The Effect of In Vivo IL-7 Deprivation on T Cell Maturation

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Summary

A number of previous studies have suggested a key role for interleukin 7 (IL-7) in the maturation of T lymphocytes. To better assess the function of IL-7 in lymphopoiesis, we have deprived mice of IL-7 in vivo by long-term administration of a neutralizing anti-IL-7 antibody. In a previous report (Grabstein, K. H., T. J. Waldschmidt, F. D. Finkelman, B. W. Hess, A. R. Alpert, N. E. Boiani, A. E. Namen, and P. J. Morrissey. 1993. *J. Exp. Med.* 178:257–264), we used this system to demonstrate the critical role of IL-7 in B cell maturation. After a brief period of anti–IL-7 treatment, most of the pro–B cells and all of the pre–B and immature B cells were depleted from the bone marrow. In the present report, we have injected anti–IL-7 antibody for periods of up to 12 wk to determine the effect of in vivo IL-7 deprivation on the thymus. The results demonstrate a >99% reduction in thymic cellularity after extended periods of antibody administration. Examination of thymic CD4- and CD8- defined subsets revealed that, on a proportional basis, the CD4+, CD8+ subset was most depleted, the CD4 and CD8 single positive cells remained essentially unchanged, and the CD4–, CD8– compartment actually increased to ∼50% of the thymus. Further examination of the double negative thymocytes demonstrated that IL-7 deprivation did, indeed, deplete the CD3–, CD4–, CD8– precursors, with expansion of this subset being interrupted at the CD44+, CD25+ stage. The proportional increase in the CD4–, CD8– compartment was found to be due to an accumulation of CD3+, T cell receptor α,β + double negative T cells. Additional analysis revealed that anti–IL-7 treatment suppressed the audition/selection process of T cells, as shown by a significant reduction of single positive cells expressing CD69 and heat stable antigen. Finally, the effects of IL-7 deprivation on the thymus were found to be reversible, with a normal pattern of thymic subsets returning 4 wk after cessation of treatment. The present results thus indicate a central role for IL-7 in the maturation of thymic-derived T cells.

IL-7 has been demonstrated to play a key role in the maturation of B lymphocytes (for review see reference 1). In addition to directly inducing growth of early B cells (2, 3), studies have shown that the absence of IL-7 severely compromises B cell maturation (4–6). Consistent with these results, we recently demonstrated that short-term in vivo administration of an anti–IL-7 antibody profoundly blocks B cell development, with cells failing to progress beyond the S7 (CD43)+, heat stable antigen (HSA)[1]lo pro–B cell stage (7).

IL-7 has also been proposed to promote the maturation of T cells, although its mode of action is less clear. Experiments have documented the presence of both IL-7 messenger RNA and protein in the thymus (2, 8, 9). In addition, in

vitro studies have demonstrated the capacity of IL-7 to promote the viability or growth of early T cells obtained from adult and fetal thymus (10–17), as well as costimulate thymocytes when cultured with IL-2 or mitogens (11, 13–15). Subset analysis of thymic T cells has indicated that IL-7 preferentially stimulates CD4–, CD8– double negative (DN) T cells, especially those expressing IL-2 receptor (12, 16, 17). Additional studies have suggested an effect of IL-7 on TCR rearrangement. When cultured with IL-7, T cell precursors from thymus or fetal liver were found to express rearranged beta or gamma chain transcripts (18–20).

In vivo studies have further indicated a role for IL-7 in T cell development. Infusion of recombinant IL-7 into normal adult mice results in an increase of peripheral T cells and accelerates recovery of T cells in mice treated with irradiation or chemotherapy (21–24). Similar increases in T cell numbers are observed in IL-7 transgenic mice (25). When injecting an anti–IL-7 receptor antibody for 14 d, Nishikawa and co-

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[1] *Abbreviations used in this paper:* BSS, balanced salt solution; DN, double negative; DP, double positive; HSA, heat stable antigen; SP, single positive; TN, triple negative; TSLP, thymic stromal-derived lymphopoietin.

workers observed a marked reduction in thymic cellularity, as well as a partial loss of peripheral T cells (26). Anti–IL-7 antibody was also found to suppress the capacity of thymic implants to reconstitute conventional T cells in athymic mice (27). In our previous study examining the effects of injected anti–IL-7 antibody on normal mice, a loss of thymic content was likewise observed after short-term treatment (7). Taken together, the various studies suggest an important function for IL-7 in T cell maturation.

In the present report, we have further assessed the requirement for IL-7 in T cell maturation by injecting normal adult mice with high dose anti–IL-7 antibody for up to 12 wk. By neutralizing the cytokine in this manner, we were able to document the dependence of thymocyte maturation on IL-7 and characterize the effect of IL-7 deprivation on the various thymic subsets. The results demonstrate a profound loss of thymic cellularity upon anti–IL-7 antibody injection, with a >99% depletion of thymocytes at 12 wk of treatment. When examining the CD4- and CD8- defined subsets, it was found that on a proportional basis, the double positive (DP) population exhibited a substantial loss, the CD4 and CD8 single positive (SP) subsets were unchanged, and the DN subset actually increased. The increase of DN cells was due to a greater proportion of TCR α,β+ CD4–, CD8– T cells, and a reduction of the CD3–, CD4–, CD8– triple negative (TN) precursor population. Thus, in addition to playing a central role in B cell maturation, the present results strongly imply a similar role for IL-7 in T cell development.

