Allelic Exclusion in pTα-deficient Mice: No Evidence for Cell Surface Expression of Two T Cell Receptor (TCR)-β Chains, but Less Efficient Inhibition of Endogeneous Vβ→(D)Jβ Rearrangements in the Presence of a Functional TCR-β Transgene

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Summary

Although individual T lymphocytes have the potential to generate two distinct T cell receptor (TCR)-β chains, they usually express only one allele, a phenomenon termed allelic exclusion. Expression of a functional TCR-β chain during early T cell development leads to the formation of a pre-T cell receptor (pre-TCR) complex and, at the same developmental stage, arrest of further TCR-β rearrangements, suggesting a role of the pre-TCR in mediating allelic exclusion. To investigate the potential link between pre-TCR formation and inhibition of further TCR-β rearrangements, we have studied the efficiency of allelic exclusion in mice lacking the pre-TCR-α (pTα) chain, a core component of the pre-TCR. Staining of CD3+ thymocytes and lymph node cells with antibodies specific for Vβ6 or Vβ8 and a pool of antibodies specific for most other Vβ elements, did not reveal any violation of allelic exclusion at the level of cell surface expression. This was also true for pTα-deficient mice expressing a functionally rearranged TCR-β transgene. Interestingly, although the transgenic TCR-β chain significantly influenced thymocyte development even in the absence of pTα, it was not able to inhibit fully endogeneous TCR-β rearrangements either in total thymocytes or in sorted CD25+ pre-T cells of pTα→β mice, clearly indicating an involvement of the pre-TCR in allelic exclusion.

Functional TCR genes are assembled by a program of somatic gene rearrangements from variable (V), diversity (D), and joining (J) gene segments, diversity (D) genes, and joining (J) elements at the TCR-β loci and from Vα and Jα elements at the TCR-α loci. At the TCR-β locus, Dβ→Jβ rearrangements precede Vβ→DJβ rearrangements. Although this process of V(D)J recombination could theoretically give rise to cells with two in-frame TCR rearrangements at corresponding alleles, and thus two functional α or β TCR genes, virtually all T lymphocytes of the αβ lineage express only one particular TCR-β chain, a phenomenon referred to as allelic exclusion. Analysis of an increasing number of T cell clones and hybridomas has revealed that allelic exclusion at the TCR-β locus is largely due to the fact that αβ T cells carry as a rule only one productive TCR-β rearrangement, whereas the rearrangement on the other allele is either incomplete (DJβ) or out of frame (1). These findings are in line with the notion that a productive TCR-β rearrangement can somehow prevent further rearrangements at the TCR-β locus. Strong support for this hypothesis has been obtained in mice expressing productively rearranged TCR-β transgenes (2, 3), which enforce almost complete inhibition of endogeneous Vβ→DJβ rearrangements, whereas Dβ→Jβ rearrangements were essentially unimpaired. In contrast, no inhibition of endogeneous TCR-β rearrangements could be observed in mice expressing a nonproductive TCR-β transgene (3).

In mature T cells, the rearrangement status of the TCR-α locus differs from that of the TCR-β locus in that usually both alleles carry Vα→Jα rearrangements and cells with two functional TCR-α alleles are easily detectable (1, 4, 5). In fact, in TCR-α transgenic mice there is no or only very inefficient inhibition of endogeneous Vα→Jα rearrangements (6, 7, 8). Thus, it appears that rearrangements in the TCR-α locus continue on both alleles until a receptor is formed that can bind to thymic MHC molecules and induce positive selection, an event that leads to downregu-
tion of RAG expression and complete termination of all TCR gene rearrangements (6, 9, 10, 11).

