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Bcl-2 Can Rescue T Lymphocyte Development in Interleukin-7 Receptor–Deficient Mice but Not in Mutant *rag-1*$^{-/-}$ Mice

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Summary

Signals from cytokine and antigen receptors play crucial roles during lymphocyte development. Mice lacking interleukin-7 receptor are lymphopenic, due to a defect in cell expansion at an early stage of differentiation, and the few mature T cells that develop in IL-7R$^{-/-}$ animals are functionally impaired. Both defects were rescued completely by overexpression of the anti-apoptosis protein Bcl-2. T cell progenitors lacking antigen receptor molecules are also blocked in differentiation and die, presumably because they fail to receive a positive signal via their pre-T cell receptor. Surprisingly, Bcl-2 did not promote survival or differentiation of T cells in *rag-1*$^{-/-}$ mice. These results provide evidence that blocking apoptosis is the essential function of IL-7R during differentiation and activation of T lymphocytes and that pre-TCR signaling blocks a pathway to apoptosis that is insensitive to Bcl-2.

Introduction

The magnitude of hematopoietic cell production is determined by the rates of cell division, cell death, and cell differentiation (Metcalf, 1989; Raff, 1996). Signals from cytokine receptors and antigen receptor molecules are known to be critical for development and function of hematopoietic and lymphoid cells (Metcalf, 1989; Godfrey and Zlotnik, 1993; Rajewsky, 1996). For many developmental processes that are regulated by these receptors, it is unclear which of their functions is the essential one: inducing cell division, promoting differentiation, or inhibiting apoptosis.

Differentiation of T lymphocytes of the TCRα/β lineage is initiated in the thymus from fetal liver– or bone marrow–derived hematopoietic stem cells, and CD3$^-$CD4^{lo} CD8$^-$Thy-1(CD90)$^+$ thymocytes have been identified as the earliest recognizable T cell progenitors (Shortman and Wu, 1996) (Figure 1). These cells progressively lose their multipotentiality and become committed to the T lineage after acquiring the CD3$^-$CD4$^-$CD8$^-$ (here called pro-T) phenotype. Pro-T cells can be subdivided into four subpopulations according to expression of the cell surface markers CD25 (IL-2 receptor α chain) and CD44 (Pgp-1), which define the presently accepted developmental pathway: CD25$^-$CD44$^+$ (pro-T1) → CD25$^+$CD44$^+$ (pro-T2) → CD25$^+$CD44$^-$ (pro-T3) → CD25$^-$CD44$^-$ (pro-T4) (Godfrey and Zlotnik, 1993) (Figure 1). An estimated 9–10 cell divisions occur between stem cell immigration into the thymus and the pro-T3 stage (Penit et al., 1995; Shortman and Wu, 1996); this presumably serves to generate a large pool of pro-T3 cells for initiation of TCRβ gene rearrangement. Expression of *rag-1* and *rag-2* genes, which encode essential components of the rearrangement machinery, is first induced at the pro-T2 → pro-T3 transition (Godfrey and Zlotnik, 1993). Expression of the pre-TCR complex (Groettrup et al., 1993) is necessary and sufficient for sustained survival, proliferative expansion, and progression of T cell progenitors via the pro-T4 stage into CD4$^+$CD8$^+$ cortical thymocytes (Mombaerts et al., 1992a, 1992b; Shinkai et al., 1992; Fehling et al., 1995) (Figure 1). These pre-T cells undergo TCRα gene rearrangement (Petrie et al., 1993) and become subject to immunological selection based on their TCRα/β specificity (von Boehmer, 1994) (Figure 1).

Interleukin-7 (IL-7) was originally identified and cloned as a cytokine that induced proliferation of B cell progenitors in the absence of stromal cells (Namen et al., 1988; Goodwin et al., 1989). Studies in mice injected with IL-7 or expressing an IL-7 transgene and experiments with IL-7-stimulated lymphocytes in tissue culture revealed that the cytokine promotes growth and survival of pro-B cells, pro-T cells and antigen-stimulated mature T cells (Murray et al., 1989; Morrissey et al., 1989, 1991; Samaridis et al., 1991; Rich et al., 1993; Fisher et al., 1995). IL-7 stimulates cells through a high affinity receptor complex composed of (at least) the IL-7R and the IL-2R γ chain, which is shared between the receptors for IL-2, IL-4, IL-7, IL-9, and IL-15 (Noguchi et al., 1993; Kondo et al., 1994). Injection of mice with neutralizing antibodies to IL-7 or IL-7R led to a rapid decline of the short-lived B and T cell progenitors, followed by a slower disappearance of the long-lived, mature lymphocytes (Grabstein et al., 1993; Sudo et al., 1993). Genetically modified animals deficient in IL-7 or IL-7R (IL-7$^{-/-}$ mice or IL-7R$^{-/-}$ mice) are severely lymphopenic, but the block in differentiation is not complete and some mature B and T cells are found in peripheral lymphoid organs (Peschon et al., 1994; von Freeden-Jeffry et al., 1995). The mature T lymphocytes from IL-7R$^{-/-}$ mice have been shown to be relatively unresponsive to mitogenic and antigenic activation (Maraskovsky et al., 1996), consistent with the aforementioned reports that showed that IL-7R signaling promotes proliferation and antagonizes apoptosis in mature T cells (Morrissey et al., 1989, 1991; Murray et al., 1989; Samaridis et al., 1991; Rich et al., 1993). This has established a nonredundant requirement for this receptor/ligand pair in the expansion of B and T cell progenitors, but it still remains unresolved whether its essential role is triggering cell division, promoting differentiation, or preventing apoptosis.

Pre-TCR signaling is implicated in regulating proliferation, survival, and differentiation of T lymphocytes at the

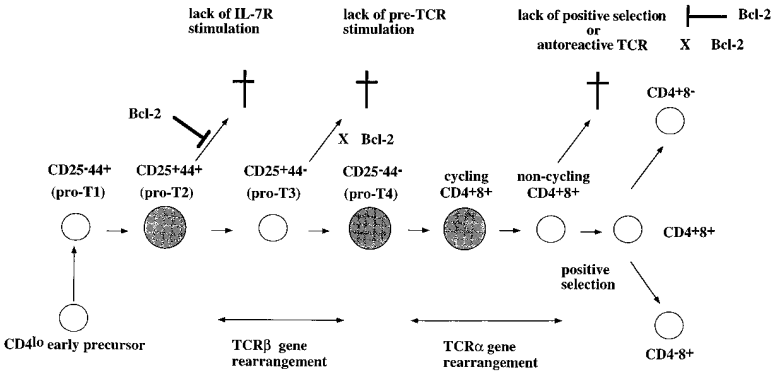

Figure 1. Current Model of the Development of TCRα/β T cells in the Thymus Indicating the Physiological Pathways to Apoptosis and Whether or Not They Can Be Inhibited by Bcl-2

Bcl-2 inhibits apoptosis caused by the absence of signals from IL-7R (this paper) or from MHC molecules (no positive selection) (Linette et al., 1994; Strasser et al., 1994b) but is a poor antagonist of the deletion of cells lacking TCR molecules (this paper) or bearing autoreactive receptors (Sentman et al., 1991; Strasser et al., 1991; Strasser et al., 1994b). Large circles represent cycling cells, small circles quiescent, noncycling cells.

transition from the pro-T3 stage to the pro-T4 stage. The pre-TCR complex is composed of the TCRβ chain, the gp33 pTα chain (which acts as a surrogate TCRα chain), and the CD3 proteins (Groettrup et al., 1993). Mice lacking either one of these components of the pre-TCR complex have a severe block in T lymphopoiesis at the pro-T3 → pro-T4 transition (Mombaerts et al., 1992a, 1992b; Shinkai et al., 1992; Fehling et al., 1995). This block is absolute in *rag*$^{-/-}$ animals, lacking TCR chains altogether (Mombaerts et al., 1992a; Shinkai et al., 1992), but leaky in pTα$^{-/-}$ mice (Fehling et al., 1995) and TCRβ$^{-/-}$ mice (Mombaerts et al., 1992b), possibly because complexes of other TCR chains can partially replace the function of the classical pre-TCR. As for the IL-7R, the relative contributions of signals for cell division, cell differentiation, or cell survival to the overall function of the pre-TCR remains obscure.