Materials and Methods

Mice. 6–8-wk old female BALB/c mice were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN) and housed in specific pathogen-free investigator access rooms throughout the study.

Antibodies for In Vivo Treatment. M25 (7), a mouse IgG2b anti–human IL-7 monoclonal antibody and Flag-M1 (7), an isotype-matched control, were purified from culture supernatants on protein A affinity columns. The purified preparations contained <10 pg of endotoxin/mg of antibody.

Antibodies for Flow Cytometry. For flow cytometric analysis, the following antibodies were used: GK1.5, rat anti–mouse CD4 (28); 53.6.72, rat anti–mouse CD8 (29); 145-2C11, hamster anti–mouse CD3 (30); H57-597, hamster anti–mouse TCR α,β (31); 7D4, rat anti–mouse IL-2Rα (32); 9F3, rat anti–mouse CD44 (33); M1/69, rat anti–mouse HSA (34); F23.1, rat anti–mouse Vβ8 (35), and 6B2, a rat anti–mouse B220 (36). Antibodies were semipurified by 50% saturated ammonium sulfate precipitation from serum-free supernatants (HB101) and were conjugated with FITC, biotin, and Cyanine 5.18, using standard procedures. PE-conjugated anti-CD3, anti-CD4, and anti-CD8 were purchased from PharMingen (San Diego, CA), as were biotin-labeled anti–murine CD69, antibodies to the Vβ3, Vβ11, and Vβ12 forms of the TCR. FITC-avidin and Texas Red-avidin were purchased from Vector Laboratories, Inc., Burlingame, CA.

Treatment Protocol. Mice were injected intraperitoneally with 1 mg of either M25 or Flag-M1 control antibody in a 100 μl vol, three times per wk. Thymic cells were analyzed at 2, 4, 8, and 12 wk of treatment.

Cell Preparation. Thymic cells were dissociated from the thymus by gently pressing the thymus glands between the frosted ends of two glass slides. The cells were suspended in balanced salt solution (BSS) and washed by centrifugation at 1,500 rpm for 7 min. Mononuclear cells were isolated by density gradient centrifugation (Lympholyte M; Cedarlane Laboratories, Ltd., Hornby, Canada). Cells were washed again in BSS and suspended in staining solution (5% fetal bovine serum in BSS + 0.1% sodium azide). Bone marrow cells were obtained by flushing out cells from the dissected femur and tibia with BSS, using a 30-gauge needle. Mononuclear cells were prepared as described above.

Flow Cytometric Analysis. Staining was performed as a two-step procedure. 1 × 10⁶ cells were suspended in staining buffer consisting of 5% serum and 0.1% NaN₃ in BSS. The cells were, first, incubated with biotin, FITC, PE, or Cyanine 5.18–conjugated antibodies for 20 min at 4°C. 25 μg 2.4G2 antibody and 10 μl normal rat serum were added to eliminate background staining due to Fc receptors. After washing, cells were further incubated with the appropriate avidin conjugate for 20 min at 4°C followed by additional washings and subsequent FACS® analysis. Cells were run on a flow cytometer (FACS® 440; Becton Dickinson & Co., Mountain View, CA) equipped with a primary argon ion laser and a rhodamine 6G CR599 dye head laser (Coherent, Inc., Palo Alto, CA), pumped by a second argon ion laser. 20 thousand cells were collected per sample. The FACS® 440 data were collected and analyzed using a VAX station 3200 computer (Digital Equipment Corp., Maynard, MA) equipped with DESK software (kindly supplied by Wayne Moore, Stanford University, Stanford, CA). Two-color contours are represented as 5% probability plots.

Results

M25 is a mouse anti–human IL-7 monoclonal antibody which cross-reacts with and neutralizes murine IL-7 (7). Since it is an autologous protein (BALB/c derived), it can be given in high concentrations to mice for long periods of time without risk of an anti-IgG immune response. The neutralizing capacity of this antibody was demonstrated in a previous study, in which short-term administration of the M25 protein completely blocked B cell maturation at an early stage (7). To similarly test the effect of IL-7 deprivation on T cell maturation, mice were treated from 2 to 12 wk with anti–IL-7 or control antibody, with each mouse receiving 1 mg of protein three times a week. In data not shown, all mice treated with anti–IL-7 antibody lacked the prominent population of B220ᵗᵒ, HSAʰⁱ (early plus immature) B cells normally found in the bone marrow, indicating the effectiveness of the treatment over extended periods.

When administrating the anti–IL-7 antibody to adult mice, the cell recovery from the thymus decreases dramatically, such that by 12 wk of treatment, a 99% reduction in thymic cellularity is observed (Fig. 1). This is in contrast to mice treated with control antibody, which maintained a normal level of thymocyte counts. Fig. 1 further demonstrates a biphasic loss of thymocytes, in that cellularity was rapidly lost during the first 2 wk of M25 administration, followed by a more gradual loss during the latter 10 wk.

To assess the effect of IL-7 deprivation on the major thymic subsets, recovered cells were stained with antibodies to CD4 and CD8, and the proportion of the four major populations was determined with flow cytometry. Although all popula-
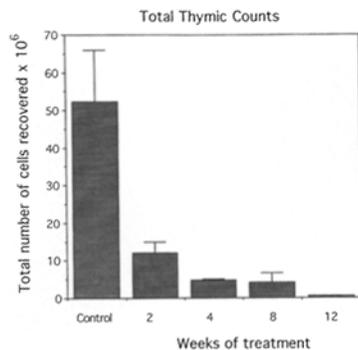
Figure 1. Thymic cell recovery is markedly decreased by the administration of anti–IL-7 antibody. Cell counts were obtained from the suspended thymus glands of adult BALB/c mice (three–five per group) intraperitoneally injected with 1 mg of monoclonal anti–IL-7 antibody three times a week for 2, 4, 8, or 12 wk. The control group represents the mean of all mice similarly injected with the isotype matched mouse IgG. (12-wk control-treated thymus averaged 58 × 10^6 recovered cells). The data are shown as mean ± SEM.

