Induction of Interleukin 2 Receptor β Chain Expression by Self-recognition in the Thymus

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Summary

1–2% of adult mouse thymocytes express the T cell receptor α/β (TCR-α/β) together with the interleukin (IL) 2Rβ (p70), but not the α (p 55) chain. We show that the previously described α/β-TCR$^+$CD4$^-$8$^-$ and the partially overlapping Ly6C$^+$ thymocytes are contained within this subset. Most IL-2Rβ$^+$ α/β-TCR$^+$ cells have a mature and activated (heat stable antigen [HSA]$^-$, thymic shared antigen 1 [TSA-1]$^-$, CD44^{high}, CD69$^+$) phenotype. Overrepresentation of Vβ8.2 in both CD4$^-$8$^-$ and CD4 and/or CD8$^+$ IL-2Rβ$^+$ thymocytes suggests that IL-2Rβ expression is induced by a TCR-mediated activation event. In mice transgenic for an H-2K^b–specific TCR, IL-2Rβ$^+$ cells were abundant under conditions of mainstream negative selection, i.e., in the presence of K^b, but absent under conditions of mainstream positive selection or in a nonselecting environment. Together, these results show that in addition to clonal deletion, self-recognition by immature thymocytes leads to phenotypic maturation of a small subset of thymocytes expressing IL-2Rβ. IL-2–deficient mice contain normal numbers of IL-2Rβ$^+$ α/β-TCR$^+$ thymocytes, indicating that like mainstream T cell development, this minor pathway of positive selection does not depend on IL-2. However, in the absence of IL-2, the CD4/CD8 subset composition of IL-2Rβ$^+$ thymocytes is skewed towards CD4$^-$8$^+$, mostly at the expense of CD4$^-$8$^-$. A possible relevance of this finding for the development of the immune pathology of IL-2–deficient mice is discussed.

In resting mature T lymphocytes, engagement of the TCR results in the expression of both the α (p55, CD25) and the β (p70, CD122) chain of the IL-2R, allowing IL-2 to exert its growth and differentiation-promoting activities on antigen-triggered T cells (for a review see reference 1). IL-2R are also found on freshly isolated mouse thymocytes, but neither the inductive stimuli leading to their expression nor their functional role, if any, are presently understood. Indeed, whereas CD25 expression is an important means of defining a subset of CD3$^-$4$^-$8$^-$ thymocytes (2–4), the functional significance of this IL-2R which lacks the β chain (5) required for signal transduction (6) remains obscure. In addition, the intrathymic generation of normal numbers of CD4 and CD8 T cells expressing the TCR-α/β in IL-2–deficient mice indicates that this cytokine is dispensable for mainstream T cell development (7).

The β chain of the IL-2R, which together with the constitutively expressed γ chain forms a functional IL-2R (8), has recently been detected by Takeushi et al. (5) on a major subset of fetal and adult γ/δ thymocytes and on a very small fraction (1–2%) of adult thymocytes that express the TCR-α/β at a level between that of immature CD4$^+$8$^+$ and mature CD4$^+$8$^-$ or CD4$^-$8$^+$ cells. These "α/β-TCR^{int}" cells, which make up ~90% of adult IL-2Rβ$^+$ thymocytes, do not express the IL-2Rα chain (5). In the rat, we recently found that in vitro stimulation of immature CD4,8 double positive cells with mAb to the TCR induces IL-2Rβ, but not α mRNA and conveys IL-2 reactivity (9). Accordingly, we hypothesize that the IL-2Rβ$^+$α/β-TCR$^+$ cells described by Takeushi et al. (5) had acquired the IL-2Rα$^-$β$^+$ phenotype as a result of self-recognition during positive and/or negative selection of the TCR repertoire.

In the present report, this hypothesis was tested by analyzing the cell surface phenotype and TCR-α/β repertoire of IL-2Rα$^-$β$^+$ thymocytes from normal mice and from TCR-transgenic mice undergoing positive or negative selection. In addition, it was tested if this subset is normally represented in IL-2–deficient mice.

Materials and Methods

Animals. BALB/c, C57Bl/6, C3H/HeJ, and DBA/2 mice were bred at the Institute of Virology and Immunobiology, University of Würzburg from offspring of pregnant females obtained from

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Iffa Credo (Domaine des Oncins, France). IL-2–deficient mice were the third generation backcross of the IL-2–deficient C57BL/6 × 129 line originally derived by Schorle et al. (7) with the C3H/HeJ strain, H-2^d, H-2^{dxk} and H-2^{bxk} mice on the C57BL/10 background transgenic for the H-$2K^b$-specific KB5.C20 TCR (10, 11) were the kind gift of Dr. B. Arnold (Deutsches Krebsforschungszentrum, Heidelberg, Germany).

