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Interferons α/β inhibit IL-7-induced proliferation of CD4$^-$ CD8$^-$ CD3$^-$ CD44$^+$ CD25$^+$ thymocytes, but do not inhibit that of CD4$^-$ CD8$^-$ CD3$^-$ CD44$^-$ CD25$^-$ thymocytes

D.-M. SU, J. WANG, Q. LIN,* M. D. COOPER* & T. WATANABE *Department of Molecular Immunology, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan, and *Howard Hughes Medical Institute, Division of Developmental and Clinical Immunology, Departments of Medicine, Pediatrics and Microbiology, University of Alabama at Birmingham, Birmingham, AL, USA*

SUMMARY

Type 1 interferons (IFN-α/β) have recently been shown to inhibit interleukin-7 (IL-7)-induced growth and survival of early B-lineage cells. The CD3$^-$ CD4$^-$ CD8$^-$ (triple negative; TN) thymocytes from normal mice strongly proliferated upon stimulation with IL-7 in suspension culture. Such an IL-7-induced proliferation was suppressed by the addition of IFN-α/β, but a fraction of the TN thymocytes still showed proliferation. The IL-7-induced growth of TN thymocytes from scid mice, which lack the CD44$^-$ CD25$^-$ subpopulation, was completely inhibited by the addition of IFN-α/β. The IL-7 induced proliferation of CD4$^-$ CD8$^-$ thymocytes from T-cell receptor (TCR) transgenic mice, the majority of which are CD3$^+$ CD44$^-$ CD25$^-$, was resistant to IFN-α/β-mediated suppression. In fetal thymus organ cultures (FTOC), the addition of IL-7 greatly increased the population of CD4$^-$ CD8$^-$ CD44$^+$ CD25$^+$ thymocytes and IFN-α/β inhibited this IL-7-driven expansion. In contrast, the addition of IL-7 markedly decreased the percentages of CD4$^-$ CD8$^-$ CD3$^-$ CD44$^-$ CD25$^-$ cells, and IFN-α/β reversed the effect and increased the subpopulations of CD44$^-$ CD25$^+$ and CD44$^-$ CD25.$^-$ Finally, IFN-β mRNA was found to be expressed in the thymus. The data suggest that type 1 interferons inhibit IL-7-driven proliferation of TN thymocytes, but do not block the normal differentiation process.

INTRODUCTION

In the thymic microenvironment, immature CD4$^-$ CD8$^-$ CD3$^-$ triple-negative (TN) thymocytes rearrange their T-cell antigen receptor (TCR) genes, express their co-receptor CD4 and CD8 (double-positive; DP) cells to undergo positive and negative selections, and thereby differentiate to become self-major histocompatibility complex (MHC)-restricted mature CD4$^+$ (helper) or CD8$^+$ (killer) T cells.[1–3] The TN immature thymocytes can be subdivided into four developmental stages by their surface expression of CD44 and CD25.[4,5] The most immature subpopulation of CD44$^+$ CD25$^-$ thymocytes differentiates into the CD44$^+$ CD25$^+$ pro-T subpopulation, in which thymocytes begin to undergo TCRβ V(D)J gene rearrangement to become CD44$^-$ CD25$^+$ pre-T cells. The most mature TN thymocytes are CD44$^-$ CD25$^-$ post-pre-T cells that have successfully completed their TCRβ gene rearrangements.[4–9] The transition of CD44$^-$ CD25$^+$ pre-T into CD44$^-$ CD25$^-$ mature TN cells requires the expression of a functional TCRβ chain in association with the pre-Tα chain to form pre-T receptors.[10–12] T-cell development is arrested at the CD44$^-$ CD25$^+$ pre-T-cell stage in scid mice[13,14] and in mice deficient in the RAG-1 or RAG-2 genes,[6,15] which are unable to rearrange their TCR genes productively.

An analogous progression occurs early in the B-cell differentiation pathway. B220$^+$ CD43$^+$ bone marrow cells that are HSA$^-$BP-1$^-$ (fraction A, corresponding to CD44$^+$ CD25$^-$ TN thymocytes) differentiate to become HSA$^+$ BP-1$^-$ (fraction B, similar to CD44$^+$ CD25$^+$ TN thymocytes) cells, which progress to the HSA$^+$ BP-1$^+$ stage (fraction C, CD44$^-$ CD25$^+$ TN counterpart) and then undergo successful immunoglobulin gene rearrangements to become HSA^{++} BP-1$^+$ cells (fraction C′, CD44$^-$ CD25$^-$ TN counterpart).[16] B-cell development is arrested between fractions C and C′ in mice that are unable to express the pre-B receptor, such as the scid mice or mice deficient in RAG-2, membrane μ heavy chain or J_H genes.[17]

A variety of cytokines are involved in the growth and differentiation of thymocytes. These include interleukin-1 (IL-1),[18] IL-2,[19] IL-4,[20] IL-6,[18,21] tumour necrosis factor-α (TNF-α),[18,22] granulocyte–macrophage colony-stimulating

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Abbreviations: Ab, antibody; DN, double-negative thymocytes (CD4$^-$ CD8$^-$); DP, double-positive thymocytes (CD4$^+$ CD8$^+$); FTOC, fetal thymus organ culture; TN, triple-negative thymocytes (CD4$^-$ CD8$^-$ CD3$^-$).