Figure 2. Anti–IL-7 treatment alters the proportion of CD4- and CD8-defined thymic subsets. Thymic cells were obtained from mice treated for 2–12 wk and processed for flow cytometry as described in Materials and Methods. The cells were stained with PE-conjugated anti-CD4 and Cyanine-conjugated anti-CD8 before flow cytometric analysis. The percentage of each population was calculated by integration. The control group represents the average of all mice injected with the isotype-matched mouse IgG. The values present the mean (three–five mice per group) ± SEM.

tions experienced a loss in absolute numbers (Table 1), the CD4+, CD8+ (DP) subset was most affected on a percentage basis. Whereas the DP population normally composes ~70% of the thymocyte content, Fig. 2 demonstrates that this subset dropped to 25% after 12 wk of anti–IL-7 treatment. The proportion of CD4 and CD8 SP cells remained relatively unchanged, although there was a trend towards increased levels of CD4 SP cells in M25-treated mice. The percentage of CD4-, CD8- DN cells was also markedly affected, with an increase to almost 50% of the thymus upon long-term M25 treatment. Histologic examination of thymus tissue from treated mice was also performed. In addition to a marked loss of mass, fixed and stained (hematoxylin and eosin) tissue revealed a loss of structure and definition in the cortical zones, with retention of medullary morphology (data not shown). This is consistent with a loss of the immature stages.

Table 1. *Total Cell Recovery per Thymic Subset*

Week	Mean total cells × 10^6			
	DN	DP	CD4 SP	CD8 SP
Control	3.4	37.9	8.5	2.9
2	1.6	5.8	3.2	1.5
4	1.4	1.6	1.3	0.4
8	0.9	0.9	1.0	0.3
12	0.1	0.06	0.08	0.02

Values were obtained by multiplying the total thymus cell recoveries at 2, 4, 8, and 12 wk of M25 treatment by the corresponding percent for each population. Percentages were obtained flow cytometrically using anti-CD4 and anti-CD8 staining. The control group values represent the mean of all mice injected with the isotype-matched mouse IgG.
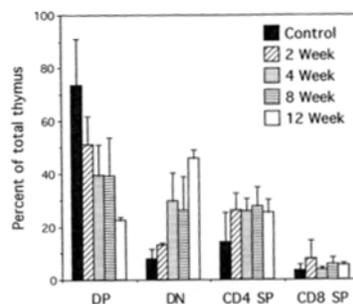
Since the DN thymic subset is known to contain the least mature cells in the thymus, it was anticipated that this population would be most sensitive to the effects of IL-7 deprivation. It was curious, therefore, that the DN population was increased on a proportional basis with M25 administration. Three-color flow cytometry was, therefore, used to assess the composition of the DN compartment, using antibodies to CD4 and CD8 in combination with a panel of other markers. Fig. 3 *A* shows the CD4 vs. CD8 contour plots of animals treated with anti–IL-7 or control antibody for 8 wk. When analyzing the phenotype of the DN subset, as demonstrated in Fig. 3 *B*, it is clear that anti–IL-7 treatment (*shaded histograms*) markedly reduced the percentage of early thymocytes as compared to controls (*open histograms*). This is demonstrated by a marked drop in the CD25+, HSA+ subset. Most of the DN cells at this time point are TCR α,β +, CD5^{hi}, and CD44^{hi}. Consistent with previous reports, the level of TCR expression on these DN thymocytes is intermediate compared to mature SP cells (37, 38). The proportion of TCR γ,δ + thymic cells is not markedly different between anti–IL-7 and control-treated mice. It appears, therefore, that the preferential increase in the percentage of DN thymocytes results from the resistance of DN TCR α,β + T cells to IL-7 deprivation. Fig. 4 illustrates the three-color flow cytometric analysis of DN thymocytes from mice treated for 2, 4, and 12 wk with anti–IL-7 (*shaded histograms*) or control (*open histograms*) antibody. Together, these data clearly show a steady loss of the early thymic CD25+, HSA+ compartment, such that by 12 wk of anti–IL-7 treatment, virtually all of the DN subset is composed of TCR α,β + T cells. Although the results indicate that long-term maintenance of the precursor population is dependent upon IL-7, the loss of these cells was progressive and not abrupt. This is in contrast to our previous studies on the B cell lineage, where IL-7 deprivation caused a rapid and complete loss of early B cells (7). Thus, whereas M25 treatment has a pronounced effect on both B and T cell precursors, the early B cell compartment appears much more sensitive to the deprivation of IL-7.
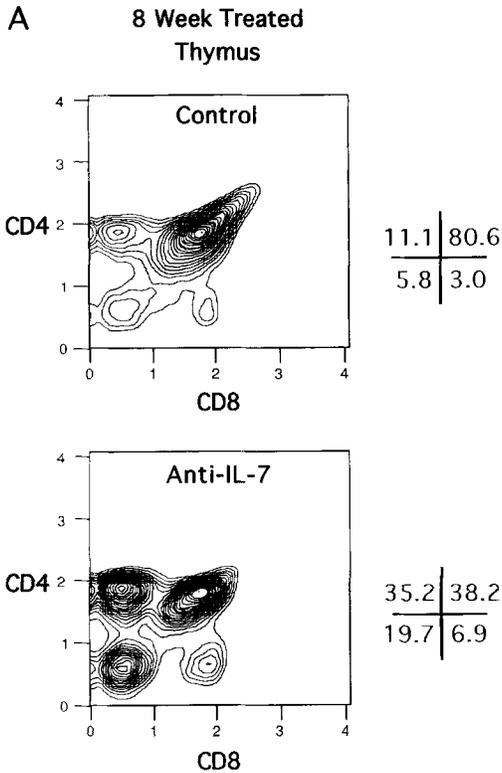
A   8 Week Treated
Thymus