Antibodies. mAb TM-β1 to the mouse IL-2R β chain (12) was the kind gift of Drs. T. Tanaka and M. Miyasaka (Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan). mAb Desirée-1 (13) specific for the transgenic TCR KB5.C20, was kindly provided by Dr. A.-M. Schmitt-Verhulst (Centre d'Immunologie, Marseille-Luminy, France) mAb F23.2 recognizing the TCR-Vβ8.2 gene segment (14) was the kind gift of Dr. U. Staerz (University of Colorado, Denver, CO). mAbs to CD25, CD44, CD69, Ly6C, thymic shared antigen (TSA-1)[1], heat stable antigen (HSA), and TCR-α/β were purchased from Pharmingen (San Diego, CA); anti-CD4 mAbs were from Becton Dickinson & Co. (San Jose, CA) or Medac (Hamburg, Germany); and anti-CD8 mAbs were from Boehringer Mannheim (Mannheim, Germany).

Immunofluorescence and Flow Cytometry. For two- or three-color FACS® analysis (Becton Dickinson & Co.), 2 × 10^6 nylon-wool–passaged thymocytes or lymph node cells from 4–8-wk-old mice were stained with saturating amounts of the respective antibodies. First-step unconjugated anti-IL-2Rβ mAb TM-β1 was visualized with donkey anti–rat-Ig-PE (Medac) and after blocking with normal rat Ig (Sigma Chemical Co., St. Louis, MO), biotinylated and FITC-conjugated mAb to the other markers were added. Finally, biotinylated mAbs were developed with streptavidin-RED^{670} (GIBCO, Eggenstein, Germany). Alternatively, mAb F23.2 to Vβ8.2 or mAb Desirée-1 were stained indirectly with donkey anti–mouse Ig-PE (Jackson ImmunoResearch, distributed through Dianova, Hamburg, Germany) or rabbit anti–mouse Ig-FITC prepared at the Institute for Virology and Immunology, Würzburg, blocked with normal mouse Ig (Sigma Chemical Co.), and stained with biotinylated TM-β1 and directly PE- or FITC-conjugated mAb to the markers indicated. Where necessary, preincubation with anti-Fc-receptor mAb 24G2 (15) preceded staining with biotinylated mAb TM-β1. All immunofluorescence stainings were performed on ice in PBS containing 0.1% BSA and 0.02% sodium azide with reactions washed once after each step and twice after incubation with biotinylated mAb. Flow cytometry was performed with a FACScan® flow cytometer (Becton Dickinson & Co.) and data were analyzed using the LYSYS II software. Routinely, 10,000 events were analyzed. Results are shown as $\log_{10}$ fluorescence intensities on a four-decade scale displayed as dot plots or histograms.

Results

Phenotype of IL-2R$\alpha^-\beta^+$ Thymocytes from Normal Mice. 1–2% of thymocytes from 4–8-wk-old mice react with the anti-IL-2Rβ mAb TM-β1 (12). The phenotype of this small subset was analysed in BALB/c, C57BL/6, C3H/HeJ, and DBA/2 mice. The marker profile shown in Fig. 1 for IL-2Rβ^+ thymocytes from C3H/HeJ mice is representative for the four stains tested. As reported by Takeuchi et al. (5), most (~90%) of IL-2Rβ^+ cells in the adult thymus are α/β-TCR^{int}, i.e., they express the TCR at a level between that of most immature CD4$^+$8$^+$ and mature CD4$^+$ or CD8$^+$ thymocytes. We also confirmed that these cells do not express the IL-2Rα chain. Adult IL-2Rβ^+ thymocytes are of the size of mature lymphocytes, and 80–90% express low to undetectable amounts of HSA and TSA-1, both of which mark immature thymocytes (16, 17). IL-2Rβ^+ thymocytes are uniformly CD44$^+$, expressing this marker at the highest level found on any thymocyte subset. In mature T cells, this