The protein product of the proto-oncogene *bcl-2* can protect cells against a broad range of physiological or experimentally applied apoptotic stimuli, implying that multiple signaling routes converge upon a common cell death effector mechanism that is subject to inhibition by Bcl-2 (reviewed by Cory, 1995; Korsmeyer, 1995). To assess the contribution of inhibition of apoptosis to the overall functions of the IL-7R and the pre-TCR, we introduced a *bcl-2* transgene into IL-7R$^{-/-}$ mice and *rag-1*$^{-/-}$ mice. The results revealed that Bcl-2 overexpression could completely rescue T cell development and T cell function in IL-7R$^{-/-}$ mice but had no discernible impact on the production or survival of T cell progenitors in *rag-1*$^{-/-}$ mice. These findings have implications for signal transduction from cytokine receptors and antigen receptor molecules, for lymphopoiesis, and for the control of lymphocyte apoptosis.

Results

Expression of a *bcl-2* Transgene Rescues T Lymphocyte Development in IL-7R-Deficient Mice

The defects in T cell development in IL-7R-deficient mice could be a consequence of reduced cell proliferation, blocked differentiation, impaired cell survival, or a combination thereof. To determine whether an inhibitor of apoptosis could rescue the defect caused by absence of IL-7R signaling, IL-7R$^{-/-}$ mice (Peschon et al., 1994) were crossed with Eμ-*bcl-2-36* transgenic mice (Strasser et al., 1991). The latter express a human *bcl-2* cDNA constitutively at high levels in T and B lymphoid cells. The cellularity of thymus and spleen was measured for wild-type mice (IL-7R$^{+/+}$), *bcl-2* transgenic mice, mutant IL-7R$^{-/-}$ mice, and *bcl-2*/IL-7R$^{-/-}$ mice (Figure 2). Consistent with our previous observations (Peschon et al., 1994), IL-7R$^{-/-}$ mice could be divided into two subgroups: those (representing about 65% of animals) that have a thymus cellularity of <1% of that of wild-type mice (hereafter called IL-7R$^{-/-}$ <1%) and those (~35%) that have a thymus cellularity of >1% of normal controls (hereafter called IL-7R$^{-/-}$ >1%). Compared to wild-type mice or *bcl-2* transgenic mice, all IL-7R$^{-/-}$ mice contained considerably fewer T lymphoid cells in thymus (13- to 2000-fold reduction) and spleen (5- to 20-fold

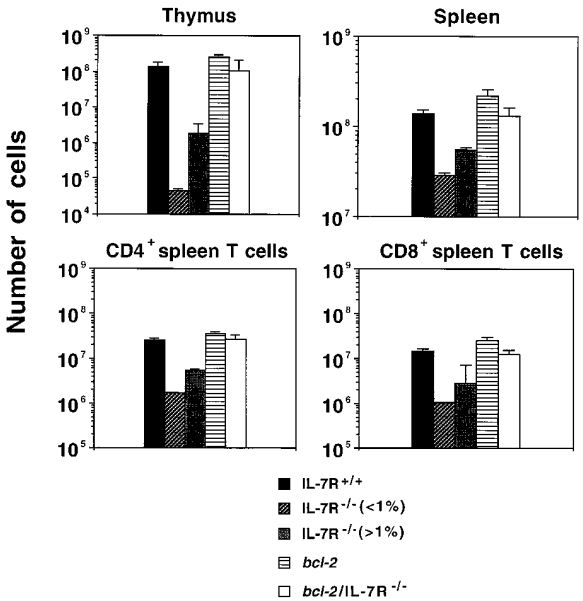

Figure 2. Expression of a *bcl-2* Transgene Rescues T Lymphocyte Production in IL-7R$^{-/-}$ Mice

Hemocytometric counts were performed on single cell suspensions prepared from thymus and spleen of wild-type mice, *bcl-2* transgenic mice, IL-7R$^{-/-}$ mice (with thymus cellularity of <1% or >1% of those of wild-type mice), and *bcl-2*/IL-7R$^{-/-}$ mice. The number of CD4$^+$ and CD8$^+$ T cells in the spleen was calculated by determining their frequency by immunofluorescence staining and flow cytometric analysis, and multiplication by the total number of spleen leukocytes. The data represent the arithmetic mean ± SD of 9 mice of each type.

reduction). In contrast, essentially all *bcl-2*/IL-7R$^{-/-}$ mice had normal numbers of thymocytes and splenic T cells (Figure 2).

The first obvious abnormality that occurs during differentiation of IL-7R$^{-/-}$ T cells is a severe reduction in the number of CD25^{+} CD3^{-}CD4^{-}CD8^{-} pro-T cells (Peschon et al., 1994). Since up to 12 cell divisions are thought to occur between this stage and the numerically dominant population in the thymus, the CD4^{+}CD8^{+} pre-T cells, it is conceivable that T lymphopenia is a direct consequence of this defect. To determine whether overexpression of Bcl-2 could prevent this early block in T cell development in the IL-7R$^{-/-}$ mice, we investigated the composition of T cell progenitor populations in *bcl-2*/IL-7R$^{-/-}$ mice. Not only did *bcl-2*/IL-7R$^{-/-}$ thymi show relatively normal cellularity (Figure 2), they also displayed a normal distribution of the four major subsets: CD4^{-}CD8^{-} progenitors, CD4^{+}CD8^{+} precursors, and CD4^{+}CD8^{-} and CD4^{-}CD8^{+} mature T cells (Figure 3). An apparently normal distribution of subsets was noted in those IL-7R$^{-/-}$ thymus glands with a cellularity of >1% of that of wild-type mice, although the absolute numbers of these cell populations were still reduced by a factor of 10- to 100-fold (Figures 2 and 3). IL-7R$^{-/-}$ thymi with a cellularity of <1% of that of wild-type mice were composed almost entirely but not exclusively of immature CD3^{-}CD4^{-}CD8^{-} progenitors (Figure 3). Consistent with previous observations (Peschon et al., 1994), detailed analysis of CD4^{-}CD8^{-} thymocytes revealed that all IL-7R$^{-/-}$ mice (both <1% and >1%) had a substantial deficit (50- to 1000-fold in absolute numbers, compared to wild-type mice) of CD25^{+} pro-T2 and pro-T3 cells (Figure 3). In contrast, *bcl-2*/IL-7R$^{-/-}$ mice contained relatively normal numbers of CD25^{+} pro-T2 and pro-T3 cells (Figure 3). Four-color immunofluorescence analysis of CD3^{-}CD4^{-}CD8^{-} pro-T cells, using antibodies to CD25 (IL-2R α chain) and CD44, confirmed that the numbers of pro-T2, pro-T3, and pro-T4 cells are reduced in IL-7R$^{-/-}$ mice but are at normal levels in *bcl-2*/IL-7R$^{-/-}$ mice (data not shown). These results demonstrate that expression of the cell death inhibitor Bcl-2 rescues T cell development in IL-7R-deficient mutant mice, indicating that in this process IL-7R signaling is essential for blocking apoptosis but is not required for proliferation or differentiation.

Expression of a *bcl-2* Transgene Rescues the Functional Defect of Antigen- or Mitogen-Stimulated IL-7R$^{-/-}$ T Lymphocytes

IL-7R deficiency does not cause a complete block in T cell differentiation, and a numerically reduced (5- to 20-fold) population of CD4^{+} and CD8^{+} T cells is present in peripheral lymphoid organs of IL-7R$^{-/-}$ mice (Peschon et al., 1994). These T cells appear phenotypically normal but are impaired in their responsiveness to mitogenic or antigenic stimulation (Maraskovsky et al., 1996). It is unclear whether this defect is caused by reduced cell division or by excessive apoptosis. Alternatively, IL-7R functions during development in the thymus are needed for expression of an immunological function at the mature stage (Maraskovsky et al., 1996). We examined the impact of *bcl-2* transgene expression on mitogen- or antigen-stimulated proliferation and functional maturation of IL-7R$^{-/-}$ T cells.