Correspondence: Dr T. Watanabe, Medical Institute of Bioregulation, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-82, Japan.

factor (GM-CSF)[23] and IL-7.[24–27] Among these, IL-7 seems to be the most crucial for the proliferation of thymocytes, since disruption of either the IL-7 or IL-7 receptor (IL-7R) genes results in a drastic decrease in the number of thymocytes.[28,29] IL-7 induces thymocyte growth and particularly the expansion of the $CD44^+$ $CD25^+$ pro-T cells within the TN thymocyte compartment.[30] The pro-T cells are analogous to the pro-B cells ($B220^+$ $CD43^+$ HSA^+ $BP\text{-}1^-$), which undergo the immunoglobulin heavy chain gene rearrangement and are also highly IL-7 responsive. IL-7 is thus a growth factor for both early B- and T-lineage cells, regardless of the rearrangement status of their antigen (Ag)-receptor genes.

We have observed that the type 1 interferons (IFN-α/β) selectively inhibit the IL-7-induced proliferation of early B-lineage cells, and that pro-B cells are more sensitive to IFN-α/β-induced cell death than are pre-B cells.[31] Since IL-7 is also a crucial growth factor for thymocytes, we investigated the effect of IFN-α/β on the growth and differentiation of thymocytes in suspension and fetal thymus organ cultures (FTOC). These studies indicate that IFN-α/β inhibits the IL-7-induced expansion of TN thymocytes, but does not interfere with their differentiation to become mature $CD4^+$ or $CD8^+$ T cells. In addition, IFN-β was found to be expressed in the thymus and thus may represent a physiological regulator of thymocyte development.

MATERIALS AND METHODS

Mice

Four- to 6-week-old BALB/c mice were purchased from Kyudo (Kumamoto, Japan). Six-week-old scid mice (Fox Chase Scid C.b.-17 strain) were purchased from Fox Chase Cancer Center (Philadelphia, PA). Four- to 6-week-old ovalbumin T-cell receptor (OVA-TCR) transgenic (Tg) mice were kindly provided by Dr D. Y. Loh,[32] and maintained in specific pathogen-free (SPF) conditions. Fetuses were C57BL/6 mice obtained from the Laboratory of Embryonic and Genetic Engineering, Medical Institute of Bioregulation (Kyushu University, Japan). The day that fertilized eggs were transplanted into female mice was designated as day 0 of fetal age.

Cytokines and antibodies

Mouse α and β fibroblast IFN, which were produced in mouse L929 cell cultures by stimulation with Poly I:C, were purchased from Sigma (St Louis, MO). Recombinant IL-7 was purchased from Genzyme (Boston, MA). The IL-7R antibody (Ab), A7R34, was kindly provided by Dr S.-I. Nishikawa.[33] RL172 (anti-CD4) and HO2.2 (anti-CD8) Abs were the ascitic fluids of the hybridoma cells. Anti-CD3ε hybridoma 145-2C11 and 500A2 were the culture supernatant fluids. Fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated anti-CD8 (clone 53.6.7), PE-conjugated anti-CD4 (clone GK1.5), PE-conjugated anti-CD44 and biotin–anti-CD44 (anti-pgp-1, clone 1M7), FITC-conjugated anti-CD25 (anti-IL-2 receptor α-chain, clone 7D4), FITC-conjugated, PE-conjugated and biotin-conjugated anti-CD3ε antibodies were purchased from Pharmingen (San Diego, CA). RED 670-conjugated streptavidin (Avi-RED 670) was purchased from Life Technologies (Tokyo, Japan).