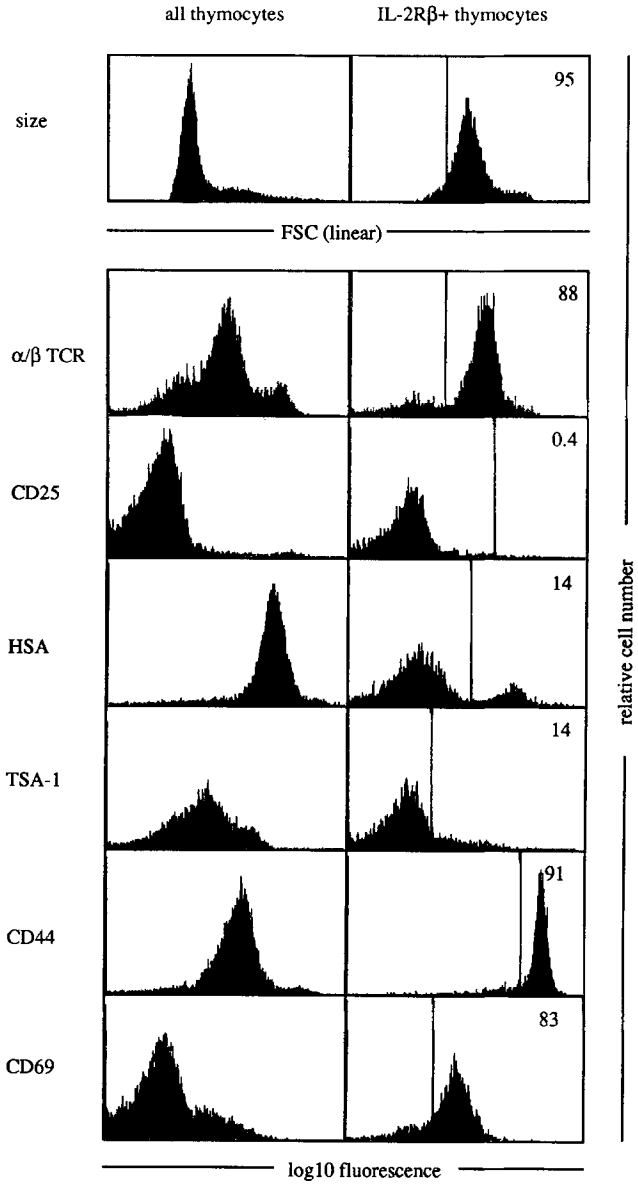


Figure 1. Cell surface phenotype of IL-2Rβ^+ thymocytes. Nylon-wool–passaged thymocytes of C3H/HeJ mice were stained with anti-IL-2Rβ mAb TM-β1 and with the mAb indicated as described in Materials and Methods. (*Left*) Marker profiles for all living thymocytes; (*right*) marker profiles for cells gated for expression of TM-β1. Numbers display the percentage of IL-2Rβ^+ thymocytes showing higher expression of the marker than indicated by the bars. Histograms represent $\log_{10}$ fluorescence intensities on a four-decade scale.

[1] *Abbreviations used in this paper:* FTOC, fetal thymic organ culture; HSA, heat stable antigen; TSA-1, thymic shared antigen 1.

Table 1. *CD4/CD8 Subset Composition of IL-2Rβ⁺ Thymocytes from Inbred Mouse Strains*

	C57Bl/6		C3H/HeJ		BALB/c		DBA/2	
	x	s	x	s	x	s	x	s
$CD4^+CD8^-$	29.0	3.6	27.9	1.7	21.6	7.9	13.5	0.6
$CD4^-CD8^+$	8.9	1.5	7.0	2.5	10.3	4.3	57.6	2.1
$CD4^+CD8^+$	10.4	3.8	7.5	0.2	5.5	2.6	8.2	3.1
$CD4^-CD8^-$	51.8	2.1	57.6	3.6	62.6	2.9	20.7	3.1

Numbers given are mean percentage values (x) and standard deviations (s) from three animals.

$CD44^{high}$ phenotype marks antigen-experienced cells (18, 19). Finally, the activation marker CD69 is found on the majority of IL-2Rβ⁺ thymocytes.

IL-2Rβ⁺ Thymocytes Contain the $CD4^-8^-$ α/β-TCR⁺ Subset in Addition to other CD4, 8 phenotypes. IL-2Rβ⁺ thymocytes contain a high frequency (50–60%) of $CD4^-8^-$ cells (5, and Table 1). The three other subsets defined by CD4 and CD8 expression are, however, also represented, although at lower frequencies (20–30% $CD4^+8^-$, 7–11% $CD4^-8^+$, and 5–10% $CD4^+8^+$). An exception is the DBA/2 strain, in which about half of IL-2Rβ⁺ thymocytes are $CD4^-8^+$ cells. The IL-2Rβ⁺ $CD4^+8^+$ population is HSA^+, since the frequencies of $CD4^+HSA^+$, $CD8^+HSA^+$, and $CD4^+$ and/or $CD8^+$ HSA^+ cells among IL-2Rβ⁺ thymocytes were indistinguishable (data not shown).

Overrepresentation of Vβ8.2 in Subsets of IL-2Rβ⁺ Thymocytes. To probe whether the composition of the TCR repertoire would point to a TCR-mediated activation event responsible for the induction of the IL-2Rβ⁺ phenotype, the contribution of Vβ 6, 8.1, 8.2, 8.3, 11, and 14 to the repertoire of IL-2Rβ⁺ thymocytes and peripheral T cells was analyzed. A clear-cut skewing of Vβ usage was only observed with regard to Vβ8.2 (Fig. 2), which is expressed at several-fold higher frequencies in IL-2Rβ⁺ thymocytes than in peripheral lymph node T cells. The increased frequency of $V\beta8.2^+$ cells was observed both in the major $CD4^-8^-$ subset and in IL-2Rβ⁺ cells expressing CD4 and/or CD8. The only exception was the unusually large subset of $CD8^+$ IL-2Rβ⁺ thymocytes from DBA/2 mice.