Control

11.1 | 80.6

5.8 | 3.0

CD4

CD8

Anti-IL-7

35.2 | 38.2

19.7 | 6.9

CD4

CD8

Figure 3. Anti–IL-7 treatment depletes a DN CD25+, CD44–, HSA+, CD5–, TCR α,β + subset. Thymic cells from adult BALB/c mice treated for 8 wk with anti–IL-7 or isotype-matched control antibody were stained with conjugated antibodies and analyzed by three-color flow cytometry as described in Materials and Methods. Cells were stained with PE-conjugated anti-CD4, Cyanine-conjugated anti-CD8, and biotin-conjugated antibodies to CD25, CD44, HSA, CD5, TCR α,β, or TCR γ,δ plus FITC-avidin. (A) Two-color contours of CD4- and CD8-stained thymocytes. (B) Phenotype of CD4–, CD8– gated thymocytes from anti–IL-7 (*shaded histograms*)– and control IgG (*open histograms*)–treated mice. In A, the percentages for the DN, DP, CD4 SP, and CD8 SP subsets are shown to the right of each contour plot.

B   Double Negative Thymocytes
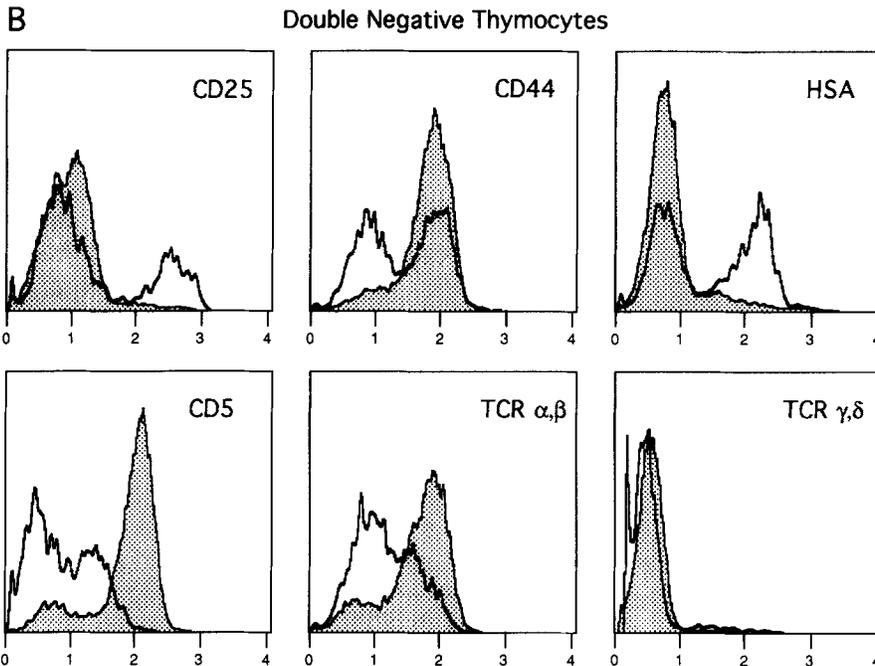
Due to the predominance of TCR α,β + T cells in the DN thymic subset of M25-treated mice, assessment of early precursors within the CD4–, CD8– compartment proved difficult. Further flow cytometric experiments, in which the CD3–, CD4–, CD8– (TN) thymic population was examined, were thus performed. As demonstrated by several in-vestigators, the precursor cells within the TN subset can be divided into progressive stages of maturation based upon CD44 and CD25 expression (39–41). The earliest cells are reported to be CD44+, CD25–, followed by the acquisition of CD25, the subsequent loss of CD44, and finally the loss of CD25. The CD44–, CD25– cells then progress into the DP stage
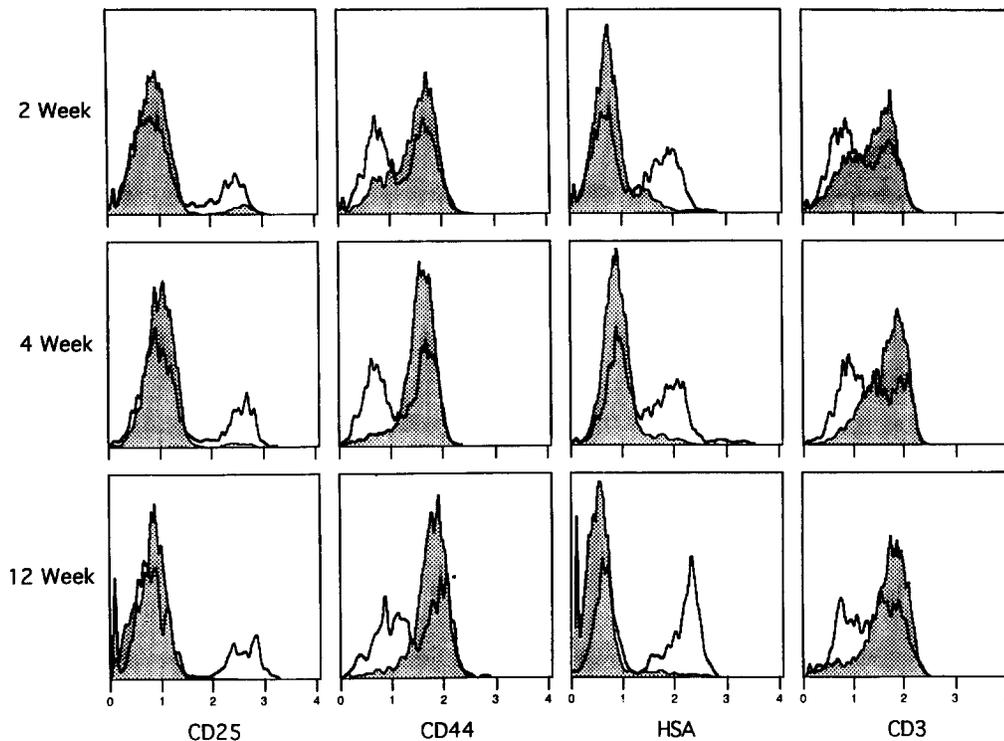
Figure 4. Early thymocytes are progressively lost with anti–IL-7 treatment. Thymocytes from mice treated with M25 for 2, 4, and 12 wk (*shaded histograms*) or mice similarly treated with control antibody (*open histograms*) were stained with PE-conjugated anti-CD4, Cyanine-conjugated anti-CD8, and biotin-conjugated antibodies to CD25, CD44, HSA, CD5, or CD3 plus FITC-avidin. The histograms are derived from the CD4–, CD8– gated populations.