Isolation of thymocyte subpopulations

To isolate $CD4^-$ $CD8^-$, $CD4^+$ $CD8^+$, $CD4^+$ $CD8^-$ and $CD4^-$ $CD8^+$ subpopulations, thymocytes stained with PE–anti-CD4 and FITC–anti-CD8 were sorted with a FACSort flow cytometer (Becton Dickinson, Mountain View, CA). To prepare $CD4^-$ $CD8^-$ DN thymocytes from OVA-TCR transgenic mice, thymocytes (10^7/ml, 10 ml) in fetal calf serum (FCS)-free RPMI-1640 medium were incubated with RL172 and HO2.2 Ab (ascitic fluid diluted 100-fold with RPMI-1640 medium) for 45 min on ice, spun down, resuspended in FCS-free RPMI-1640 medium containing 1/10 diluted Low-Tox-M rabbit complement (Cedarlane, Ontario, Canada) and incubated for another 45 min at 37° in a water bath. Viable cells were then separated by centrifuging the cells on Lympholyte M (Cedarlane) at 600 *g* for 20 min at room temperature. The purity of the DN cells was found to be greater than 98% in most experiments. To prepare $CD4^-$ $CD8^-$ $CD3^-$ TN thymocytes from BALB/c mice, DN cells prepared as described above were incubated further in a plate coated with a mixture of two different anti-CD3ε antibodies (145-2C11, 500A2) at 4° for 45 min. The floating cells were harvested and incubated with anti-CD3ε Ab-conjugated magnetic beads (Dynal, Great Neck, NY). After removal of positive cells with a magnet, the purity of TN cells (about 0·2–0·4% of total cells) was tested by staining with PE–anti-CD4 plus FITC–anti-CD8 and FITC–anti-CD3, respectively. There were less than 2% of $CD4^{lo}$, less than 0·5% of $CD8^+$ and less than 3% of $CD3^{lo}$ cells in DN population contamination.

Cell proliferation assay

In 96-well flat-bottomed plates, cells (1×10^5/well in 200 μl of RPMI-1640 containing 5×10^{-5} M 2-mercaptoethanol, supplemented with 10% heat-inactivated FCS) were cultured for 2 days, pulsed with 1 μCi/well of [^{3}H]thymidine for the last 8 hr, and then harvested onto glass fibre filters. Thymidine uptake was assessed with a β-counter.

Flow cytometric analysis

To evaluate IL-7R expression by the different subsets of $CD4^-$ $CD8^-$ $CD3^-$ TN thymocytes, TN cells (10^5–10^6) were stained with biotinylated IL-7R Ab on ice for 20 min, washed with staining buffer (phosphate-buffered saline containing 2% FCS and 0·05% NaN_3), and then incubated with PE–CD44 Ab, FITC–CD25 Ab and Avi-RED 670. TN cells stained only with PE–CD44 Ab, FITC–CD25 Ab and Avi-RED 670 served as controls. The stained cells were analysed with a FACScan flow cytometer (Becton Dickinson).

Fetal thymus organ culture (FTOC)

Fetal thymi from C57BL/6 mice were cultured according to a procedure described previously.[34] In brief, day 14·5 fetal thymus lobes (2–3 lobes/well) were cultured in 1 ml of medium (RPMI-1640 containing 5×10^{-5} M 2-mercaptoethanol, supplemented with 10% heat-inactivated FCS) or in the presence of IL-7, IL-7 plus IFN-α/β or IFN-α/β alone, in Falcon 48-well plates in a 5% CO_2, 15% N_2, 80% O_2 (high oxygen culture) atmosphere at 37°. The thymocytes were harvested by pressing the lobes through a nylon mesh filter, and then stained with PE–CD4 Ab and FITC–CD8 Ab, or stained with biotin–CD44 Ab, PE–CD4+CD8 Ab and FITC–CD25 Ab, followed by

Avi-RED 670. Cells were analysed with a FACScan flow cytometer (Becton Dickinson).

IFN-β mRNA expression

Total RNA was extracted from approximately 10^6 thymus cells by an acid–guanidium–thiocyanate method and treated with RNase-free DNase I at 37° for 1 hr to digest the contaminated genomic DNA, followed by two rounds of phenol/chloroform/isoamyl alcohol extraction. First-strand cDNA was synthesized using AMV reverse transcriptase and oligo dT as primers in a 20-μl reaction. The polymerase chain reaction (PCR) was performed for 35 cycles in 50 μl buffer containing 200 μM each of dNTP, 25 pmol of each primer, 2·5 U Taq polymerase (Gibco, Grand Island, NY) and 5 μl of the first-strand cDNA. The forward and reverse primers for the IFN-β gene were 5′-CCTGGAGCAGCTGAATGGAA-3′ and 5′-GTACCTTTGCACCCTCCAGT-3′. The PCR reaction was performed at 94° for 1 min, 62° for 1·5 min and 72° for 2 min. The PCR products were hybridized with a ^{32}P-labelled internal primer (5′-AGCACTGGGTGGAATGAGAC-3′). For β actin gene expression, the forward (5′-TAGACTTCGAGCAGGAGGAGATG-3′) and the reverse (5′-CGTACTCCTGCTTGCTGATCCA-3′) primers were used for amplification at 94° for 1 min, 65° for 1·5 min and 72° for 2 min. Similarly, the actin PCR products were hybridized with a ^{32}P-labelled internal primer (5′-TCCATCATGAAGTGTGACGT-3′). As a control for these PCR reactions, PCR products were amplified from the first-strand cDNA in the same way except that reverse transcriptase was omitted.