While, in general, TCR-α rearrangements occur relatively late during thymocyte development, primarily at the transition from the double-negative (DN) to the double-positive (DP) stage and during the DP stage itself (12, 13), TCR-β rearrangements are initiated and completed much earlier, namely at a CD4+8− (DN) stage defined by the expression of the IL-2 receptor α chain (CD25) (12, 14). Any model postulating a negative feedback of functional TCR-β chains on rearrangement at the second β allele therefore presumes a signaling function of TCR-β in the absence of TCR-α. A similar situation is encountered in B cells where IgH chains are thought to inhibit further rearrangements at the IgH locus, well before mature IgL chains become available. The discovery of the pre-B cell receptor (BCR) (15, 16) and pre-TCR (17, 18) provided likely candidates for the signaling machinery mediating allelic exclusion at the corresponding loci in the absence of mature light chains or TCR α chains, respectively, because these receptors consist, in the case of the pre-BCR, of a conventional IgH chain paired with surrogate light chains α5 and VpreB (along with signal-transducing Igα [mb-1] and Igβ [B29] proteins) (19) and, in the case of the pre-TCR, of a conventional TCR-β chain disulfide-linked to the invariant pre-TCR-α (pTα) chain (in association with components of the CD3 complex) (20). Surprisingly, however, analysis of the limited number of mature B cells that develop in α5-deficient and therefore pre-BCR-defective mice did not provide any evidence for violation of allelic exclusion (21). On the other hand, more recent experiments seem to indicate that allelic exclusion is not fully operating in the absence of α5 when precursor rather than mature B cells are studied (22).

The role of the pre-TCR in allelic exclusion of the TCR-β locus has been investigated lately in mouse chimeras that were generated by injecting TCR-β-transgenic, pTα−/− embryonic stem (ES) cells into RAG−/− blastocysts (23). Analysis of ES cell–derived thymocytes in these chimeric mice revealed equivalent inhibition of endogenous Vβ→DJβ rearrangements in the presence and absence of the pTα chain and provided no evidence for the existence of T lymphocytes with more than one TCR-β chain (23). Here, we report on allelic exclusion in stable lines of pTα−/− and TCR-β-transgenic, pTα−/− mice rather than chimeric animals which permitted a more rigorous analysis. Our results indicate an involvement of the pre-TCR in allelic exclusion at the TCR-β locus, but they also show that TCR-β signaling is not completely compromised in the absence of pTα, a finding that may explain why it has been difficult so far to demonstrate an effect of the pre-TCR, and by inference of the pre-BCR, on allelic exclusion when analyzing pTα−/− (23) or α5-deficient mice (21).

Materials and Methods

Mice. C57BL6 (B6) mice, which were used in most experiments as wild-type controls, were purchased from IFFA-Credo (France). All other genetically modified mice (pTα−/−, TCR-β-transgenic, RAG-2−/−, and the respective intercross offspring) were bred and maintained in a specific pathogen-free facility of the Basel Institute for Immunology (Switzerland). All animals used were 6–12 wk of age. pTα−/− mice were identified by Southern blotting of PstI-digested tail DNA using as a probe a genomic PstI–Xhol fragment (610 bp) corresponding to positions 5,432 to 6,046 of the pTα gene (numbering according to reference 24). TCR-β-transgenic mouse lines expressing functionally rearranged, Vβ8.2 transgenes have been described previously (2). In our experiments, we have used the line with ~20 copies. Transgenic mice were identified by PCR with 1 μg of genomic tail DNA as template and primers specific for Vβ8.2 (5′-GCATGGGCTAGGCTCCATT-3′) and a region located immediately 3′ of the β2 cluster (5′-TGAGAGCTGTCTCCTACTCTGATT-3′). Cycling conditions were as follows: 1 min at 94°C (denaturation); 30 s at 94°C; 1 min at 55°C; and 1 min 30 s at 72°C; 25 cycles. Amplification of a ~920-bp fragment indicated the presence of TCR-β transgenes. Breeding stocks of RAG-2–deficient mice have been provided by Dr. F.W. Alt (Boston, MA). RAG-2−/− animals were identified based on the absence of B220 surface IgM lymphocytes as evidenced by double staining of peripheral blood cells with B220–PE and sheep anti-mouse Ig–FITC antibodies.