during which they complete TCR gene rearrangement, express the TCR, and undergo selection and final differentiation (for review see references 39 and 40). Thymocyte suspensions from mice treated for 4 wk with anti–IL-7 or control antibody were stained with antibodies to CD3, CD4, CD8, as well as CD25 and CD44. Mice given antibody for only 4 wk were used to ensure sufficient cell numbers for flow cytometric analysis. As shown in Fig. 5, the four CD25- and CD44- defined populations within the TN compartment of control mice are easily demonstrable, with the CD25+, CD44+ subset being the least numerous. When examining the thymus of M25-treated mice, three of the four populations are greatly reduced. Whereas the earliest CD44+, CD25– subset is still easily seen, the remaining TN populations are diminished. These data thus indicate that IL-7 is used for thymocyte expansion, beginning at the CD44+, CD25+ stage.

In addition to analyzing the DN and TN subsets of the thymus, the phenotype of the CD4+,CD8–, CD4–,CD8+, and the DP populations was assessed subsequent to anti–IL-7 treatment. Although the DP compartment was greatly reduced in M25-treated mice, the overall phenotype of these cells did not markedly differ when compared with that of control treated animals. It was noted, however, that the DP cells of IL-7–deprived mice typically lacked the population of CD4^{hi}, CD8^{hi} cells seen in control mice, and consisted

primarily of CD4^{lo}, CD8^{lo} cells (Fig. 3 *A*). This result became more pronounced with increasing duration of M25 treatment.

When examining the CD4 and CD8 SP subsets, it was noted that anti–IL-7 treatment reduced or eliminated the HSA+ cells normally found within these populations. Since HSA+ cells within the SP populations are thought to represent recently auditioned cells (42), we examined the coordinate expression of HSA and CD69 on the CD4+, CD8– and CD4–, CD8+ subsets. Cells coexpressing HSA and CD69 are those recently derived from the DP pool after having undergone TCR-mediated positive selection (43, 44). Thymocytes from mice treated for 4 wk with anti–IL-7 and control antibody were, therefore, stained in a four-color protocol with antibodies to CD4, CD8, CD69, and HSA. The results shown in Fig. 6 demonstrate that IL-7 deprivation markedly reduced the HSA+, CD69+ subset present within each of the SP populations. This suggests that the lack of IL-7 either retards the rate at which cells progress through the selection process, or, more likely, reduces the number of T cells available for selection events. Fig. 6 also shows the elimination of the HSA^{hi}, CD69– cells within the CD8 SP population upon M25 treatment. This subset is thought to represent cells in transit from the TN to the DP stage (45, 46), and thus appears to be highly sensitive to IL-7 deprivation. In data not shown, the CD4 and CD8 SP populations were also tested
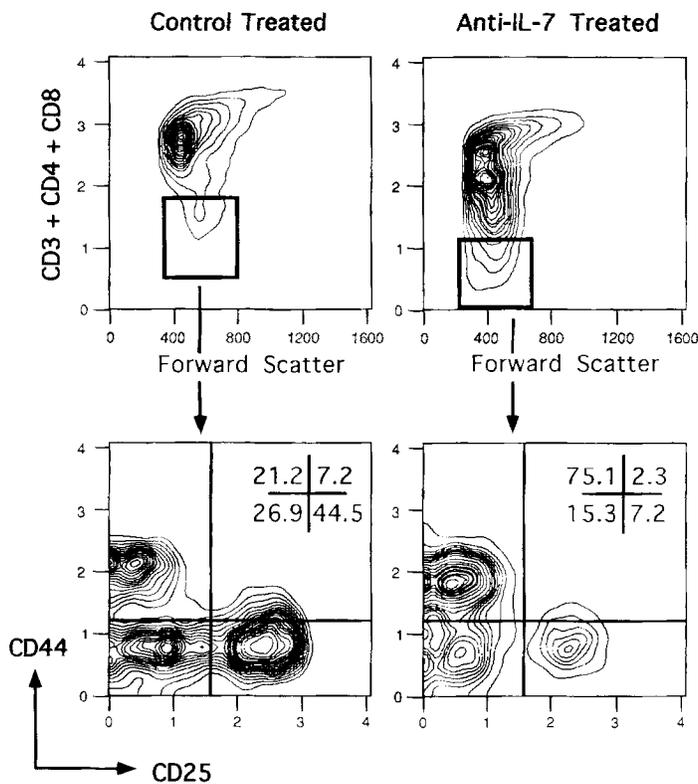
Control Treated   **Anti-IL-7 Treated**