Upon stimulation by antibody-mediated cross-linking of the TCR/CD3 complex, CD18 (LFA-1 β chain), and either CD4 or CD8 coreceptors, T cells from IL-7R$^{-/-}$ mice yielded 4- to 10-fold fewer lymphoblasts in comparison to T cells from normal mice or *bcl-2* transgenic

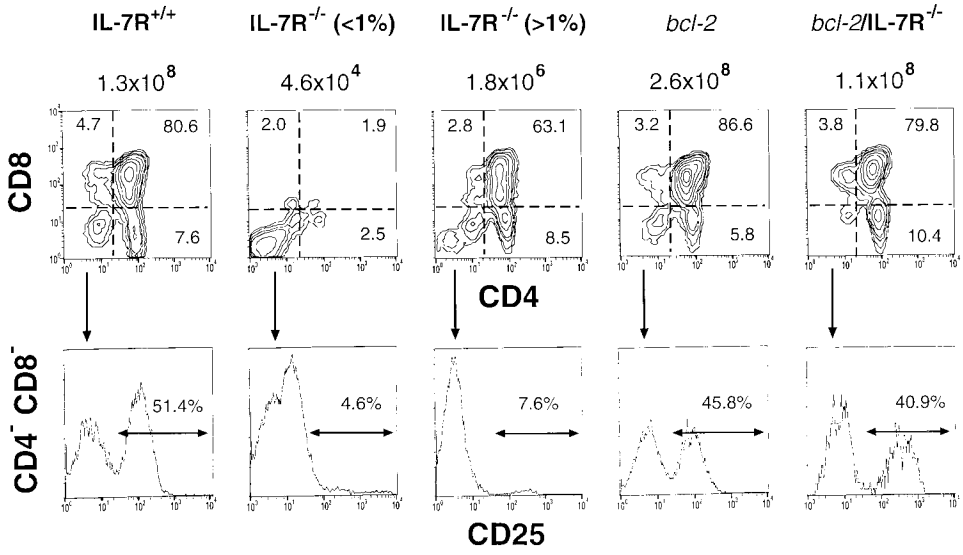

Figure 3. Expression of a *bcl-2* Transgene Rescues T Lymphocyte Differentiation in the Thymus of IL-7R$^{-/-}$ Mice

Composition of thymocyte subsets was analyzed in wild-type mice, IL-7R$^{-/-}$ mice (cellularity <1% of wild-type mice), IL-7R$^{-/-}$ mice (cellularity >1% of wild-type mice), *bcl-2* transgenic mice and *bcl-2*/IL-7R$^{-/-}$ mice by immunofluorescence staining and flow cytometric analysis. The top row presents two-color immunofluorescence contour plots of the expression of CD4 (FITC) and CD8 (R-PE) on all thymocytes. The bottom row presents a histogram analysis of the expression of CD25/IL-2R α chain (APC) on CD4^{-}CD8^{-} thymocytes. The results shown are representative of data from 9 or more mice of each type.

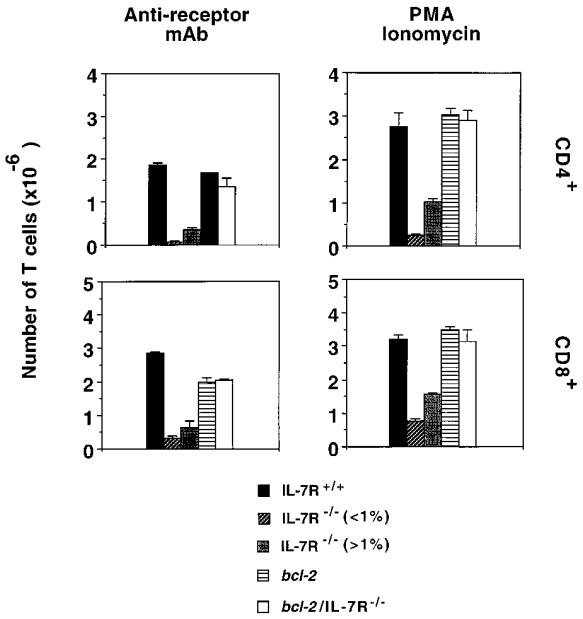

Figure 4. Expression of a *bcl-2* Transgene Rescues Proliferation of Mitogen-Stimulated T Lymphocytes in IL-7R$^{-/-}$ Mice

Purified CD4$^+$ (5×10^4) and CD8$^+$ (1×10^4) spleen cells from wild-type mice, IL-7R$^{-/-}$ mice (cellularity <1% of wild-type mice), IL-7R$^{-/-}$ mice (cellularity >1% of wild-type mice), *bcl-2* transgenic mice, and *bcl-2*/IL-7R$^{-/-}$ mice were activated in vitro in the presence of rhIL-2 with immobilized antibodies to CD3, CD18 (LFA-1 β chain), and either CD4 or CD8, or with PMA and ionomycin. Total numbers of viable T cells were determined by hemocytometric counting on day 6. The data represent the mean ± SD of 9 mice from each group.

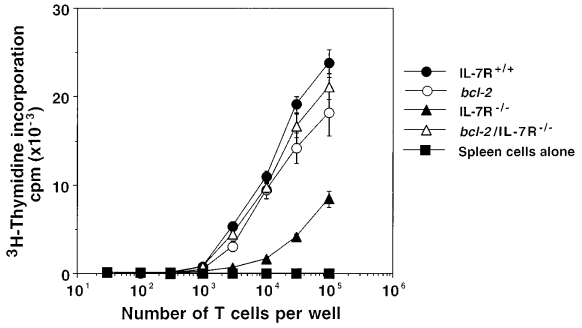

Figure 5. Expression of a *bcl-2* Transgene Rescues Proliferation of Antigen-Stimulated T Lymphocytes in IL-7R$^{-/-}$ Mice

Purified CD4$^+$ T cells from the spleen of wild-type mice, IL-7R$^{-/-}$ mice (cellularity <1% of wild-type mice), *bcl-2* transgenic mice and *bcl-2*/IL-7R$^{-/-}$ mice were activated in vitro with irradiated allogeneic spleen cells from DBA/2 mice. Cultured cells were pulsed for 6 hr with 0.5 μCi ^{3}H-thymidine on day 4. The data represent the arithmetic mean ± SD of 9 mice from each group.

mice (Figure 4). In contrast, CD4$^+$ and CD8$^+$ T cells from *bcl-2*/IL-7R$^{-/-}$ mice expanded normally in numbers in response to this treatment (Figure 4). Similar results were obtained when purified T lymphocytes were instead polyclonally activated with phorbol ester and ionomycin (Figure 4).

To assess responsiveness to antigenic stimulation, graded numbers of purified CD4$^+$ T cells from mice of the various genotypes were activated in cocultures with irradiated allogeneic DBA/2 spleen cells (Figure 5). Unlike IL-7R$^{-/-}$ T cells that were 10-fold less responsive to alloantigen, *bcl-2*/IL-7R$^{-/-}$ T cells proliferated similarly to wild-type or *bcl-2* transgenic T cells (Figure 5).

We wished to determine whether the relatively normal responsiveness of *bcl-2*/IL-7R$^{-/-}$ T cells in vitro reflected normal immunoreactivity to antigenic challenge in vivo. Mice from the different strains were therefore challenged with P815 allogeneic mastocytoma cells, and tumor growth was monitored. All wild-type mice and *bcl-2* transgenic mice rejected P815 tumor cells within three weeks of challenge, whereas most (>80%) IL-7R$^{-/-}$ mice were unable to do so and had to be sacrificed. In contrast, all *bcl-2*/IL-7R$^{-/-}$ mice were able to efficiently reject allogeneic tumor transplants.

These results reveal that expression of the cell death inhibitor Bcl-2 rescues the proliferative and functional defect of IL-7R-deficient T cells, thereby restoring normal T lymphocyte–mediated immunity. It therefore appears that IL-7R signaling plays an essential role in blocking apoptosis of antigenically stimulated T lymphocytes and does so through a mechanism that can be replaced by expression of a *bcl-2* transgene.