IL-2Rβ⁺ Thymocytes Include the α/β-TCR⁺ $CD4^-8^-$ and $Ly6C^+$ Subsets. Earlier studies have shown that in α/β-TCR^+ $CD4^-8^-$ (20–22) and the partially overlapping $Ly6C^+$ (23) subsets, up to 50% of thymocytes express Vβ8 family members, primarily Vβ8.2 (23–25). Since about half of IL-2Rβ⁺ thymocytes are α/β-TCR^+ $CD4^-8^-$ (Table 1), and $Ly6C^+$ thymocytes also contain CD4- and/or CD8-expressing cells, the overlap of these two subsets with IL-2Rβ⁺ cells was examined. As shown in Fig. 3 *A* for C57BL/6 mice, virtually all α/β-$TCR^+CD4^-8^-$ and ~90% of the $Ly6C^+$ thymocytes express IL-2Rβ. However, ~20% of IL-2Rβ⁺ thymocytes express CD4 and/or CD8 but not Ly6C (Fig. 3 *B*), indicating that IL-2Rβ marks some additional cells not contained within these two previously defined subsets.

Generation of IL-2Rβ⁺ Thymocytes in TCR Transgenic Mice Undergoing Positive or Negative Selection. The possibility that IL-2Rβ expression is the result of TCR stimulation during positive and/or negative repertoire selection was analyzed in transgenic mice with a K^b-specific TCR (KB5.C20) (11) that is identified by the anti-idiotypic mAb Desirée-1 (13). As shown in Fig. 4, virtually no idiotype-positive IL-2Rβ⁺ thymocytes were detected in Desirée-1-transgenic mice with a nonselecting H-2 haplotype ($H\text{-}2^d$), or in a positively

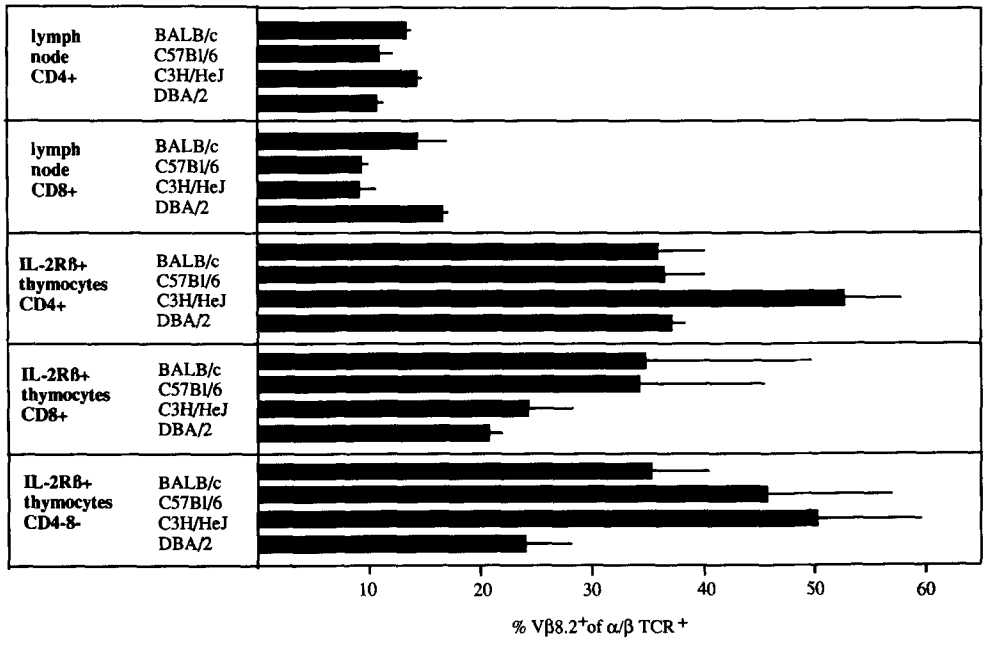


Figure 2. Vβ8.2 expression in lymph node T cells and IL-2Rβ⁺ thymocytes. Nylon-wool-passaged cells from mice of the indicated strains were stained indirectly with pan-anti-TCR-α/β or anti-Vβ8.2, with biotinylated anti-IL-2Rβ mAb TM-β1, and fluorochrome-conjugated anti-CD4 or CD8 mAb. Bars show the relative frequencies and standard deviations of $V\beta8.2^+$ cells (n = 3) among all TCR^+ cells expressing or lacking CD4 and/or CD8.