Figure 5. TN thymoctyes become IL-7 responsive at the CD44+, CD25+ stage. Thymocytes from mice treated for 4 wk with anti–IL-7 or control IgG were stained with PE-conjugated antibodies to CD3, CD4, and CD8, Cyanine conjugated anti-CD25, and FITC-conjugated anti-CD44. The TN population was gated as shown (*top*), to reveal the CD44- and CD25-defined subsets within this compartment. The CD3-, CD4-, CD8- gate in the anti–IL-7–treated mouse was set lower to eliminate the large proportion of the CD3ˡᵒ, DN TCR α,β+ cells. CD25+ cells were not eliminated due to the lower gating. The values refer to the percentage of cells within each quadrant of the TN compartment.

for the presence of Vβ3-, Vβ11-, and Vβ12-expressing cells after 4 wk of treatment. This was done to test whether anti–IL-7 administration affected the deletion of Mls reactive T cells, which normally occurs in I-E+ BALB/c mice. In both M25- and control-treated mice, Vβ3, Vβ11, and Vβ12 posi-

tive T cells were absent in the SP populations, with a normal representation of Vβ8-expressing cells. Under these conditions, therefore, it appears that neutralization of IL-7 does not affect thymic negative selection.

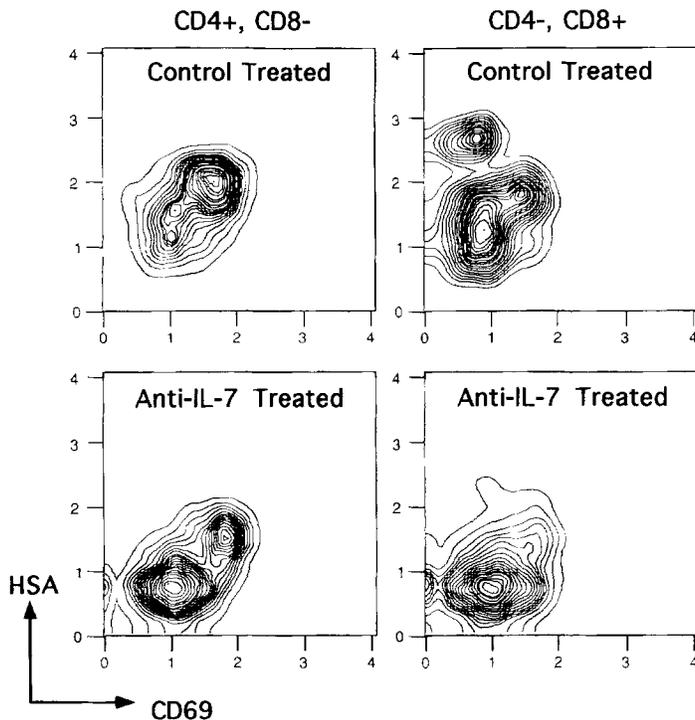Given the striking reduction of thymic cellularity upon

Figure 6. Anti–IL-7 treatment markedly reduces thymocytes undergoing selection events. Thymoctyes from mice treated for 4 wk with M25 or control IgG were stained with PE-conjugated anti-CD4, Cyanine-conjugated anti-CD8, FITC-conjugated anti-HSA, and biotin-conjugated anti-CD69 plus Texas Red-avidin. The CD69 vs. HSA contours are derived from the CD4+, CD8- and CD4-, CD8+ gated populations.