RESULTS

IFN-α/β inhibits exogenous IL-7-induced growth and survival of immature thymocytes

Adult murine thymocytes were sorted into four subpopulations according to their CD4 and CD8 expression. IL-7-induced proliferation of sorted $CD4^-$ $CD8^-$ DN thymocytes was found to be inhibited by IFN-α/β (data not shown). We then examined the effect of IFN-α/β on IL-7-induced proliferation of $CD4^-$ $CD8^-$ $CD3^-$ TN thymocytes from scid and BALB/c control mice, and $CD4^-$ $CD8^-$ DN cells from OVA-TCR transgenic mice. We used DN cells from TCR transgenic mice because the majority (96%) of the DN cells expressed CD3 and therefore TN cells could not be purified. While IL-7-induced proliferation of the TN cells from scid mice was completely inhibited by IFN-α/β (Fig. 1b), the proliferation of the DN cells from OVA-TCR transgenic mice was not significantly inhibited by the IFN-α/β treatment (Fig. 1c). On the other hand, the proliferation of the TN cells from BALB/c normal control mice was only partially inhibited (Fig. 1a).

IL-7 receptor expression by TN cells from scid and normal mice, and by DN cells from TCR transgenic mice

Since differential sensitivity to IFN-α/β-induced growth inhibition was observed among TN thymocytes from normal and scid mice, and DN thymocytes from OVA-TCR transgenic mice, we surveyed the different subpopulations of the TN and DN cells for differences in the expression of IL-7 receptor. We found that the $CD44^+$ $CD25^-$, $CD44^+$ $CD25^+$ and $CD44^-$ $CD25^+$ subpopulations from normal control, TCR transgenic and scid mice all expressed IL-7R, although the percentages of $IL\text{-}7R^+$ cells and the expression levels differed (Fig. 2). The most striking difference in the scid, normal and TCR transgenic thymocytes was found in the $CD44^-$ $CD25^-$ population, which contained cells that had successfully undergone TCR gene rearrangement. In normal control mice, *c.* 28% of the TN cells was the $CD44^-$ $CD25^-$ population, but this population was absent among the TN thymocytes from scid mice, which were unable to rearrange their TCR and immunoglobulin genes correctly. In OVA-TCR transgenic mice, the $CD44^-$ $CD25^-$ population consisted of *c.* 70% of the DN thymocytes. The TN or DN cells from normal control or TCR transgenic mice that survived in the presence of IL-7 and IFN-α/β may thus mainly correspond to the $CD44^-$ $CD25^-$ $IL\text{-}7R^+$ population, although other subpopulations of DN cells from control mice could also be resistant to IFN-α/β treatment.

IFN-α/β inhibits IL-7-induced expansion of TN $CD44^+$ $CD25^+$ cells without affecting the TN $CD44^-$ $CD25^-$ subpopulation of thymocytes in FTOC

Additional evidence for the resistance of the $CD44^-$ $CD25^-$ population to the inhibitory effects of IFN-α/β was obtained in experiments employing FTOC. While IFN-α/β inhibited the IL-7-induced proliferation of $CD44^+$ $CD25^+$ cells dose dependently, it did not affect the $CD44^-$ $CD25^-$ and $CD44^-$ $CD25^+$ subpopulations of TN cells in FTOC (Fig. 3). These results suggest that IFN-α/β primarily inhibits

