several cytokines may result in abnormal patterns of thymocyte maturation.

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REFERENCES

1. Burgio GR, Ugazio A, Nespoli L, Maccario R 1983 Down syndrome: a model of immunodeficiency. Birth Defects 19:325–327
2. Epstein CJ 1986 The Consequence of Chromosome Imbalance: Principles, Mechanisms, and Models. Cambridge University Press, New York
3. Levin S 1987 The immune system and susceptibility to infections in Down syndrome. In: McCoy EE, Epstein CJ (eds) Oncology and Immunology of Down Syndrome. Alan R Liss, New York, pp 143–162
4. Larocca LM, Piantelli M, Valitutti S, Castellino F, Maggiano N, Musiani P 1988 Alterations in thymocyte subpopulations in Down's syndrome (trisomy 21). Clin Immunol Immunopathol 49:175–186
5. Murphy M, Epstein LB 1990 Down syndrome (trisomy 21) thymuses have a decreased proportion of cells expressing high levels of TCRα,β and CD3. A possible mechanism for diminished T cell function in Down syndrome. Clin Immunol Immunopathol 55:453–467
6. Benda CE, Strassman GS 1965 The thymus in mongolism. J Ment Defic Res 9:109–117
7. Levin S, Schlesinger M, Handzel Z, Hahn T, Altman Y, Czernobilsky B, Boss J 1979 Thymic deficiency in Down's syndrome. Pediatrics 63:80–87
8. Murphy M, Epstein LB 1992 Down syndrome (DS) peripheral blood contains phenotypically mature $CD3^+TCR\alpha,\beta^+$ cells but abnormal proportions of $TCR\alpha,\beta^+$, $TCR\gamma,\delta^+$, and $CD4^+CD45RA^+$ cells: evidence for an inefficient release of mature T cells by the DS thymus. Clin Immunol Immunopathol 62:245–251
9. Jung V, Rashidbiagi A, Jones C, Tisenfield JA, Shows TB, Pestka S 1987 Human chromosome 6 and 21 are required for sensitivity to human interferon γ. Proc Natl Acad Sci USA 84:4151–4155
10. Fischer T, Rehm A, Aguet M, Pfizenmaier K 1990 Human chromosome 21 is necessary and sufficient to confer human IFNγ responsiveness to somatic cell hybrids expressing the cloned human IFNγ receptor gene. Cytokine 2:157–161
11. Epstein CJ, Epstein LB 1983 Genetic control of the response to interferon in man and mouse. In: Pick E (ed) Lymphokines, Vol 8. Alan R Liss, New York, pp 277–301
12. Epstein CJ, Weil J, Epstein LB 1986 Abnormalities in the interferon response and immune systems in Down syndrome: studies in human trisomy 21 and mouse trisomy 16. In: McCoy EE, Epstein CJ (eds) Oncology and Immunology of Down Syndrome. Alan R Liss, New York, pp 191–208
13. Carding SR, Hayday AC, Bottomly, K 1991 Cytokines in T-cell development. Immunol Today 12:239–245
14. Howard M, Farrar J, Hilfiker M, Johnson B, Takatsu K, Hamaoka T, Paul WE 1982 Identification of a T cell-derived B cell growth factor distinct from interleukin 2. J Exp Med 155:914–923
15. Palacios R, Sideras P, von Boehmer H 1987 Recombinant interleukin 4/BSF-1 promotes growth and differentiation of intrathymic T cell precursors from fetal mice *in vitro*. EMBO J 6:91–95
16. Zlotnik A, Ransom J, Frank G, Fischer M, Howard M 1987 Interleukin 4 is a growth factor for activated thymocytes: possible role in T-cell ontogeny. Proc Natl Acad Sci USA 84:3856–3860
17. Severinson E, Naito T, Tokumoto H, Fukushima D, Hirano A, Hama K, Honjo T 1987 Interleukin 4 (IgG1 induction factor): a multifunctional lymphokine acting also on T cells. Eur J Immunol 17:67–72
18. Carding SR, Bottomly K 1988 IL4 (B cell stimulatory factor 1) exhibits thymocyte growth factor activity in the presence of IL-2. J Immunol 140:1519–1526
19. Takei F 1988 IL-2 and IL-4 stimulate different subpopulations of double-negative thymocytes. J Immunol 141:1114–1119
20. Gause WC, Takashi T, Mountz JD, Finkelman FD, Steinberg AD 1988 Activation of CD4-CD8-thymocytes with IL4 vs. IL1 + IL2. J Immunol 141:2240–2245