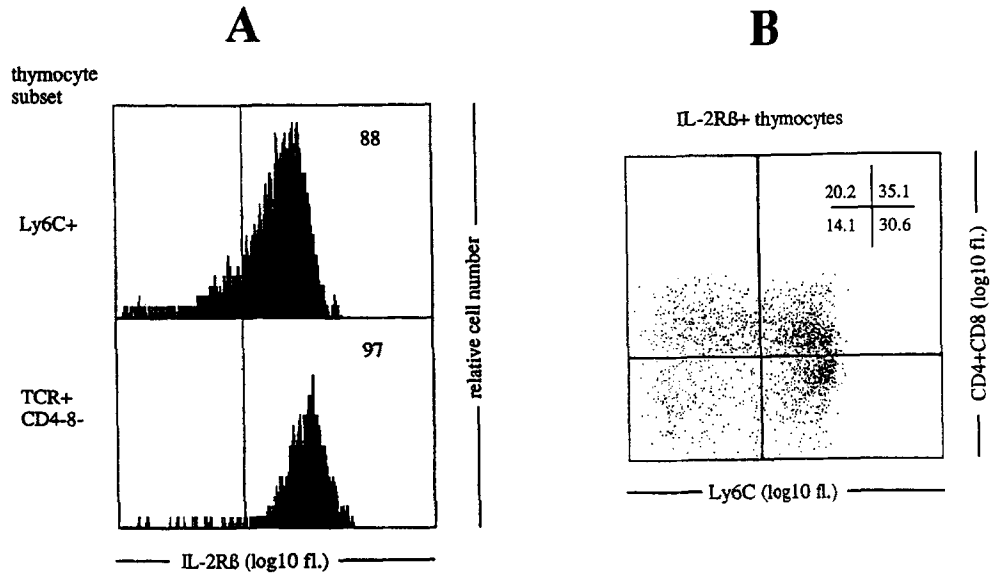
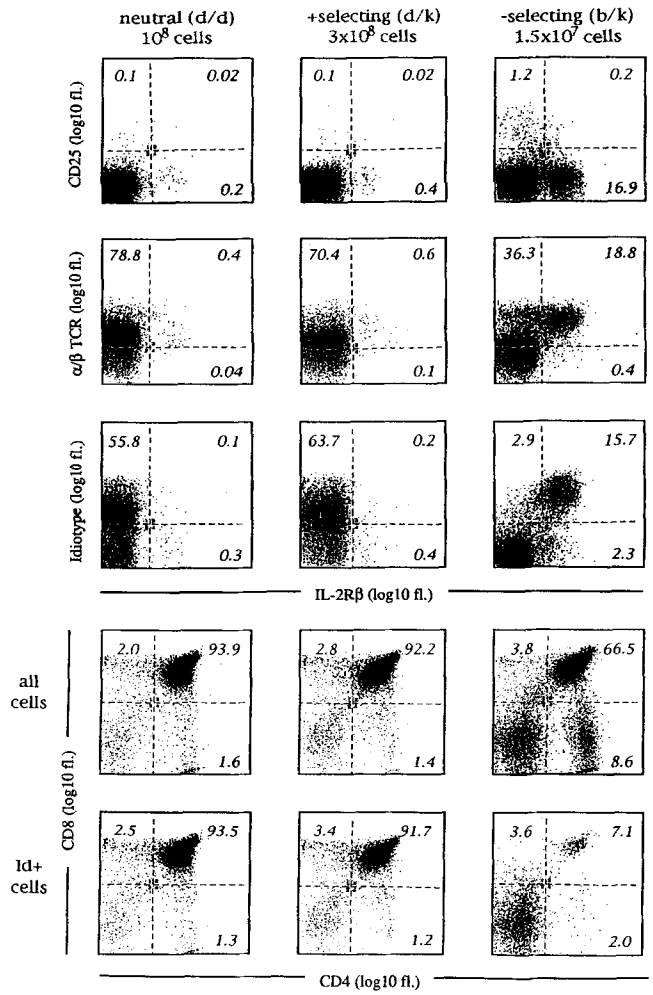


Figure 3. IL-2Rβ^+ thymocytes contain TCR$^+$CD4$^-$CD8$^-$ thymocytes, Ly6C$^+$ cells and others. (*A*) IL-2Rβ expression by Ly6C$^+$ (*top*) and by TCR$^+$ CD4$^-$8$^-$ (*bottom*) C57Bl/6 thymocytes. (*B*) Ly6C vs CD4 and/or CD8 profile of IL-2Rβ^+ thymocytes. Histograms and dot plots display $\log_{10}$ fluorescence intensities on a four-decade scale.



selecting (H-2^{dxk}) thymus. In contrast, most idiotype-positive thymocytes present in negatively selecting H-2^{bxk} animals expressed the IL-2Rβ, but not α chain. Although the thymuses of such negatively selecting animals are much smaller than those expressing only positively selecting H-2 antigens, there was an absolute (at least 10-fold) increase in the total number of idiotype-positive, IL-2Rβ^+ thymocytes from a negatively selecting as compared to a nonselecting thymus. In fact, the few idiotype-positive IL-2Rβ^+ events recorded in the thymus of positively selecting animals are likely to be due to nonspecific background staining (data not shown). As also shown in Fig. 4 and confirming earlier results (26), the IL-2Rβ^+ cells that survived negative selection were mostly CD4$^-$8$^-$, but also contained a small but well-defined CD4$^+$ 8$^+$ subset. CD4$^-$8$^-$ cells also constitute the major population of idiotype-positive cells in the periphery of H-2^{bxk} KB5.C20 TCR-transgenic mice (11). As in the thymus, these cells express IL-2Rβ without α (data not shown). In summary, self-recognition in this TCR-transgenic model leads to an accumulation of IL-2R$\alpha^-\beta^+$ CD4$^-$8$^-$ thymocytes that escaped negative selection, whereas in the absence of the cognate antigen, IL-2Rβ^+ thymocytes with the transgenic TCR are absent.