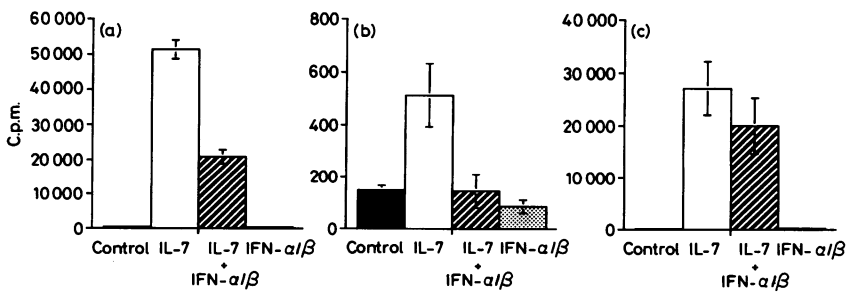

Figure 1. Inhibition of IL-7-induced proliferation of TN or DN thymocytes by IFN-α/β. TN thymocytes (1×10^5/well) from (a) control BALB/c mice, (b) BALB/c scid mice, or (c) DN thymocytes (1×10^5/well) from BALB/c OVA-TCR transgenic mice, were cultured for 2 days in media alone, in the presence of 100 IU/ml of IL-7 or 1000 IU/ml of IFN-α/β, or with both IL-7 and IFN-α/β. The cell cultures were pulsed with 1 μCi/well of [^{3}H]thymidine ([^{3}H]TdR) for the last 8 hr and the mean [^{3}H]TdR incorporation and standard errors were calculated from triplicate cultures.

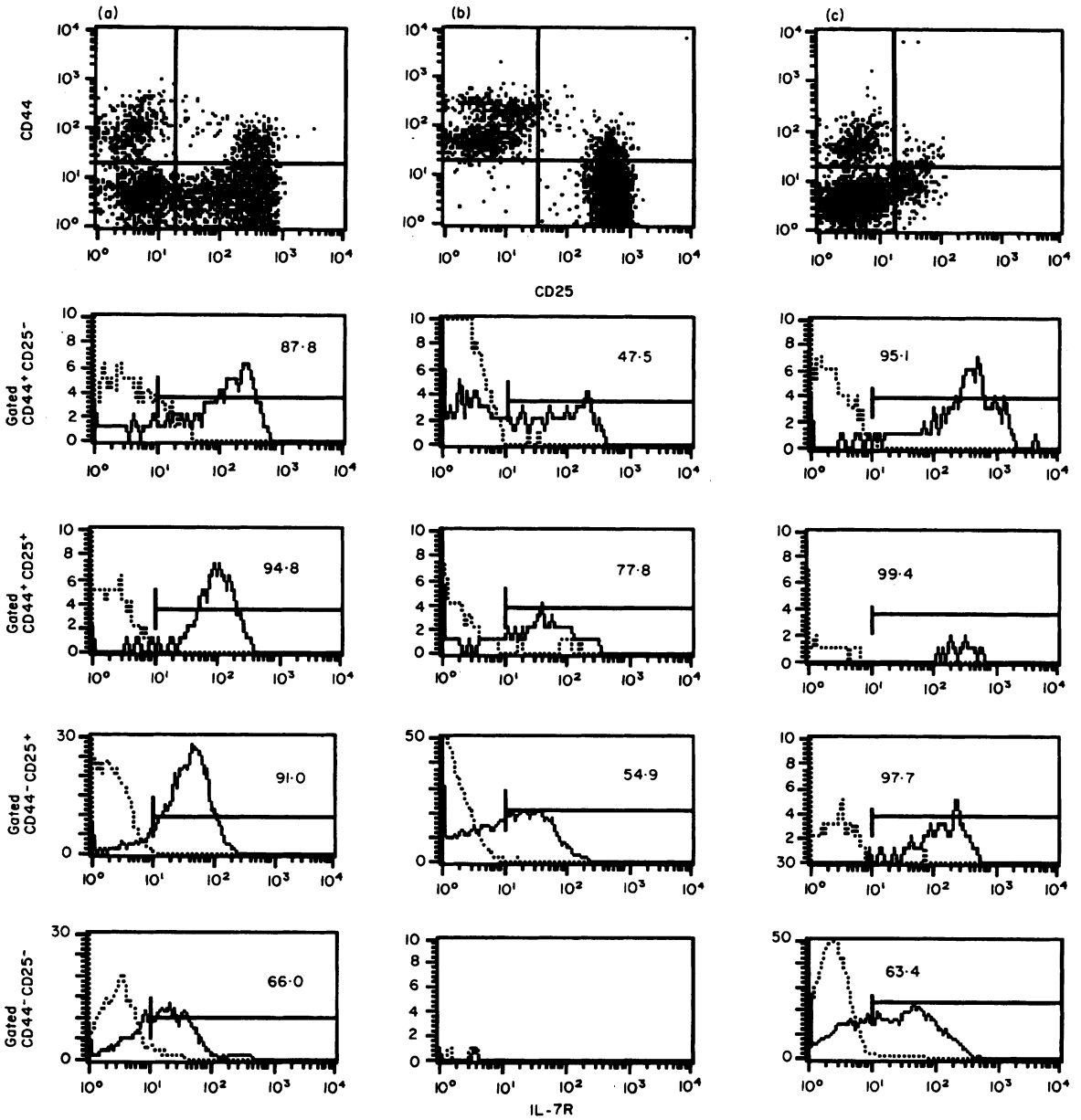

Figure 2. IL-7R expression by thymocyte subpopulations. Purified TN thymocytes from (a) normal, (b) scid and (c) DN thymocytes from OVA-TCR transgenic mice were stained with FITC-conjugated anti-CD25, PE-conjugated anti-CD44, biotin-conjugated anti-IL-7R antibody, followed by Avi-RED 670. Solid lines in the histograms indicate IL-7R staining, and dotted lines depict control staining.