21. Chen W-F, Fischer M, Frank G, Zlotnik A 1989 Distinct patterns of lymphokine requirement for the proliferation of various subpopulations of activated thymocytes in a single-cell assay. J Immunol 143:1598–1605
22. Spits H, Yssel H, Takebe Y, Arai N, Yokota T, Lee F, Arai KI, Banchereau J, de Vries J 1987 Recombinant interleukin 4 promotes the growth of human T cells. J Immunol 139:1142–1147
23. Dalloul AH, Mossalayi MD, Dellagi K, Bertho J-M, Debre P 1989 Factor requirements for activation and proliferation steps of human $CD2^+CD3^-CD4^-CD8^-$ early thymocytes. Eur J Immunol 19:1985–1990
24. Ueno Y, Boone T, Uittenbogaart CH 1989 Selective stimulation of human thymocyte subpopulations by recombinant IL-4 and IL-3. Cell Immunol 118:382–393
25. Barcena A, Toribio ML, Pezzi L, Martinez C 1990 A role for interleukin 4 in the differentiation of mature T cell receptor γ/δ^+ cells from human intrathymic T cell precursors. J Exp Med 172:439–446
26. Barcena A, Sanchez MJ, Luis de la Pompa J, Toribio ML, Kroemer G, Martinez C 1991 Involvement of the interleukin 4 pathway in the generation of functional γδ T cells from human pro-T cells. Proc Natl Acad Sci USA 88:7689–7693
27. Lowenthal JW, Ransom J, Howard M, Zlotnik A 1988 Up-regulation of interleukin 4 receptor on immature ($Lyt\text{-}2^-/L3T4^-$) thymocytes. J Immunol 140:474–478
28. Tentori L, Pardoll DM, Zuniga JC, Hu-Li J, Paul WE, Bluestone JA, Kruisbeek AM 1988 Proliferation and production of IL-2 and B cell stimulatory factor 1/IL-4 in early fetal thymocytes by activation through Thy-1 and CD3. J Immunol 140:1089–1094
29. Ransom J, Fischer M, Mosmann T, Yokota T, DeLuca D, Schumacher J, Zlotnik A 1987 Interferon-γ is produced by activated immature mouse thymocytes and inhibits the interleukin 4-induced proliferation of immature thymocytes. J Immunol 139:4102–4108
30. Carding SR, Jenkinson EJ, Kingston R, Hayday AC, Bottomly K, Owen JTT 1989 Developmental control of lymphokine gene expression in fetal thymocytes during T-cell ontogeny. Proc Natl Acad Sci USA 86:3342–3345
31. Reem GH, Cook LA, Henriksen DM, Vilcek J 1982 Gamma interferon induction in human thymocytes activated by lectins and B cell lines. Infect Immun 37:216–221
32. Reem GH, Duggan A, Schleuning M 1989 Immunoregulation and production of tumor necrosis factor α by human thymocytes. Cancer Res 49:3568–3573
33. Suda T, Murray R, Fischer M, Yokota T, Zlotnik A 1990 Tumor necrosis factor-α and P40 induce day 15 fetal thymocyte proliferation in combination with IL-2. J Immunol 144:1783–1787
34. Ranges GE, Zlotnik A, Espevik T, Dinarello CA, Cerami A, Palladino MA 1988 Tumor necrosis factor α/cachectin is a growth factor for thymocytes. Synergistic interactions with other cytokines. J Exp Med 167:1472–1478
35. Hurme M 1988 Both interleukin 1 and tumor necrosis factor enhance thymocyte proliferation. Eur J Immunol 18:1303–1306
36. Ehrke MJ, Ho RLX, Hori K 1988 Species-specific TNF induction of thymocyte proliferation. Can Immunol Immunother 27:103–108
37. Spits H, Borst J, Tax W, Capel P, Terhorst C, deVries J 1985 Characteristics of a monoclonal antibody (WT31) that recognizes a common epitope on the human T cell receptor for antigen. J Immunol 135:1922–1928
38. Band J, Hochstenbach F, McLean J, Hata S, Krangel MS, Brenner MB 1987 Immunochemical proof that a novel rearranging gene encodes the T cell receptor delta subunit. Science 238:682–684
39. Boyum FH 1968 Isolation of lymphocytes, granulocytes, and macrophages. Scand J Clin Lab Invest 21(suppl 97):77–89
40. Harris CA, Derbin KS, Hunte-McDonough B, Krauss MR, Chen KT, Smith DM, Epstein LB 1991 Manganese superoxide dismutase is induced by IFN-γ in multiple cell types. Synergistic induction by IFN-γ and tumor necrosis factor or IL-1. J Immunol 147:149–154