Expression of a *bcl-2* Transgene Does Not Affect T Cell Development in Mutant *rag-1*$^{-/-}$ Mice

T cell differentiation is arrested at the pro-T3 stage in mutant *rag-1*$^{-/-}$ mice that are defective in antigen receptor gene rearrangement (Godfrey et al., 1992; Mombaerts et al., 1992b). The lifespan of pro-T3 cells is limited to approximately 3–4 days in the absence of productive TCRβ gene rearrangement, and such cells die, presumably because they fail to receive a positive signal through the pre-TCR (Penit et al., 1995). As a consequence, *rag-1*$^{-/-}$ mice contain normal numbers of pro-T1, pro-T2, and pro-T3 cells but are devoid of pro-T4 cells, CD4$^+$CD8$^+$ cortical thymocytes, and mature T cells. Expression of a *bcl-2* transgene had little impact on T cell development in *rag-1*$^{-/-}$ mice. Thymus cellularity was not significantly increased (3–7 × 10^6 cells as in control *rag-1*$^{-/-}$ mice) and differentiation remained arrested at the pro-T3 T cell progenitor stage (Figure 6). To investigate the impact of Bcl-2 on production and death of T cell progenitors, we measured the distribution within the cell cycle (Figure 6) and the rate of BrdU incorporation (Figure 7) of pro-T cells in *bcl-2*/*rag-1*$^{-/-}$ mice and their transgene-negative littermates.

T cell progenitors from mutant *rag-1*$^{-/-}$ mice, like those from normal animals (Penit et al., 1995), had a high turnover rate; almost 60% were labeled with BrdU after two days, and around 90% by day 4 (Figure 7). The frequency of BrdU-positive Thy-1$^+$ thymocytes in *bcl-2*/*rag-1*$^{-/-}$ mice did not differ significantly from that of *rag-1*$^{-/-}$ controls (Figure 7). Consistent with these data, *bcl-2* transgene expression did not alter the distribution within the cell cycle of pro-T1, pro-T2, or pro-T3 cells in *rag-1*$^{-/-}$ mice (Figure 6).

These results show that overexpression of Bcl-2 cannot promote survival of T cell progenitors that have failed to undergo productive TCRβ gene rearrangement. This

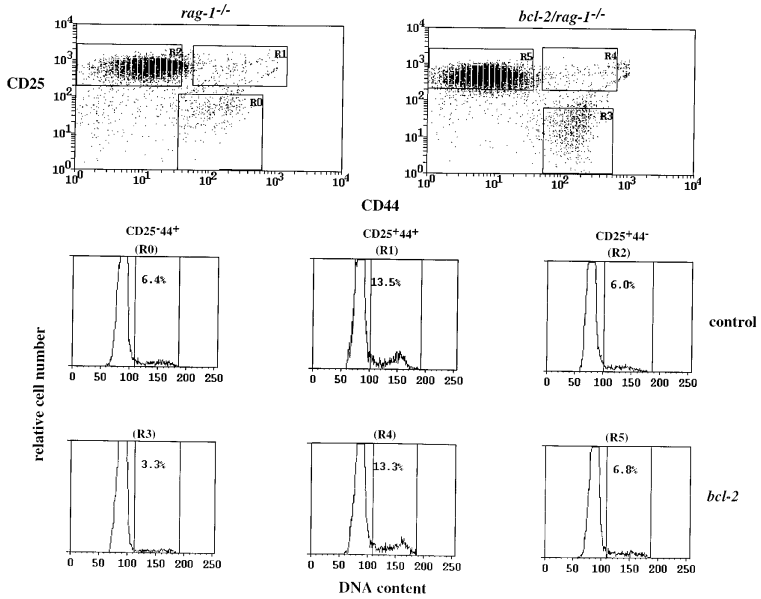

Figure 6. Expression of a *bcl-2* Transgene Does Not Alter T Cell Development in *rag-1*-Deficient Mice

Pro-T1 ($CD25^-CD44^+$), pro-T2 ($CD25^+CD44^+$), and pro-T3 ($CD25^+CD44^{low}$) cells from the thymus of *rag-1*$^{-/-}$ and *bcl-2*/*rag-1*$^{-/-}$ mice were purified by FACS, permeabilized, and stained for flow cytometric analysis of DNA content. The top row shows two-color immunofluorescence dot plots of the expression of CD25 and CD44 on thymic T cells gated for expression of Thy-1. The bottom two panels show the DNA content of the purified T cell progenitors. The percentage of cells with a DNA content >2C is indicated. The data shown are representative of those from 4 mice of each genotype.

physiological cell death must therefore be activated by a signaling cascade that is insensitive to Bcl-2.

Discussion

T cell differentiation proceeds through a succession of genotypically and phenotypically identifiable stages and is regulated by the interaction of several processes (von Boehmer, 1994). These include cell expansion prior to initiation of antigen receptor gene rearrangement (Penit et al., 1995; Shortman and Wu, 1996), proliferation of clones with productive TCRβ gene rearrangements (Godfrey and Zlotnik, 1993), growth arrest during TCRα gene rearrangement (Petrie et al., 1993), apoptosis of cells lacking TCR chains or bearing TCRα/β complexes that have insufficient affinity for major histocompatibility complex (MHC) molecules (von Boehmer, 1994), deletion of thymocytes bearing autoreactive TCR (Nossal, 1994), and positive selection of cells with potentially useful antigen receptor specificities into the pool of long-lived, mature, recirculating T lymphocytes (von Boehmer, 1994). The IL-7R and TCR chains play crucial roles in the regulation of these processes, but it is unclear whether it is their effect on cell division, cell survival or differentiation, or a combination thereof that is responsible for their activity. We wanted to dissect these processes to see whether differentiation and proliferation require active signaling via IL-7R or TCR chain–containing surface receptors or is merely a consequence of sustained T cell survival. This was achieved by constructing novel strains of mice bearing either mutations in the IL-7R or mutations that block antigen receptor gene rearrangement (*rag-1*$^{-/-}$) and expressing in their T cells a *bcl-2* transgene to inhibit apoptosis. We found that overexpression of Bcl-2 was sufficient to rescue T cell development (Figures 2 and 3) and T cell function (Figures 4 and 5) in IL-7R-deficient mice but could not rescue *rag-1*$^{-/-}$ pro-T cells, which are unable to express pre-TCR molecules, from programmed cell death (Figures 6 and 7). A fundamental difference therefore exists in the developmental potential of pro-T cells in *bcl-2*/IL-7R$^{-/-}$ mice compared to those from *bcl-2*/*rag-1*$^{-/-}$ mice. This indicates that stimulation of IL-7R and pre-TCR must initiate distinct sets of signaling cascades for regulation of apoptosis, proliferation, and differentiation.

Implications for Signal Transduction from Cytokine Receptors

Our studies on *bcl-2*/IL-7R$^{-/-}$ mice indicate that the essential effects of IL-7R signaling for T cell development and activation can be replaced by overexpression of the anti-apoptosis protein Bcl-2 (Figures 2–5). Since Bcl-2 does not promote proliferation (in fact, it can slow it under certain circumstances) (Linette et al., 1996; Mazel et al., 1996; O'Reilly et al., 1996), and since Bcl-2 does not facilitate differentiation of thymocytes that failed to receive signals from MHC molecules (Linette et al., 1994; Strasser et al., 1994b), it appears that the essential function of IL-7R signaling in T cell development must be to promote cell survival. Consistent with this conclusion, it has been shown that expression of a *bcl-2* transgene can substitute for IL-7R-transduced survival signals in pre-B cell lines growing on stromal layers (Rolink et al., 1993). Other studies did, however, indicate that IL-7R signaling is needed not only for inhibiting apoptosis but also for proliferation (Namen et al., 1988; Goodwin et al., 1989; Murray et al., 1989) and differentiation (Muegge et al., 1993) of lymphoid cells. What might be the explanation for this apparent discrepancy? In the tissue culture experiments that demonstrated a proliferative effect of IL-7, other growth stimuli, such as antigen receptor cross-linking or signals from stromal cells, were usually present (Morrissey et al., 1989; Murray et al., 1989) or cell growth was short-term and was initiated by cells that were cycling in vivo at the time of isolation (Namen et al., 1988; Goodwin et al., 1989). The experiments that implicated signals from IL-7R as an initiating