A

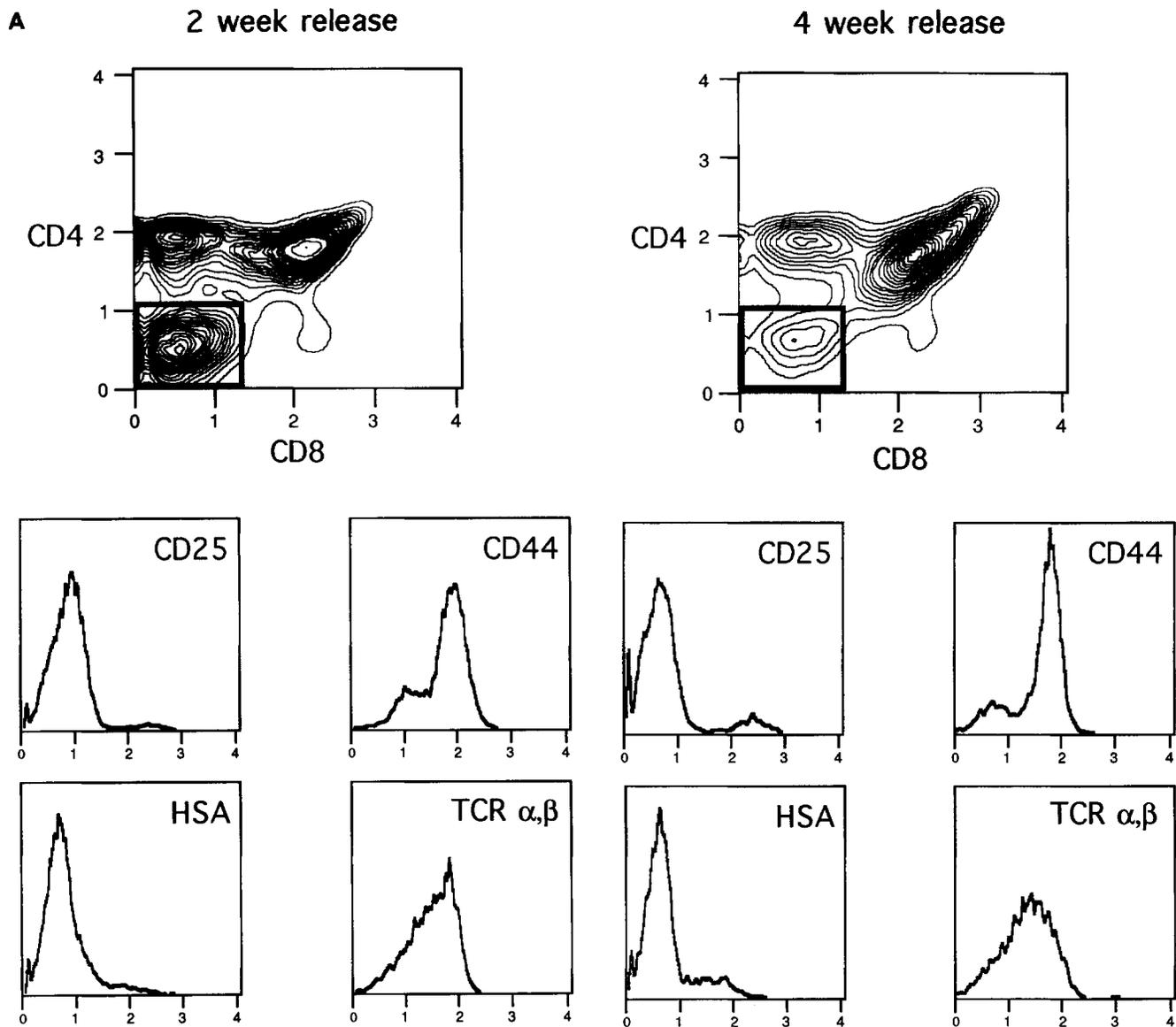**2 week release**   **4 week release**

Figure 7. The effects of IL-7 deprivation on thymic maturation are reversible. Mice injected with M25 for 8 wk, followed by withdrawal of treatment for 2 wk (*A*) or 4 wk (*B*). Thymocytes were stained with PE-conjugated anti-CD4, Cyanine-conjugated anti-CD8, and biotin-conjugated antibodies to CD25, CD44, HSA, or TCR α,β, plus FITC-avidin. The histograms are derived from the CD4−, CD8− gated populations, as shown (*top*). The percentages for each of the populations are as follows: 2-wk thymus; DN = 32.2%, DP = 39.1%, CD4 SP = 24.1%, CD8 SP = 4.5%. 4-wk thymus; DN = 7.4%, DP = 72.6%, CD4 SP = 17.6%, CD8 SP = 2.2%.

anti–IL-7 injection, we next sought to examine the reversibility of IL-7 deprivation. After 8 wk of M25 treatment, mice were allowed to rest for either 2 or 4 wk, with subsequent flow cytometric analysis of the thymus. Fig. 7 *A* demonstrates that after 2 wk of release, the thymus is still abnormal with a skewed proportion of the CD4- and CD8-defined subsets and a reduced level of precursors. Also noteworthy is the continued reduction of the CD4^{hi}, CD8^{hi} subset in the DP population. By 4 wk after the last M25 injection, however, the thymus has returned to a normal phenotype (Fig. 7 *B*). In addition to a greater number of CD25+, HSA+ DN precursors, the CD4- and CD8-defined subsets appear normal,

and the range of CD4 and CD8 expression levels in the DP population is now similar to that of control mice. Interestingly, thymic recovery averaged only 2.5 × 10^6 total cells after 4 wk of release. These findings indicate that, although normal patterns of thymic maturation reappear in a matter of weeks, greater periods of time may be required for reestablishing normal cell numbers upon withdrawal of the neutralizing antibody.

Discussion

A number of previous studies have demonstrated the capacity of IL-7 to support the viability or growth of thymo-

cytes in culture (10–17). While these experiments implied a role for IL-7 in the maturation of T cells, it was unclear as to whether this cytokine is central to the process of early T cell expansion. The present report directly examines the issue by depriving IL-7 in vivo and following the fate of the thymic compartment. After extended administration of an anti–IL-7 antibody, >99% of the thymic cellularity was lost, compared to control treated mice. The drop in thymic cell counts was accompanied by a preferential loss of the TN precursor and DP populations, and retention of mature TCR α,β+ subsets. Together with previous results showing a critical need for IL-7 in B cell maturation (7), these data demonstrate IL-7 to be a key cytokine in the establishment and replenishment of the lymphoid system.