IL-7-induced cell proliferation of the early-stage DN cells while having little effect on that of the late-stage DN thymocytes.

IFN-α/β treatment does not inhibit the differentiation of immature thymocytes into mature CD4$^+$ or CD8$^+$ cells in FTOC

The above data suggested that IFN-α/β serve as negative regulators of IL-7-induced proliferation, but may not provide a cell death signal for cells that have succeeded in their TCR gene rearrangements (CD44$^-$ CD25$^-$ population). When the effect of IFN-α/β alone on the generation of CD4$^+$ or CD8$^+$ mature cells in FTOC was examined, the number of mature CD4$^+$ or CD8$^+$ cells generated in FTOC was not significantly decreased (Fig. 4). These data further attest that IFN-α/β does not induce growth inhibition in fetal thymocytes that attain the capacity to differentiate into mature thymocytes.

IFN-β mRNA expression in the thymus

We have shown that IFN-α/β treatment affects the growth and survival of thymocytes at certain stages in their development. As a first step to determine whether IFN-α/β may represent physiological regulators, we examined the expression of IFN-β mRNA in the mouse thymus by reverse transcriptase (RT)-

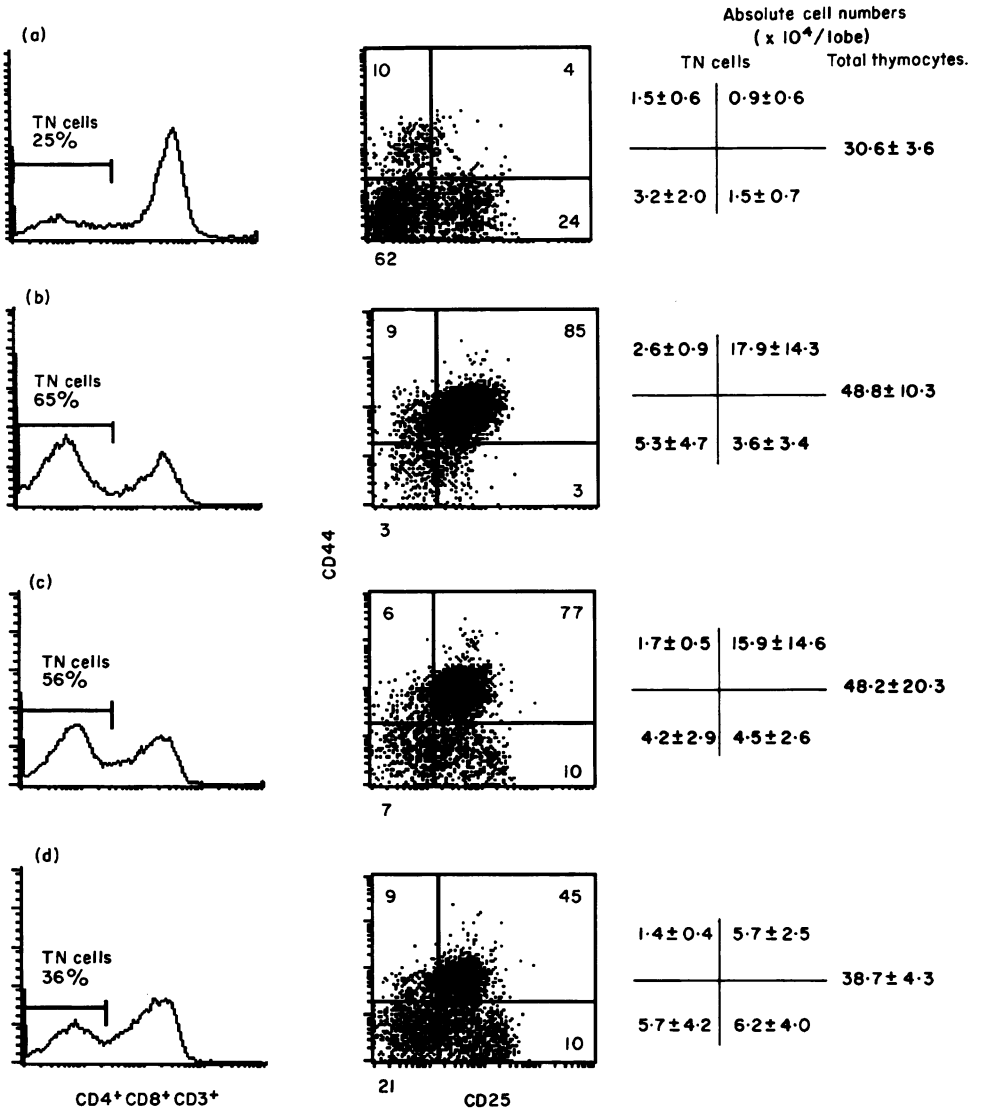

Figure 3. Analysis of TN subpopulations of gated CD4⁻ CD8⁻ CD3⁻ thymocytes in FTOC of C57BL/6 mice reacted with IL-7 and/or IFN-α/β. The left column shows the percentages of TN cells. The profiles of CD44 and CD25 expression in the TN cells are shown in the middle column. The absolute cell numbers of each subpopulation in TN cells and total cell number of thymus lobes are shown in the right column. FTOC was carried out in media alone (a), in the presence of 100 IU/ml IL-7 (b), with IL-7 plus 500 IU/ml of IFN-α/β (c), or with IL-7 plus 1000 IU/ml of IFN-α/β (d). On culture day 10, the cells were analysed with three-colour staining of PE-conjugated anti-CD4, anti-CD8 and anti-CD3ε, FITC-conjugated anti-CD25 and biotin–anti-CD44 followed by Avi-RED 670. The absolute cell number (mean cell number ± SD) in the quadrants was estimated based on the percentage of each subpopulation among total cells that was counted under a microscope with a haemacytometer (data from three independent experiments).

