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 (p55) 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]+, CD44hi, 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-2Kb-specific TCR, IL-2Rβ+ cells were abundant under conditions of mainstream negative selection, i.e., in the presence of Kb, 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.

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...
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-2b, H-2(m) and H-2*m mice on the C57BL/10 background transgenic for the H-2Kk-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-2Rβ 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-VB8.2 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), 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 × 10⁶ 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 VB8.2 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 FACSScan® flow cytometer (Becton Dickinson & Co.) and data were analyzed using the LYSYS II software. Routinely, 10,000 events were analyzed. Results are shown as log₁₀ fluorescence intensities on a four-decade scale displayed as dot plots or histograms.

Results

Phenotype of IL-2Rα-β+ 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-TCRint, 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.](image-url)
CD4/CD8 Subset Composition of IL-2Rβ+ Thymocytes from Inbred Mouse Strains

| Strain    | C57Bl/6 | C3H/HeJ | BALB/c | DBA/2 |
|-----------|---------|---------|--------|-------|
|           | x       | x       | x      | s     |
| CD4+CD8-  | 29.0    | 3.6     | 27.9   | 1.7   |
| CD4-CD8+  | 8.9     | 1.5     | 7.0    | 2.5   |
| CD4+CD8+  | 10.4    | 3.8     | 7.5    | 0.2   |
| CD4-CD8-  | 51.8    | 2.1     | 57.6   | 3.6   |

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

CD4^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 Vβ8.2+ 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 Kb-specific TCR (KB5.C20) (11) that is identified by the anti-idiotypic mAb Desiree-1 (13). As shown in Fig. 4, virtually no idiotype-positive IL-2Rβ+ thymocytes were detected in Desiree-1-transgenic mice with a nonselecting H-2 haplotype (H-2d), or in a positively

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**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 Vβ8.2+ 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+CD8- (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^{bd}) thymus. In contrast, most idiotype-positive thymocytes present in negatively selecting H-2^{bk} 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^{bk} 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-2Rα-β+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 \\

Figure 4. IL-2Rβ expression on thymocytes from mice expressing a H-2Kb-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^{bk}) thymus were stained for expression of the markers indicated. Total cell numbers are given on top of each column.
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-2Kb-specific TCR in which no IL-2Rαβ+ idiotypic-positive thymocytes were detected in the absence of Kb, whereas in its presence, the idiotypic-positive thymocytes are IL-2Rαβ+. 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-2Rαβ+ thymocytes. Skewing towards Vβ8, and mostly towards Vβ8.2, has previously been reported for the CD4-8α/β-TCR+ and for the partially overlapping Ly6C+ (20-25) thymocyte subsets. We show here that both are contained within the 1-2% of IL-2Rαβ+ 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-2Rαβ+ population along with the striking over-representation 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-

Ly6C and CD69 (data not shown). Analysis of the CD4/8 subset distribution among IL-2Rαβ+ 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-2Rαβ+ thymocytes, this expanded CD4+8- population expressed both the CD8α and β chains (data not shown). To date, the distorted subset composition of IL-2Rαβ+ cells is the first abnormality in thymocyte subset distribution we have observed in IL-2-/- mice.
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 conclu-
sion was obtained in H-2^{abk} 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^+ 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^- thymo-
cytes (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 sug-
gest 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 thymo-
cytes 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^+ thymo-
cyte selection is reminiscent of the recently described “posi-
tive selection” of intraepithelial lymphocytes with a tran-
genic 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 het-
erogeneous 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-1^- HSA-
CD4^-8^- cells. It is also in line with the rapid induction of
IL-2Rβ, but not α mRNA, and concomitant downregula-
tion 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','-CD4^-8^- intermediate without transitional expres-
sion 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 thy-
mocytes 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 appear-
ance 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-2Rβ 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 possi-
bility 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^{abk} mice presently investigated that express a tran-
genic 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. Al-
ternatively, their function could be to suppress anti-self re-
ponses of T cells having undergone mainstream repertoire
selection. Either scenario could be of relevance to the immu-
nopathology 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 au-
toantibodies (44) points to a malfunctioning of counter-
regulatory mechanisms in these animals. The abnormal pheno-
typic 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-
2^-/- mice.
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