IL-2 Is Not Required for the In Vivo Generation of IL-2Rβ^+ α/β-TCR^{int} Thymocytes. The functional relevance of IL-2R$\alpha^-\beta^+$ expression on thymocytes with self-specific TCR-

Figure 4. IL-2Rβ expression on thymocytes from mice expressing a H-2K^b-specific transgenic TCR. Thymocytes from mice expressing the transgenic TCR KB5.C20 in a neutral (H-2$^{d/d}$), positively selecting (H-2$^{d/k}$) or negatively selecting (H-2$^{b/k}$) thymus were stained for expression of the markers indicated. Total cell numbers are given on top of each column.

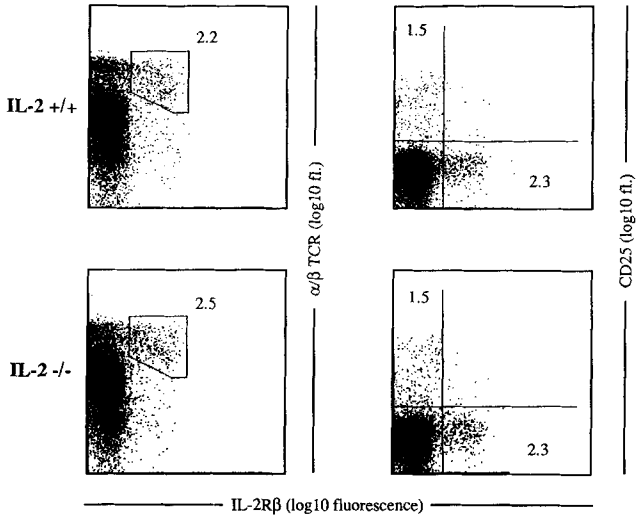


Figure 5. Generation of IL-2Rβ^+ thymocytes in IL-2-deficient mice. Representative stainings of IL-2Rβ vs TCR-α/β (*left*) and IL-2Rβ vs CD25 (*right*) are shown for thymocytes from IL-2-deficient mice (*bottom*) and their wild-type littermates (*top*). 3.2 ± 1.8 million IL-2Rβ^+ cells (n = 6) were contained within thymuses of control mice. Thymuses from IL-2-deficient mice contained 4.4 ± 1.7 million (n = 7) IL-2Rβ^+ thymocytes.

α/β is unclear. To investigate the possibility that an IL-2-mediated signal delivered through this receptor is required for the generation of these cells, we compared their frequency in mice with an inactivated IL-2 gene and their wild-type littermates. As shown in Fig. 5, both IL-$2^{+/+}$ and IL-$2^{-/-}$ mice contained approximately the same frequency of IL-2Rβ^+ α/β-TCR^{int} cells. Therefore, IL-2 is not required for the generation of this subset. Also, IL-2Rβ^+ cells from IL-2-deficient mice were indistinguishable from those of their wild-type littermates with regard to lack of CD25 expression (Fig. 5), uniformly high expression of CD44, and the distribution of Ly6C and CD69 (data not shown). Analysis of the CD4/8 subset distribution among IL-2Rβ^+ thymocytes did, however reveal a two- to threefold skewing towards the CD4$^-$8$^+$ subset, mostly at the expense of CD4$^-$8$^-$ cells (Fig. 6). Like all CD8$^+$IL-2Rβ^+ thymocytes, this expanded CD4$^-$8$^+$ population expressed both the CD8α and β chains (data not shown). To date, the distorted subset composition of IL-2Rβ^+ cells is the first abnormality in thymocyte subset distribution we have observed in IL-$2^{-/-}$ mice.