PCR analysis (the mice were kept under SPF conditions). We chose to analyse IFN-β because there is only one IFN-β gene in contrast to more than a dozen IFN-α genes. IFN-β mRNA expression was indeed detectable in the thymus (Fig. 5), thus supporting the possibility that constitutively produced IFN-β could be a physiological regulator of thymocyte development in the thymus microenvironment.

DISCUSSION

In the present study, we have demonstrated that type 1 interferons inhibit the IL-7-induced growth of immature TN thymocytes. The growth inhibition is attributable to apoptosis, at least in part (data not shown). We have previously shown that IFN-α/β similarly inhibits the IL-7-induced growth and survival of early B-lineage cells.[31] The type 1 interferons are thus negative regulators for early T and B cells. In contrast to the complete growth inhibition of scid thymocytes by IFN-α/β, a fraction of the TN thymocytes from normal mice and most of the DN thymocytes from OVA-TCR transgenic mice consistently survived in the presence of IFN-α/β. The presence of a CD44⁻ CD25⁻ IL7R⁺ subpopulation in thymocytes from wild-type and TCR transgenic mice, but not in scid mice, suggested that this might represent the IFN-α/β-resistant population. The idea was supported by experiments employing FTOC, in which IFN-α/β treatment inhibited the IL-7-induced

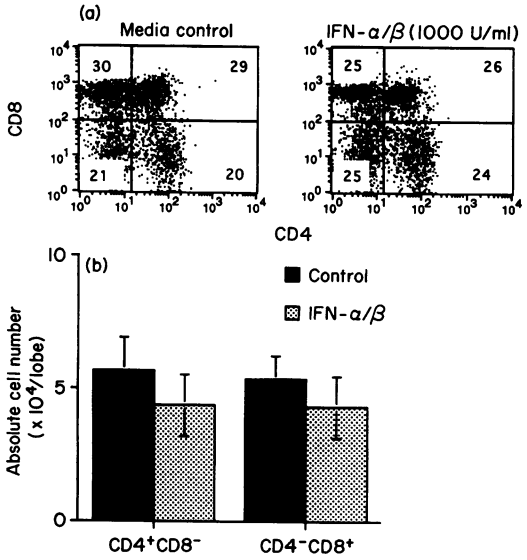

Figure 4. IFN-α/β treatment does not demonstrably inhibit thymocyte differentiation into the mature $CD4^+$ and $CD8^+$ single-positive populations. Profiles of thymocytes (a) and absolute cell numbers of $CD4^+$ or $CD8^+$ single-positive cells (b) from 10-day FTOC of C57BL/6 mice. FTOC was carried out in media control or in the presence of IFN-α/β (1000 IU/ml). Mean cell number $\pm$ SEM calculated from three independent experiments.