41. Groh V, Porcelli S, Fabbi M, Lanier L, Picker LJ, Anderson T, Warnke RA,

Bhan AK, Strominger JL, Brenner MB 1989 Human lymphocytes bearing T cell receptor γ/δ are phenotypically diverse and evenly distributed throughout the lymphoid system. J Exp Med 169:1277–1297
42. Bucy RP, Chen C-L H, Cooper MD 1989 Tissue localization and CD8 accessory molecule expression of T$\gamma\delta$ cells in humans. J Immunol 142:3045–3049
43. Porcelli S, Brenner MB, Band H 1991 Biology of the human $\gamma\delta$ T-cell receptor. Immunol Rev 120:137–183
44. Claassen JL, Levine AD, Buckley RH 1990 Recombinant human IL-4 induces IgE and IgG synthesis by normal and atopic donor mononuclear cells. Similar dose response, time course, requirement for T cells, and effect of pokeweed mitogen. J Immunol 144:2123–2130
45. Coffman RL, Ohara J, Bond MW, Carty J, Zlotnik A, Paul WE 1986 B cell stimulatory factor-1 enhances the IgE response of lipopolysaccharide-activated B cells. J Immunol 136:4538–4541
46. Helle M, Brakenhoff JPJ, De Groot ER, Aarden LA 1988 Interleukin 6 is involved in interleukin 1-induced activities. Eur J Immunol 18:957–959
47. Elias JA, Trinchieri G, Beck JM, Simon PL, Seghal PB, May LT, Kern JA 1989 A synergistic interaction of IL-6 and IL-1 mediates the thymocyte-stimulating activity produced by recombinant IL-1-stimulated fibroblasts. J Immunol 142:509–514
48. Denning SM, Kurtzberg J, Le PT, Tuck DT, Singer KH, Haynes BF 1988 Human thymic epithelial cells directly induce activation of autologous immature thymocytes. Proc Natl Acad Sci USA 85:3125–3129
49. Mizushima Y, Saitoh M, Ogata M, Kosaka H, Tatsumi Y, Kiyotaki C, Hamoaka T, Fujiwara H 1989 Thymic stroma-derived T cell growth factor (TSTGF). IV. Capacity of TSTGF to promote the growth of L3T4-Lyt-2- thymocytes by synergy with phorbol myristate acetate or various IL. J Immunol 142:1195–1202
50. Murphy M, Friend DS, Pike-Nobile L, Epstein LB 1992 TNF-α and IFN-γ expression in human thymus: localization and overexpression in Down syndrome (Trisomy 21). J Immunol (in press)
51. Aparicio P, Alonso JML, Toribio ML, Marcos MA, Pezzi L, Martinez-A C 1989 Isolation and characterization of (γ,δ) CD4$^+$ T cell clones derived from human fetal liver cells. J Exp Med 170:1009–1014
52. Saito H, Hatake K, Dvorak AM, Leiferman KM, Donnenberg AD, Arai N, Ishizaka K, Ishizaka T 1988 Selective differentiation and proliferation of hematopoietic cells induced by recombinant human interleukins. Proc Natl Acad Sci USA 85:2288–2292
53. Tepper RI, Levinson DA, Stanger BZ, Campos-Torres J, Abbas AK, Leder P 1990 IL-4 induces allergic-like inflammatory disease and alters T cell development in transgenic mice. Cell 62:457–467
54. Lewis DB, Yu CC, Forbush KA, Carpenter J, Sato TA, Grossman A, Liggitt DH, Perlmutter RA 1991 Interleukin 4 expressed *in situ* selectively alters thymocyte development. J Exp Med 173:89–100
55. Plum J, Smedt MD, Leclercq G, Tison B 1990 Inhibitory effect of murine recombinant IL-4 on thymocyte development in fetal thymus organ culture. J Immunol 145:1066–1073
56. Plum J, Smedt MD, Billiau A, Heremans H, Leclercq G, Tison B 1991 IFN-γ reverses IL-4 inhibition of fetal thymus growth in organ culture. J Immunol 147:50–54
57. Spits H, Yssel H, Paliard X, Kastelein R, Figdor C, de Vries J 1988 IL-4 inhibits IL-2-mediated induction of human lymphokine-activated killer cells, but not the generation of antigen-specific cytotoxic T lymphocytes in mixed leukocyte cultures. J Immunol 141:29–36
58. Martinez OM, Gibbons RS, Garovoy MR, Aronson FR 1990 IL-4 inhibits IL-2 receptor expression and IL-2-dependent proliferation of human T cells. J Immunol 144:2211–2215