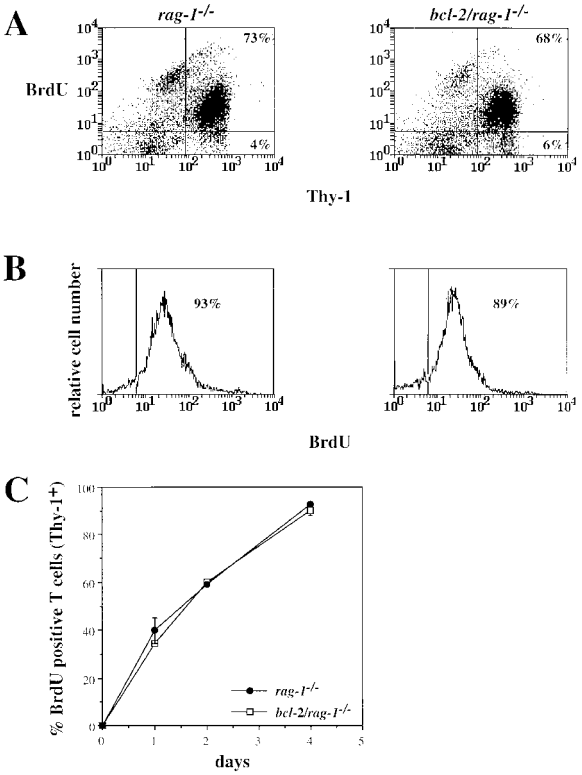

Figure 7. Expression of a *bcl-2* Transgene Does Not Affect the Turnover Rate of Pro-T Cells in *rag-1*-Deficient Mice

Cohorts of *rag-1*$^{-/-}$ and *bcl-2*/*rag-1*$^{-/-}$ mice were fed continuously for 1, 2, or 4 days with BrdU to label proliferating cells. Thymocytes were stained with monoclonal antibodies specific to Thy-1 (*y* axis: Tricolor) and BrdU (x axis: FITC) to determine the percentage of T lymphocytes that had incorporated BrdU. The top panels show results of 4 days of continuous BrdU labeling as two-color dot plots (A) and histograms of the BrdU labeling profile of Thy-1$^+$ T cells (B). The bottom panel (C) shows the fraction of BrdU-labeled T cells as a function of time of continuous labeling. Values are arithmetic means ± SD from 2 mice of each genotype at each time point.

event for TCR gene rearrangement were performed on thymocytes in tissue culture (Muegge et al., 1993). Since IL-7 promotes survival of thymocytes in vitro (Murray et al., 1989), it is unclear whether IL-7 was directly responsible for initiating rearrangement or merely facilitated it by inhibiting apoptosis. In any case, the finding that some mature lymphocytes are formed in IL-7$^{-/-}$ mice and IL-7R$^{-/-}$ mice is inconsistent with the notion of an indispensable role for IL-7R signaling in differentiation. In conclusion, there appears to be no evidence contradictory to the hypothesis that IL-7R signaling promotes T lymphocyte growth and development solely by inhibiting apoptosis.

What signaling cascades might be responsible for IL-7-induced cell survival? It is likely that the IL-2R γ chain and the Jak-3 kinase are critically involved, since mutant mice lacking these molecules have a phenotype similar to that of IL-7R$^{-/-}$ animals (Cao et al., 1995; Nosaka et al., 1995). It will therefore be interesting to test whether expression of a *bcl-2* transgene can also rescue T cell development and T cell function in IL-2R γ chain$^{-/-}$ mice and Jak-3$^{-/-}$ mice. The survival signal from IL-7R could function by inducing expression of Bcl-2 or one of its homologs. Consistent with this idea, Bcl-2 and IL-7R are coexpressed at high levels in CD3$^-$CD4$^-$CD8$^-$ pro-T cells and mature T lymphocytes and absent from CD4$^+$CD8$^+$ pre T cells (Sudo et al., 1993; Veis et al., 1993). We are currently investigating whether loss of IL-7R leads to reduced levels of Bcl-2 in pro-T cells. Alternatively, IL-7R signaling could promote cell survival at a step upstream of the cell death effector machinery by interfering with a default pathway to apoptosis that is normally activated in situations of limiting supply of growth factors.

Implications for Signal Transduction from the Pre-T Cell Receptor

Our analysis of *bcl-2*/*rag-1*$^{-/-}$ mice shows that Bcl-2 overexpression has no discernible effect on the block to T cell development caused by the absence of TCR molecules (Figures 6 and 7). This is consistent with previous observations that expression of a *bcl-2* transgene does not significantly increase thymus cellularity in mutant *scid* or *rag-2*$^{-/-}$ mice (Linette et al., 1994; Strasser et al., 1994a). Neither of these two studies, however, investigated the effect of Bcl-2 on the pro-T3 → pro-T4 differentiation step or on the in vivo lifespan of pro-T cells. Our detailed analysis of cell surface markers (Figure 6) and cell turnover (Figure 7) established that Bcl-2 is unable to promote survival or differentiation of pro-T cells lacking signals from the pre-TCR. It is unlikely that the lack of an effect of Bcl-2 is due to insufficient levels of transgene expression at the pro-T stage, since it is capable of rescuing those cells from lack of IL-7R signaling (Figures 2 and 3) and since it prolongs the survival of purified *scid* and *rag-1*$^{-/-}$ pro-T cells in tissue culture (data not shown).

What signaling cascades might be responsible for pre-TCR induced survival, proliferation, and differentiation of cells? The *lck* tyrosine kinase is a likely candidate for signal transduction, since *lck*$^{-/-}$ mice (Mollina et al., 1992) have a phenotype similar to that of pTα$^{-/-}$ mice (Fehling et al., 1995). The structure of the pre-TCR complex is similar to that of the TCRα/β complex; by analogy to TCRα/β stimulation, it is therefore expected that the pre-TCR activates a range of signal transduction pathways, such as the ras/MAP kinase pathway and the Rel/NF-κB family of transcription factors. These signaling molecules presumably act in concert to regulate passage of developing T cells through the pre-TCR checkpoint (Godfrey and Zlotnik, 1993).

Implications for the Control of T Cell Development

TCR chains are critical regulators of the later stages of T cell differentiation, at the time when antigen receptor gene rearrangement occurs (Figure 1). Expression of the pre-TCR is a prerequisite for passage of T lymphoid cells through the pro-T3 → pro-T4 developmental checkpoint and for clonal expansion of thymocytes with productive TCRβ gene rearrangements (Mombaerts et al., 1992a; Fehling et al., 1995) (Figure 1). Differentiation from the CD4$^+$CD8$^+$ pre-T cell stage to mature cells (CD4$^+$CD8$^-$ or CD4$^-$CD8$^+$) and immunological repertoire selection are regulated by TCRα/β heterodimers (Mombaerts et

al., 1992a; Philpott et al., 1992) (Figure 1). What is the nature of the surface receptors that regulate differentiation and cell cycling during the early stages of T lymphopoiesis prior to antigen receptor gene rearrangement? IL-7R appears to be required for cell survival but not needed for cell cycling and differentiation (Figures 2 and 3). Indeed, it appears that none of the receptors that utilize the IL-2R γ chain (IL-2R, IL-4R, IL-7R, IL-9R, and IL-15R) are indispensable for differentiation, since mutant mice lacking this molecule can produce some mature T cells, albeit at reduced numbers (Cao et al., 1995). Receptors for other stromal cell–derived factors may be crucial regulators of early T lymphopoiesis, and c-Kit and Flk2/Flt3 are likely candidates, since mice bearing mutations in these genes have reduced numbers of pro-T cells (Mackarehtschian et al., 1995; Rodewald et al., 1995).