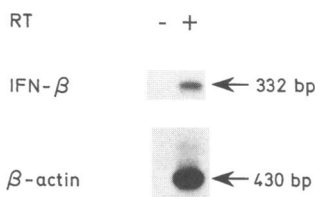

Figure 5. Expression of IFN-β RNA in the thymus. Thymus were obtained from 4–6-week-old BALB/c mice, the constituent cells were mechanically disrupted and the expression of IFN-β as well as β-actin mRNA was evaluated by RT-PCR analysis as described in the Materials and Methods.

expansion of $CD44^+$ $CD25^+$ cells without affecting the $CD44^-$ $CD25^-$ population. The $CD44^-$ $CD25^-$ population includes cells that have undergone successful TCRβ gene rearrangement[6] and thus correspond to the fraction C′ cells in B-cell development.[16] Using IL-7-dependent early B-cell lines representing different stages in the B-cell differentiation pathway, we have shown previously that surface μ^+ cell lines are relatively resistant to IFN-α/β-induced apoptosis compared with surface μ^- cell lines. The relative IFN-α/β resistance of the $CD44^-$ $CD25^-$ population ($TCR\beta^+$) is thus consistent with the observations in early B cells suggesting that μ^+ cells are less sensitive than μ^- cells to IFN-α/β treatment. Thus pre-B and pre-T cells may become less susceptible to IFN-α/β-induced apoptosis on expressing a functional pre-B- or pre-T-cell receptor.

The IFN-α/β inhibition of the IL-7-induced expansion of the $CD44^+$ $CD25^+$ subpopulation of thymocytes suggests that IFN-α/β may act to constrain IL-7-induced growth at this stage in thymocyte differentiation. The apparent lack of inhibition by IFN-α/β on the generation of mature $CD4^+$ or $CD8^+$ thymocytes in FTOC suggests that IFN-α/β may selectively affect cells with defective TCR gene rearrangement by inhibiting their proliferation and inducing apoptosis. The function of IFN-α/β within the thymic microenvironment thus could be to prevent IL-7-induced over expansion and to eliminate cells with a non-functional TCRβ gene rearrangement. In this regard, it should be interesting to study IFN-α/β receptor knock-out mice to see whether there is an accumulation of cells with non-productive gene rearrangements.[35]

It is surprising that the generation of mature $CD4^+$ or $CD8^+$ thymocytes in the FTOC system was not greatly impaired by IFN-α/β treatment. This contrasts with our observation that IFN-α/β treatment efficiently inhibited the generation of pro-/pre-B cells in long-term bone marrow cultures (LTBMC).[31] The contrasting nature of these results may be attributable to the differences in the conditions of FTOC and LTBMC. LTBMC is essentially limited to the generation of pro-/pre-B cell, and the progression of the murine B-cell differentiation pathway is highly dependent on IL-7. IL-7R Ab completely suppresses the growth of pro-B and pre-B cells in LTBMC,[31] whereas IL-7R Ab treatment of FTOC only partially inhibits the generation of mature $CD4^+$ or $CD8^+$ thymocytes (data not shown), suggesting that growth factors other than IL-7 are involved in the FTOC system, as has been shown by others.[9] Another important difference is that while long-term bone marrow culture does not support efficient differentiation of B cells, FTOC supports both DN thymocyte growth and differentiation into $CD4^+$ and $CD8^+$ T cells. The failure of IFN-α/β to inhibit the development of $CD4^+$ or $CD8^+$ mature thymocytes in FTOC suggests that IFN-α/β can not block the differentiation of $CD44^-$ $CD25^-$ cells to become mature T cells.

A number of cytokines have been identified as growth-promoting factors for thymocytes,[36] while few studies have focused on negative regulators of thymocyte proliferation. TGFβ has been shown to inhibit thymocyte proliferation induced by IL-1 or IL-7, although it was unclear as to which subpopulation was inhibited, and TGFβ inhibited the proliferative response to IL-1 more efficiently than the response induced by IL-7.[37] TGFβ has also been shown to affect CD8 expression[38] and to regulate the progression of thymocytes from immature $CD4^-$ $CD8^+$ cells to become $CD4^+$ $CD8^+$ DP cells.[39] Our results add type 1 interferons to the list of negative regulators for IL-7-induced proliferation of $CD4^-$ $CD8^-$ $CD3^-$ TN thymocytes. They also indicate the expression of IFN-β transcripts in the thymus. Since the generation of mature $CD4^+$ and $CD8^+$ thymocytes was not greatly affected by IFN-α/β treatment, we suggest that IFN-α/β could be involved in the preferential elimination of cells with defective TCRβ gene rearrangements. It remains to be determined which cell type(s) in the thymus expresses IFN-β and precisely how this cytokine might act in concert with other cytokines to regulate the growth and differentiation of thymocytes.

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