The present results clearly agree with earlier in vitro studies showing the capacity of IL-7 to support early T cell growth (10–17). The finding that in vivo anti–IL-7 treatment depletes the TN thymic compartment, beginning at the CD44+, CD25+ stage (Fig. 5), confirms the previous reports of Watson et al. (12) and Plum et al. (17) demonstrating the preferential growth of TN cells of this phenotype in IL-7–driven fetal thymic cultures. Since the earliest CD44+, CD25– TN subset was retained in mice given M25, it would appear that early T cells become responsive to IL-7 at the CD44+, CD25+ TN stage. This responsiveness could be due to the surface expression of the IL-7 receptor, or to the acquisition of the IL-2 receptor γ-chain, a component necessary for IL-7 receptor–mediated signal transduction (47, 48). The latter possibility is consistent with cells of this stage expressing CD25, the α-chain of the IL-2 receptor complex.

Several reports describing in vivo experiments are, likewise, consistent with our data. Kenai et al. demonstrated that administration of an anti–IL-7 antibody reduces the capacity of fetal thymic tissue, implanted in diffusion chambers, to promote T cell maturation in athymic mice (27). Nishikawa and co-workers further showed that injection of a rat anti–mouse IL-7 receptor antibody results in a marked reduction of thymic cellularity after 2 wk of administration (26). As in the present study, these investigators found the thymic DP population to be most affected upon disruption of IL-7 use. Our findings, thus, confirm and extend these previous studies.

Although all thymic subsets were reduced in number upon M25 administration, the CD4 SP, CD8 SP and DN TCR α,β+ T cells were least affected on a proportional basis. Four-color flow cytometric analysis showed most of the SP cells in anti–IL-7–treated mice to be HSA and CD69 negative. This indicates that the majority of the SP thymocytes in treated animals are either postselected cells ready for export (42–44), or mature cells returned from the periphery. The persistence of the DN TCR α,β+ thymocytes is also of interest. Previous investigators have shown these cells to appear late in ontogeny (38, 49), express a skewed Vβ repertoire (38, 49), and exhibit cytolitic activity (50, 51). Additional studies have suggested a portion of these cells to be extrathymically derived, perhaps arising in the liver or gut (for review see reference 52). The observation that DN TCR α,β+ T cells compose most of the DN compartment in anti–IL-7–treated mice suggests either that maturation of these cells is insensitive to the effects of M25, or that these may also be mature cells derived from the periphery. On a proportional basis, the content of DN TCR γ,δ+ thymic T cells did not change. Whether this indicates a minimal role for IL-7 in γ,δ T cell growth is presently unclear. A careful examination of peripheral T cell sites in M25-treated mice will be necessary to fully assess the importance of IL-7 on the persistence of the γ,δ T cell population.

Whereas a brief treatment with M25 resulted in a complete block of B cell maturation (7), significant depletion (>90%) of early T cells required extended periods of anti–IL-7 administration. The reason for this difference is presently unknown, but several possibilities exist. It could be argued that the murine IgG2b antibody has greater accessibility to critical sites in the bone marrow than in the thymus and, hence, requires an extended injection schedule for thymic depletion. Earlier studies have shown, however, that injection of mouse IgG2b antibodies against MHC class I (53) or class II (54) molecules is very effective in interfering with CD4+ and CD8+ T cell development, indicating full access of these antibodies to thymic tissue. A second possibility is that IL-7 is simply an expansion factor for early T cells, and, in its absence, T cells rapidly proceed through their maturational program. Should this be the case, the progressive loss of early and intermediate T cell blasts, but not a complete shutdown of development would be observed. Finally, the possibility arises that, in the absence of IL-7, thymocytes may be able to use another cytokine during the critical TN stage, albeit to a lesser extent. Recent investigation has shown significant overlap of the biologic activities of certain cytokine pairs. Examples of this include the redundant activities of IL-4 and IL-13 (for review see reference 55), as well as the activities of IL-2 and IL-15 (56, 57). It is interesting to note that a new factor, termed thymic stromal-derived lymphopoietin (TSLP), has been cloned and does, indeed, exhibit some of the same activities as IL-7 (58). The role of TSLP in lymphocyte maturation awaits further testing.

Together with studies on human SCID and experiments with cytokine-deficient mice, the data in this report clearly demonstrate a central role for IL-7 in T cell maturation. It is now understood that manifestations of human X-linked SCID are due to mutations in the γ-chain of the IL-2 receptor (for review see reference 59). To date, IL-2, IL-4, IL-7, and IL-15 are known to require the γ-chain for signal transduction. The inability of SCID patients to use these cytokines thus contributes to their lack of T cell development. The question arises as to which of the lost activities is primarily responsible for the defect in maturation. In mice made genetically deficient for IL-2 (60) or IL-4 (61), development of the T cell compartment proceeds normally. This even holds true in offspring of IL-2–deficient × IL-4–deficient parents (62). Thus, while IL-2 and IL-4 are not crucial for lymphocyte development, the profound effects of in vivo M25 treatment suggest IL-7 to be a key signal in early T cell maturation. The effects of IL-15 on early lymphocytes remain to be determined. The present results are also in good agreement with studies on mice genetically deficient for the IL-7 receptor

(63). These mice exhibit thymic cellularity ranging from 0.01 to 10% of normal thymus, and similarly display a deficit of early CD25+ T cells. Finally, it is well established that the thymus undergoes significant atrophy upon aging. Since extended deprivation of IL-7 in young adult mice results in a similar phenomenon, it is possible that the natural process of thymic atrophy results from a local shutdown of IL-7 production.

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