Implications for the Control of Lymphocyte Apoptosis

Cell death is a common fate of developing T cells: approximately 97% of all cells generated within the thymus undergo apoptosis in situ (Egerton et al., 1990; Surh and Sprent, 1994). Thymocytes lacking productive TCR gene rearrangements or expressing a TCRα/β with insufficient avidity for MHC molecules die because they fail to receive a survival signal ("death by neglect") (Surh and Sprent, 1994), while those bearing autoreactive receptors are deleted after receiving a signal through the TCRα/β (Murphy et al., 1990). Bcl-2 can inhibit apoptosis of thymocytes that failed to receive a positive signal from MHC (Linette et al., 1994; Strasser et al., 1994b), but it is a poor antidote to deletion of cells bearing autoreactive receptors (Sentman et al., 1991; Strasser et al., 1991; Strasser et al., 1994b) (Figure 1). Our studies of *bcl-2*/IL-7R$^{-/-}$ mice indicate that deprivation of IL-7 induces in pro-T cells "death by neglect" by a mechanism that can be blocked by Bcl-2. We expected that pro-T cells lacking TCR chains also die by an apoptotic mechanism that can be antagonized by Bcl-2, particularly since Bcl-2 promotes survival and accumulation of B lymphoid cells that are unable to express immunoglobulin chains in *bcl-2*/*scid* and *bcl-2*/*rag*$^{-/-}$ mice (Strasser et al., 1994a; Young et al., 1997; D. Tarlinton et al., submitted). Our analysis of the development (Figure 6) and turnover (Figure 7) of T cells in *bcl-2*/*rag-1*$^{-/-}$ mice demonstrated, however, that absence of pre-TCR signaling triggers a Bcl-2-insensitive pathway to cell death. What might the nature of this death signal be? Activation of receptors of the tumor necrosis factor receptor family (TNF-R) is a possibility since it has been shown that p55 TNF-RI and CD95 (Fas/APO-1) can trigger apoptosis in some cell types in a manner that is insensitive to Bcl-2 (Vanhaesebroeck et al., 1993; Memon et al., 1995; Strasser et al., 1995).

Experimental Procedures

Mice

The IL-7R$^{-/-}$ mice (Peschon et al., 1994), the *rag-1*$^{-/-}$ mice (Spanopoulou et al., 1994), and the Eμ-*bcl-2-36* transgenic strain (Strasser et al., 1991), expressing a human *bcl-2* cDNA constitutively at high levels in B and T lymphocytes, have been described previously. Prior to these studies, all of these strains had been backcrossed for 5 or more generations with C57BL/6 mice. The *bcl-2*/IL-7R$^{-/-}$ mice and *bcl-2*/*rag-1*$^{-/-}$ mice were generated by crossing Eμ-*bcl-2-36* mice for two or more generations with IL-7R$^{-/-}$ mice or *rag-1*$^{-/-}$ mice, respectively. Inheritance of the mutated IL-7R allele, the mutated *rag-1* allele, and the Eμ-*bcl-2-36* transgene was determined by Southern blotting or PCR analysis (Peschon et al., 1994; Spanopoulou et al., 1994; Strasser et al., 1995). All animals were analyzed at 4–8 weeks of age.

Immunofluorescence Staining, Flow Cytometric Analysis, and Cell Sorting

Isolation of CD4$^+$ and CD8$^+$ T cells from spleen was carried out as described previously (Maraskovsky et al., 1996). Erythrocyte-depleted spleen cells (2×10^7) were incubated for 20 min at 4°C with a cocktail of the following monoclonal antibodies: anti-CD45R(B220), anti-Gr-1, anti-NK1.1, and anti-Mac-1 (Pharmingen). After washing in PBS–FCS, the cells were incubated for 20 min at 4°C with anti-rat Ig-coated magnetic beads (Dynal) in a slurry of PBS–FCS. The mixture was then diluted in 5 ml PBS–FCS, cells were coated with antibodies, and beads were removed with a magnet (Dynal). The remaining cells were stained with fluorescein isothiocyanate (FITC) anti-CD4 (GK-1.5) and phycoerythrin (R-PE) anti-CD8 (53-6.7) for 30 min at 4°C in PBS–FCS. To reduce nonspecific binding of antibodies to Fcγ receptors, 50 μg/ml of rat monoclonal antibody 2.4G2 (anti-mouse FcγRII) was included during staining with fluorochrome-coupled antibodies. Cells were resuspended in PBS–FCS containing 0.5 μg/ml propidium iodide (Sigma) before sorting on a FACStar$^+$ (Becton Dickinson). Dead cells and damaged cells were excluded on the basis of low angle scatter and red fluorescence due to propidium iodide uptake. Routine analysis of sorted cells for CD4 or CD8 expression revealed a cell population of 96%–99% purity. CD3$^-$CD4$^-$CD8$^-$ T cell progenitor subpopulations from thymus of *rag-1*$^{-/-}$ and *bcl-2*/*rag-1*$^{-/-}$ mice were purified according to a published procedure (Godfrey et al., 1992). Thymocytes were stained in the presence of 2% normal rat serum with R-PE-conjugated anti-CD25 (Caltag), FITC-conjugated anti-CD44 (IM71), Texas Red- or Cy-5-conjugated anti-Thy-1 (T24.31.2), and propidium iodide (PI at 0.5 μg/ml, Sigma). All monoclonal antibodies for which no commercial source is indicated were purified in our laboratories from hybridoma supernatant on protein G–Sepharose (Pharmacia) and conjugated with biotin (Pierce), FITC, Texas Red (both from Molecular Probes), or Cy-5 (Amersham) according to each supplier's instructions. Viable (PI$^-$), CD3$^-$CD4$^-$CD8$^-$Thy-1$^+$ T cell progenitors were sorted into the three subpopulations: CD25$^-$CD44^{hi} (pro-T1), CD25$^+$CD44^{hi} (pro-T2), and CD25$^+$CD44^{low} (pro-T3) using a FACStar$^+$ or a modified FACSII (Becton Dickinson) cell sorter. Multiparameter analysis of fluorescent antibody–stained cells was performed on a FACScan (Becton Dickinson).

Tissue Culture

Single cell suspensions from thymus, spleen, or lymph nodes were prepared in phosphate buffered saline (PBS) containing 5% heat-inactivated fetal calf serum (PBS–FCS) and cultured in Dulbecco's modified Eagle's (DME) medium, supplemented with 216 mg/L L-glutamine, 50 μM 2-mercaptoethanol, antibiotics, and 10% heat-inactivated FCS (DMEM–FCS). Erythrocytes were removed by lysis in NH_4Cl. The following monoclonal antibodies were used for mitogenic stimulation of purified T cells in tissue culture: 145-2C11 (hamster anti-mouse CD3), GK1.5 (rat anti-mouse CD4), 53-6.7 (rat anti-mouse CD8), and I21/7.7 (rat anti-mouse LFA-1 β chain/CD18). All antibodies were purified from hybridoma supernatant by chromatography on protein A or protein G columns (Pharmacia). Tissue culture plates were coated with these antibodies at a concentration of 10–15 μg/ml in PBS (4 hr at 37°C or overnight at 4°C). Recombinant human interleukin 2 (rhIL-2) produced in E. coli was purchased from Cetus Corporation. For low density cultures, 5×10^4 purified CD4$^+$ or 1×10^4 purified CD8$^+$ T cells were cultured in 24-well flat-bottom culture trays (Falcon) either with immobilized mitogenic antibodies or with PMA (1 ng/ml) and ionomycin (125 ng/ml; Sigma) in 1 ml of DMEM–FCS with 30 ng/ml rhIL-2. At various days after initiation of culture, cells were harvested and counted in a hemocytometer. Mixed lymphocyte reactions were performed in 96-well round-bottom trays (Nunc). Graded numbers of purified CD4$^+$ splenic T cells

were cultured with γ-irradiated (20 Gy) allogeneic DBA/2 spleen cells (1×10^5/well). Cultures were pulsed after 4 days for 6 hr with 0.5 μCi of ^{3}H-thymidine (Amersham).