Discussion

The present findings indicate that in contrast to IL-2Rα found on immature CD3$^-$4$^-$8$^-$ thymocytes, IL-2Rβ expression on adult thymocytes of the α/β TCR lineage is not part of a developmental program but rather is induced by self-recognition. This is most clearly seen in mice with a transgenic H-2K^b-specific TCR in which no IL-2R$\alpha^-\beta^+$ idiotype-positive thymocytes were detected in the absence of K^b, whereas in its presence, the idiotype-positive thymocytes are IL-2R$\alpha^-\beta^+$. In the normal mice analyzed, skewing of the repertoire towards Vβ8.2 also indicates an antigen-specific selection or activation event in the generation of IL-2R$\alpha^-\beta^+$ thymocytes. Skewing towards Vβ8, and mostly towards Vβ8.2, has previously been reported for the CD4$^-$8$^-\alpha/\beta$-TCR$^+$ and for the partially overlapping Ly6C$^+$ (20–25) thymocyte subsets. We show here that both are contained within the 1–2% of IL-2R$\alpha^-\beta^+$ cells of the adult mouse thymus which, as described for Ly6C$^+$ thymocytes (23), can be further subdivided into the four subsets defined by CD4 and CD8. The major contribution of CD4$^+$8$^-$ cells (~25%) of the IL-2R$\alpha^-\beta^+$ population along with the striking overrepresentation of Vβ8.2 in their repertoire raises the possibility that the recently described Thy0 thymocytes, defined by the absence of the 3G11 determinant on a subset of CD4$^+$8$^-$

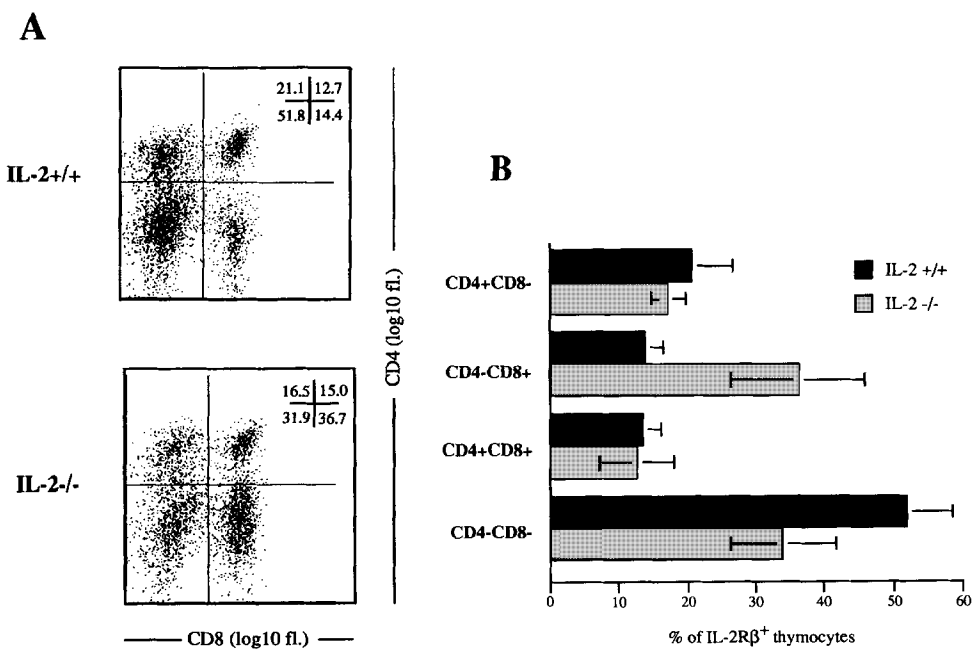


Figure 6. (*A*) Representative CD4/CD8 subset composition of IL-2Rβ^+ thymocytes from control (*top*) or IL-2-deficient (*bottom*) mice. (*B*) CD4/CD8 distribution among IL-2Rβ^+ thymocytes from wild-type (n = 6, *solid bars*) or IL-2-deficient (n = 7, *hatched bars*) mice. Bars indicate mean values and standard deviations.

cells and enriched in Vβ8 usage (27), also express IL-2Rβ. Finally, $CD4^-8^-$ (28) and $CD4^+8^-$ (29) $NK1.1^+$ $CD44^{high}$ thymocytes with a Vβ8-dominated TCR repertoire have been described. These cells are at least partially included within the IL-2Rβ$^+$ population (5, and own unpublished observations).

The self-antigen responsible for the generation of IL-2Rβ$^+$ double negative cells in the TCR-transgenic situation analyzed is obviously K^b. Additional proof for this conclusion was obtained in H-2^{dxk} mice transgenic for both the K^b-specific TCR and K^b (under the control of the mouse CD2 promoter) in which the IL-2Rβ$^+$ $CD4^-8^-$ population was similarly prominent (data not shown). $CD4^-8^-$ and $CD4^-8^{low}$ TCR-transgenic thymocytes and peripheral T cells have also been observed in other TCR-transgenic models in negatively selecting situations (30–32), although IL-2Rβ expression was not investigated. In nontransgenic mice, Vβ8.2 overselection in $CD4^-8^-$α/β-TCR^+ thymocytes (shown here to express IL-2Rβ) has recently been shown by Bix et al. (33) to depend on MHC class I expression on cells of hematopoietic origin. Together, these findings suggest that self-antigen recognition, which leads to negative selection in mainstream intrathymic T cell maturation, is the common trigger that induces IL-2Rβ expression in thymocytes of the TCR-α/β lineage, and that Vβ8.2 overselection represents a special case of a Vβ-specific interaction with a self-antigen. The antigen dependence of IL-2Rβ$^+$ thymocyte selection is reminiscent of the recently described "positive selection" of intraepithelial lymphocytes with a transgenic HY + D^b-specific TCR, which, unlike mainstream intrathymic repertoire selection, requires expression of both, the MHC restriction element and the antigenic peptide (34).