59. Hern-Ku L, Xia X, Choi YS 1990 IL-4 blocks the up-regulation of IL-2 receptors induced by IL-2 in normal human B cells. J Immunol 144:3431–3436
60. Jenkinson EJ, Kingston R, Owen JJT 1987 Importance of IL-2 receptors in intra-thymic generation of cells expressing T cell receptors. Nature 329:160–162
61. Tentori L, Longo DL, Zuniga-Pflucker JC, Wing C, Kruisbeek AD 1988 Essential role of the interleukin-2 receptor pathway in thymocyte maturation *in vivo*. J Exp Med 168:1741–1747
62. Toribio ML, De la Hera A, Borst J, Marcos MAR, Marquez C, Alonso JM, Barcena A, Martinez-A C 1988 Involvement of the interleukin 2 pathway in the rearrangement and expression of both α/β and γ/δ T cell receptor genes in human T cell precursors. J Exp Med 168:2231–2249
63. Gause WC, Takashi T, Mountz JD, Finkelman FD, Steinberg AD 1988 Activation of CD4$^-$CD8$^-$ thymocytes with IL4 vs. IL1 + IL2. J Immunol 141:2240–2245
64. Obata Y, Taguchi O, Matsufiara Y, Hasegawa H, Hamasima N, Takahashi T 1991 Abnormal thymic development, impaired immune function and $\gamma\delta$ T cell lymphomas in a TL transgenic mouse strain. J Exp Med 174:351–362
65. Spits H, Yssel H, Brockelhurst C, Krangel M 1991 Evidence for controlled gene rearrangements and cytokine production during development of human TCR$\gamma\delta^+$ lymphocytes. Curr Top Microbiol Immunol 173:47–55
66. Hodgkin PD, Cupp J, Zlotnik A, Howard M 1990 IL-2, IL-6 and IFN-γ have distinct effects on the IL-4 plus PMA-induced proliferation of thymocyte subpopulations. Cell Immunol 126:57–68
67. Heller RA, Song K, Onasch MA, Fischer WH, Chang D, Ringold GM 1990 Complementary DNA cloning of a receptor for tumor necrosis factor and demonstration of a shed form of the receptor. Proc Natl Acad Sci USA 87:6151–6155
68. Smith CA, Davis T, Anderson D, Solam L, Beckmann MP, Jerzy R, Dower SK, Cosman D, Goodwin RC 1990 A receptor for tumor necrosis factor defines an unusual family of cellular and viral proteins. Science 248:1019–1023
69. Schall TJ, Lewis M, Koller KJ, Lee A, Rice GC, Wong GHW, Gatanaga T, Granger GA, Lentz R, Raab H, Kohr WH, Goeddel DV 1990 Molecular cloning and expression of a receptor for human tumor necrosis factor. Cell 61:361–370
70. Loetscher H, Pan H-YC, Lahm LW, Gentz R, Brockhaus M, Tabuchi H, Lesslauer W 1990 Molecular cloning and expression of the human 55 kd tumor necrosis factor receptor. Cell 61:351–359
71. Lewis M, Tartaglia LA, Lee A, Bennett GL, Rice GC, Wong GHW, Chen EY, Goeddel DV 1991 Cloning and expression of cDNAs for two distinct murine tumor necrosis factor receptors demonstrate one receptor is species specific. Proc Natl Acad Sci USA 88:2830–2834
72. Tartaglia LA, Weber RE, Figari IS, Reynolds C, Palladino Jr MA, Goeddel DV 1991 The two different receptors for tumor necrosis factor mediate distinct cellular responses. Proc Natl Acad Sci USA 88:9292–9296
73. Milatovich A, Song K, Heller RA, Francke U 1991 Tumor necrosis factor receptor genes, TNFR1 and TNFR2, on human chromosomes 12 and 1. Somatic Cell Mol Genet 17:519–523
74. Kalina U, Damlin K, Dembic Z, Fountoulakis M, Stueber D, Ozmen L, Garotta G 1991 Definition of the human IFNγ receptor regions(s) that interacts with the transducing factor encoded by human chromosome 21. J Interferon Res 11:S82(abstr)
75. Hibino Y, Kumar CS, Mariano TM, Lai D, Pestka S 1991 Chimeric interferon gamma receptors demonstrate an accessory factor required for activity interacts with the extracellular domain. J Interferon Res 11:S62(abstr)
76. Hemmi S, Aguet M 1991 Functional characterization of mouse/human IFNγ receptor hybrids: evidence for species specific interaction of the extracellular receptor domain with a putative signal transducer. J Interferon Res 11:S81(abstr)