Cell Turnover Analysis

Cell turnover was measured by labeling proliferating cells in vivo with the thymidine analog bromodeoxyuridine (BrdU; Sigma), which was provided continuously for 1, 2, or 4 days in the drinking water (1 mg/ml plus 2% glucose to overcome taste aversion). Drinking water bottles were shielded from light and refreshed at three-day intervals. The cellularity of thymus, spleen, bone marrow, and lymph nodes did not alter significantly during the course of this treatment, indicating that it was not toxic, as shown before (Förster et al., 1989; Fulcher and Basten, 1994). BrdU incorporated into cellular DNA was detected by immunofluorescence staining with a specific FITC-labeled monoclonal antibody, BU-1 (Becton Dickinson), and flow cytometric analysis according to published procedures (Förster et al., 1989; Fulcher and Basten, 1994). Briefly, cells were fixed in 0.5% paraformaldehyde in PBS (20 min at room temperature). As the BU-1 antibody only binds to BrdU in single-stranded DNA, the DNA was denatured with 3N HCl containing 0.5% Tween-20 to permeabilize cell membranes (20 min at room temperature). The acid was neutralized with 0.1 M di-sodium tetraborate, and cells were then stained (30 min) with FITC-BU-1 in 0.5% Tween-20. Combined analysis of incorporated BrdU and cell surface antigen expression to identify T lymphocytes was performed by staining cells with FITC-BU-1 and biotin-labeled anti-Thy-1 (T24.31.2), which was revealed with Tricolor-streptavidin (Caltag). Flow cytometric analysis (10,000 cells per sample) was performed on a FACScan (Becton Dickinson) using the software provided. As a negative control for the BrdU staining, we included thymocytes from a mouse that had not received BrdU in all experiments. Routinely, fewer than 2% of such cells were stained by FITC-BU-1. As a positive control for both BrdU uptake into the animal and for staining with BU-1-FITC, we routinely confirmed that bone marrow granulocytes were strongly labeled.

Cell Cycle Analysis

Purified thymocyte subpopulations were analyzed for their distribution among the phases of the cell cycle by flow cytometry. DNA was labeled by staining cells for >30 min at 4°C with 50 μg/ml PI in 0.1% sodium acetate, 0.2% Triton X-100 (BDH Chemicals). Flow cytometric analysis (10,000 cells per sample) was performed on a FACScan (Becton Dickinson) at a low flow rate (100–300 cells/sec). Cell cycle distribution was determined with the FACScan Cellfit program or by manual gating.

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References

Cao, X., Shores, E.W., Hu-Li, J., Anver, M.R., Kelsall, B.L., Russell, S.M., Drago, J., Noguchi, M., Grinberg, A., Bloom, E.T., et al. (1995). Defective lymphoid development in mice lacking expression of the common cytokine receptor γ chain. Immunity *2*, 223–238.

Cory, S. (1995). Regulation of lymphocyte survival by the *BCL-2* gene family. Annu. Rev. Immunol. *13*, 513–543.

Egerton, M., Shortman, K., and Scollay, R. (1990). The kinetics of immature thymocyte development in vivo. Int. Immunol. *2*, 501–507.

Fehling, H.J., Krotkova, A., Saitnruf, C., and Von Boehmer, H. (1995). Crucial role of the pre-T-cell receptor alpha gene in development of alpha-beta but not gamma-delta T cells. Nature *375*, 795–798.

Fisher, A.G., Burdet, C., Bunce, C., Merkenschlager, M., and Ceredig, R. (1995). Lymphoproliferative disorders in IL-7 transgenic mice: expansion of immature B cells which retain macrophage potential. Int. Immunol. *7*, 415–423.

Förster, I., Vieira, P., and Rajewsky, K. (1989). Flow cytometric analysis of cell proliferation dynamics in the B cell compartment of the mouse. Int. Immunol. *1*, 321–331.

Fulcher, D.A., and Basten, A. (1994). Reduced life span of anergic self-reactive B cells in a double-transgenic model. J. Exp. Med. *179*, 125–134.

Godfrey, D.I., and Zlotnik, A. (1993). Control points in early T-cell development. Immunol. Today *14*, 547–553.

Godfrey, D.I., Zlotnik, A., and Suda, T. (1992). Phenotypical and functional characterization of c-kit expression during intrathymic T cell development. J. Immunol. *149*, 2281–2285.

Goodwin, R.G., Lupton, S., Schmierer, A., Hjerrild, K.J., Jerzy, R., Clevenger, W., Gillis, S., Cosman, D., and Namen, A.E. (1989). Human interleukin 7: molecular cloning and growth factor activity on human and murine B-lineage cells. Proc. Natl. Acad. Sci. USA *86*, 302–306.

Grabstein, K.H., Waldschmidt, T.J., Finkelman, F.D., Hess, B.W., Alpert, A.R., Boiani, N.E., Namen, A.E., and Morrissey, P.J. (1993). Inhibition of murine B and T lymphopoiesis in vivo by an anti-interleukin 7 monoclonal antibody. J. Exp. Med. *178*, 257–264.

Groettrup, M., Ungewiss, K., Azogui, O., Palacios, R., Owen, M.J., Hayday, A.C., and von Boehmer, H. (1993). A novel disulfide-linked heterodimer on pre-T cells consists of the T cell receptor β chain and a 33 kd glycoprotein. Cell *75*, 283–294.

Kondo, M., Takeshita, T., Higuchi, M., Nakamura, M., Sudo, T., Nishikawa, S.-I., and Sugamura, K. (1994). Functional participation of the IL-2 receptor γ chain in IL-7 receptor complexes. Science *263*, 1453–1454.

Korsmeyer, S.J. (1995). Regulators of cell death. Trends Genet. *11*, 101–105.

Linette, G.P., Grusby, M.J., Hedrick, S.M., Hansen, T.H., Glimcher, L.H., and Korsmeyer, S.J. (1994). Bcl-2 is upregulated at the $CD4^+CD8^+$ stage during positive selection and promotes thymocyte differentiation at several control points. Immunity *1*, 197–205.

Linette, G.P., Li, Y., Roth, K., and Korsmeyer, S.J. (1996). Cross talk between cell death and cell cycle progression: BCL-2 regulates NFAT-mediated activation. Proc. Natl. Acad. Sci. USA *93*, 9545–9552.

Mackarehtschian, K., Hardin, J.D., Moore, K.A., Boast, S., Goff, S.P., and Lemischka, I.R. (1995). Targeted disruption of the *flk2/flt3* gene leads to deficiencies in primitive hematopoietic progenitors. Immunity *3*, 147–161.

Maraskovsky, E., Teepe, M., Morrissey, P.J., Braddy, S., Miller, R.E., Luynch, D.H., and Peschon, J.J. (1996). Impaired survival and proliferation in IL-7 receptor-deficient peripheral T cells. J. Immunol. *157*, 5315–5323.

Mazel, S., Burtrum, D., and Petrie, H.T. (1996). Regulation of cell division cycle progression by *bcl-2* expression: a potential mechanism for inhibition of programmed cell death. J. Exp. Med. *183*, 2219–2226.

Memon, S.A., Moreno, M.B., Petrak, D., and Zacharchuk, C.M. (1995). Bcl-2 blocks glucocorticoid- but not Fas- or activation-induced apoptosis in a T cell hybridoma. J. Immunol. *115*, 4644–4652.

Metcalf, D. (1989). The molecular control of cell division, differentiation commitment and maturation in haemopoietic cells. Nature *339*, 27–30.

Mollina, T.J., Kishihara, K., Siderovski, D.P., van Ewijk, W., Narendran, A., Timms, E., Wakeham, A., Paige, C.J., Hartmann, K.-U., Veillette, A., et al. (1992). Profound block in thymocyte development in mice lacking $p56^{lck}$. Nature *357*, 161–164.

Mombaerts, P., Clark, A.R., Rudnicki, M.A., Iacomini, J., Itohara, S.,

Lafaille, J.J., Wang, L., Ichikawa, Y., Jaenisch, R., Hooper, M.L., and Tonegawa, S. (1992a). Mutations in T-cell antigen receptor genes α and β block thymocyte development at different stages. Nature *360*, 225–231.

Mombaerts, P., Iacomini, J., Johnson, R.S., Herrup, K., Tonegawa, S., and Papaioannou, V.E. (1992b). RAG-1-deficient mice have no mature B and T lymphocytes. Cell *68*, 869–877.

Morrissey, P.J., Goodwin, R.G., Nordan, R.P., Anderson, D., Grabstein, K.H., Cosman, D., Sims, J., Lupton, S., Acres, B., Reed, S.G., et al. (1989). Recombinant interleukin-7, pre-B cell growth factor, has costimulatory activity on purified mature T cells. J. Exp. Med. *169*, 707–716.