At which stage along the maturation pathway of IL-2Rβ$^+$α/β- T cells is IL-2Rβ expression induced? The heterogeneous phenotype of IL-2Rβ$^+$ cells suggests that this may happen at more than one stage. With regard to the $CD4^-8^-$ subset, demethylation of the CD8α gene has been taken as evidence for prior CD8 expression at an immature $CD4^-8^+$ stage or at the subsequent $CD4^+8^+$ stage (35). This would fit with the previous description of $CD4^+8^+$ thymocytes within the Ly6C subset and our present findings that ~10% of IL-2Rα$^-$β$^+$ thymocytes are $TSA\text{-}1^+HSA^+$ $CD4^+8^+$ cells. It is also in line with the rapid induction of IL-2Rβ, but not α mRNA, and concomitant downregulation of CD4 and CD8 molecules after in vitro cross-linking of the TCR-α/β on rat $CD4^+8^+$ thymocytes (9, 36). On the other hand, direct in vitro differentiation of TCR^+ $CD4^-8^-$ cells from $HSA^+CD3^-4^-8^-$ precursors via an $HSA^+CD3^+4^-8^-$ intermediate without transitional expression of CD4 or CD8 has recently been demonstrated (37). In support of this scheme, we observed that about one third of α/β-TCR^+HSA^+IL-2Rβ$^+$ thymocytes in C57Bl/6 mice express neither CD4 nor CD8 and may thus be the in vitro correlate to this $CD4^-8^-$ intermediate stage described (data not shown).

The functional importance of IL-2Rβ expression on thymocytes with self-specific receptors remains unresolved. Clearly, as shown by the presence of equivalent numbers of IL-2Rβ$^+$ thymocytes in IL-2–deficient mice and their wild-type littermates, IL-2 is not required for their generation. This is in line with the observation by Takeushi et al. (5) that blocking anti-IL-2Rβ mAb did not prevent the appearance of TCR-α/β$^+$IL-2Rβ$^+$ thymocytes both in fetal thymic organ culture (FTOC) and in vivo. On the other hand, these authors observed an expansion of this subset when IL-2 was included in FTOC, suggesting that the IL-2R expressed are functional. Since in suspension culture, IL-2 per se does not have this effect (our own unpublished observations), TCR stimulation by a ligand present in the intact thymus but not available in suspension culture may be required for IL-2–driven expansion of IL-2Rβ$^+$ thymocytes in FTOC. The possibility that IL-2β$^+$ thymocytes are, in fact, stimulated in vivo is supported by the expression of the activation markers CD44 and CD69. It seems very unlikely, however, that antigen plus IL-2–driven clonal expansion of TCR-α/β$^+$IL-2Rβ$^+$ thymocytes is operative in vivo because: (*a*) their numeric representation is not affected by IL-2 deficiency; (*b*) their major component, the TCR-α/β$^+$$CD4^-8^-$ (20, 24, 38) and $Ly6C^+$ (23) subsets accumulate slowly with age; and (*c*) at least mature $CD4^-8^-$ thymocytes are virtually devoid of cycling cells (39).

Functional competence of IL-2Rβ$^+$ thymocytes has been demonstrated at least for its TCR-α/β$^-$$CD4^-8^-$ subset, which can be stimulated to proliferate (20, 40), produce IL-4 (41), and exert cytotoxic function (40). It remains to be seen, however, if these cells also have a physiological role in the control of immune responses. It is important to note that IL-2Rβ$^+$ $CD4^-8^-$ T cells are abundant in the periphery of the H-2^{kxb} mice presently investigated that express a transgenic Kb-specific TCR (data not shown). Since neonatal thymectomy prevents their accumulation (42), it appears that these cells can migrate to the periphery. If, as we assume, such IL-2Rβ$^+$ thymocytes with self-specific receptors are also retained in the immune system of normal mice, their subsequent activation could lead to autoimmune disease. Alternatively, their function could be to suppress anti-self responses of T cells having undergone mainstream repertoire selection. Either scenario could be of relevance to the immunopathology that develops with age in IL-2–deficient mice. The unchecked lymphoproliferation (43, 44) and massive inflammation of the colon along with the formation of autoantibodies (44) points to a malfunctioning of counterregulatory mechanisms in these animals. The abnormal phenotypic composition of IL-2Rβ$^+$ thymocytes in IL-2–deficient animals, i.e., the increased production of $CD4^-$CD8α/β$^+$ at the expense of $CD4^-8^-$ cells may thus contribute to the development of the lymphoproliferative syndrome of $IL\text{-}2^{-/-}$ mice.

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