Morrissey, P.J., Conlon, P., Charrier, K., Braddy, S., Alpert, A., Williams, D., Namen, A.E., and Mochizuki, D. (1991). Administration of IL-7 to normal mice stimulates B-lymphopoiesis and peripheral lymphadenopathy. J. Immunol. *147*, 561–568.

Muegge, K., Vila, M.P., and Durum, S.K. (1993). Interleukin 7: a cofactor for V(D)J rearrangement of the T cell receptor B gene. Science *261*, 93–95.

Murphy, K.M., Heimberger, A.B., and Loh, D.Y. (1990). Induction by antigen of intrathymic apoptosis of $CD4^+CD8^+TCR^{lo}$ thymocytes in vivo. Science *250*, 1720–1723.

Murray, R., Suda, T., Wrighton, N., Lee, F., and Zlotnik, A. (1989). IL-7 is a growth and maintenance factor for mature and immature thymocyte subsets. Int. Immunol. *1*, 526–531.

Namen, A.E., Lupton, S., Hjerrild, K., Wignall, J., Mochizuki, D.Y., Schmierer, A., Mosley, B., March, C.J., Urdal, D., Gillis, S., et al. (1988). Stimulation of B-cell progenitors by cloned murine interleukin-7. Nature *333*, 571–573.

Noguchi, M., Nakamura, Y., Russell, S.M., Ziegler, S.F., Tsang, M., Cao, X., and Leonard, W.J. (1993). Interleukin-2 receptor γ chain: a functional component of the interleukin-7 receptor. Science *262*, 1877–1880.

Nosaka, T., van Deursen, J.M., Tripp, R.A., Thierfelder, W.E., Witthuhn, B.A., McMickle, A.P., Doherty, P.C., Grosveld, G.C., and Ihle, J.N. (1995). Defective lymphoid development in mice lacking Jak3. Science *270*, 800–802.

Nossal, G.J.V. (1994). Negative selection of lymphocytes. Cell *76*, 229–239.

O'Reilly, L., Huang, D.C.S., and Strasser, A. (1996). The cell death inhibitor Bcl-2 and its homologues influence control of cell cycle entry. EMBO J. *15*, 6979–6990.

Penit, C., Lucas, B., and Vasseur, F. (1995). Cell expansion and growth arrest phases during the transition from precursor ($CD4^-8^-$) to immature ($CD4^+8^+$) thymocytes in normal and genetically modified mice. J. Immunol. *154*, 5103–5113.

Peschon, J.J., Morrissey, P.J., Grabstein, K.H., Ramsdell, F.J., Maraskovsky, E., Gliniak, B.C., Park, L.S., Ziegler, S.F., Williams, D.E., Ware, C.B., et al. (1994). Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. J. Exp. Med. *180*, 1955–1960.

Petrie, H.T., Livak, F., Schatz, D.G., Strasser, A., Crispe, N.I., and Shortman, K. (1993). Multiple rearangements in TCR-α chain genes maximize the production of useful thymocytes. J. Exp. Med. *178*, 615–622.

Philpott, K.L., Viney, J.L., Kay, G., Rastan, S., Gardiner, E.M., Chae, S., Hayday, A.C., and Owen, M.J. (1992). Lymphoid development in mice congenitally lacking T cell receptor α/β-expressing cells. Science *256*, 1448–1452.

Raff, M.C. (1996). Size control: the regulation of cell numbers in animal development. Cell *86*, 173–175.

Rajewsky, K. (1996). Clonal selection and learning in the antibody system. Nature *381*, 751–758.

Rich, B.E., Campos-Torres, J., Tepper, R.I., Moreadith, R.W., and Leder, P. (1993). Cutaneous lymphoproliferation and lymphomas in interleukin 7 transgenic mice. J. Exp. Med. *177*, 305–316.

Rodewald, H.-R., Kretzschmar, K., Swat, W., and Takeda, S. (1995). Intrathymically expressed c-*kit* ligand (stem cell factor) is a major factor driving expansion of very immature thymocytes *in vivo*. Immunity *3*, 313–319.

Rolink, A., Grawunder, U., Haasner, D., Strasser, A., and Melchers, F. (1993). Immune surface Ig^+ B cells can continue to rearrange κ and λ L chain gene loci. J. Exp. Med. *178*, 1263–1270.

Samaridis, J., Casorati, G., Traunecker, A., Iglesias, A., Gutierrez, J.C., Müller, U., and Palacios, R. (1991). Development of lymphocytes in interleukin 7-transgenic mice. Eur. J. Immunol. *21*, 453–460.

Sentman, C.L., Shutter, J.R., Hockenbery, D., Kanagawa, O., and Korsmeyer, S.J. (1991). bcl-2 inhibits multiple forms of apoptosis but not negative selection in thymocytes. Cell *67*, 879–888.

Shinkai, Y., Rathbun, G., Lam, K.-P., Oltz, E.M., Stewart, V., Mendelsohn, M., Charron, J., Datta, M., Young, F., Stall, A.M., and Alt, F.W. (1992). RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangements. Cell *68*, 855–867.

Shortman, K., and Wu, L. (1996). Early T lymphocyte progenitors. Annu. Rev. Immunol. *14*, 29–47.

Spanopoulou, E., Roman, C.A.J., Corcoran, L.M., Schlissel, M.S., Silver, D.P., Nemazee, D., Nussenzweig, M.C., Shinton, S.A., Hardy, R.A., and Baltimore, D. (1994). Functional immunoglobulin transgenes guide ordered B-cell differentiation in Rag-1-deficient mice. Genes Dev. *8*, 1030–1042.

Strasser, A., Harris, A.W., and Cory, S. (1991). Bcl-2 transgene inhibits T cell death and perturbs thymic self-censorship. Cell *67*, 889–899.

Strasser, A., Harris, A.W., Corcoran, L.M., and Cory, S. (1994a). Bcl-2 expression promotes B- but not T-lymphoid development in *scid* mice. Nature *368*, 457–460.

Strasser, A., Harris, A.W., Von Boehmer, H., and Cory, S. (1994b). Positive and negative selection of T cells in T-cell receptor transgenic mice expressing a *bcl-2* transgene. Proc. Natl. Acad. Sci. USA *91*, 1376–1380.

Strasser, A., Harris, A.W., Huang, D.C.S., Krammer, P.H., and Cory, S. (1995). Bcl-2 and Fas/APO-1 regulate distinct pathways to lymphocyte apoptosis. EMBO J. *14*, 6136–6147.

Sudo, T., Nishikawa, S., Ohno, N., Akiyama, N., Tamakoshi, M., Yoshida, H., and Nishikawa, S.-I. (1993). Expression and function of the interleukin 7 receptor in murine lymphocytes. Proc. Natl. Acad. Sci. USA *90*, 9125–9129.

Surh, C.D., and Sprent, J. (1994). T-cell apoptosis detected *in situ* during positive and negative selection in the thymus. Nature *372*, 100–103.

Vanhaesebroeck, B., Reed, J.C., de Valck, D., Grooten, J., Miyashita, T., Tanaka, S., Beyaert, R., van Roy, F., and Fiers, W. (1993). Effect of *bcl-2* proto-oncogene expression on cellular sensitivity to tumor necrosis factor-mediated cytotoxicity. Oncogene *8*, 1075–1081.

Veis, D.J., Sentman, C.L., Bach, E.A., and Korsmeyer, S.J. (1993). Expression of the Bcl-2 protein in murine and human thymocytes and in peripheral T lymphocytes. J. Immunol. *151*, 2546–2554.

von Boehmer, H. (1994). Positive selection of lymphocytes. Cell *76*, 219–228.

von Freeden-Jeffry, U., Vieira, P., Lucian, L.A., McNeil, T., Burdach, S.E.G., and Murray, R. (1995). Lymphopenia in interleukin (IL)-7 gene-deleted mice identifies IL-7 as a nonredundant cytokine. J. Exp. Med. *181*, 1519–1526.

Young, F., Mizoguchi, E., Bhan, A.K., and Alt, F.W. (1997). Constitutive Bcl-2 expression during immunoglobulin heavy chain–promoted B cell differentiation expands novel precursor B cells. Immunity *6*, 23–33.