Antibodies and Flow Cytometry. The following antibodies have been used (all purchased from PharMingen, San Diego, CA, unless stated otherwise): FITC anti-mouse CD3ε (2C11); biotin anti-mouse CD3ε (500A2); FITC anti-mouse CD4 (H129.19); R–PE anti-mouse CD4 (H129.19); GIBCO BRL, Gaithersburg, MD); FITC anti-mouse CD8α (53-6.7); R 613 anti-mouse CD8α (53-6.7; GIBCO BRL); FITC anti-mouse CD11b (M1/70); GIBCO BRL; FITC anti-mouse CD19 (1D3); GIBCO BRL; FITC anti-mouse Vα8.1/8.2 transgenes have been described previously (2). In our experiments, we have used the line with ~20 copies. Transgenic mice were identified by PCR with 1 μg of genomic tail DNA as template and primers specific for Vβ8.2 (5′-GCATGGGCTAGGCTCCATT-3′) and a region located immediately 3′ of the β2 cluster (5′-TGAGAGCTGTCTCCTACTCTGATT-3′). Cycling conditions were as follows: 1 min at 94°C (denaturation); 30 s at 94°C; 1 min at 55°C; and 1 min 30 s at 72°C; 25 cycles. Amplification of a ~920-bp fragment indicated the presence of TCR-β transgenes. Breeding stocks of RAG-2–deficient mice have been provided by Dr. F.W. Alt (Boston, MA). RAG-2−/− animals were identified based on the absence of B220 surface IgM lymphocytes as evidenced by double staining of peripheral blood cells with B220–PE and sheep anti-mouse Ig–FITC antibodies.

For flow cytometry, single cell suspensions from thymi and lymph nodes (axial, mesenteric, inguinal) were prepared in PBS containing 2% calf serum (CS). The number of viable cells was determined using a Coulter counter. Thymocytes were stained at 5 × 104 cells per ml in PBS, 2% CS containing the relevant antibodies at saturating concentrations. When pools of anti-Vβ reagents were used, it proved necessary to dialyze the antibody mix containing all first-step antibodies immediately before the staining, to eliminate the cytotoxicity of the concentrated reagents. Dialysis was performed at 4°C in ultra thimbles (Schleicher & Schuell UH 100/10; cutoff ~10,000 M M) against two changes of PBS, 2% CS for 2 × 30 min. Phenotypes and proportions of thymocyte subsets were analyzed by three-color flow cytometry using a FACScan® (Beckton Dickinson, Mountain View, CA) and the LysisII program. The data depicted in Figs. 2 and 3 were analyzed with the program CellQuest (Beckton Dickinson). Dead cells were excluded from the analysis by forward- and side-scatter gating, when analyzing numerically small subpopulations also after addition of propidium iodine (PI) and gating on PI− cells. Four-
color analyses and cell sorting were performed on a FACStar Plus® (Beckton Dickinson) equipped with a pulse processor for forward-scatter width (FSC-W) in order to gate out cell doublets.

Depletion of CD4 and CD8 expressing thymocytes. Single-cell suspensions from thymi of three individual mice with the same genotypic phenotype were pooled, resuspended in 8 ml serum-free DMEM medium, and incubated simultaneously with 1 ml of anti-CD8 (31M) and 1 ml of anti-CD4 (R172.4) antibody supernatants on ice for 30 min. The cells were then washed in 10 ml DMEM, 2% C S and resuspended in 9 ml of the same medium. After adding 1 ml of freshly dissolved rabbit complement (Low-Tox-M, Cedarlane, Hornby, Canada), the suspension was incubated at 37°C for 40 min. Surviving cells were purified by Ficoll density-gradient centrifugation, washed and resuspended in PBS, 2% C S. The efficiency of CD4/CD8 depletion was usually more than 95% as determined by staining purified thymocytes with R-PE anti-mouse CD4 (H.29.19) and R613 anti-mouse CD8α (53-67) antibodies, which bind epitopes distinct from those recognized by antibodies 31M and R172.4.

Deletion of endogenous TCR rearrangements. High molecular weight thymocyte DNA was prepared as follows: cells from two thymi (ptα−/− mice) or three thymi (ptα+/− mice) of the same genotypic were pooled and digested in proteinase K solution (50 μg/ml proteinase K, 0.5% SDS) at 50°C for 24 h. The DNA was phenol/chloroform extracted, precipitated with ethanol, and dissolved in TE buffer (10 mM Tris–HCl, pH 7.5, 1 mM EDTA) at a concentration of 100 μg/ml. PCR-based analysis of TCR-β chain rearrangements was performed using an assay originally described by van Meerwijk et al. (25) and modified by Anderson et al. (26). Vγ4-1/γ1 rearrangements (nomenclature according to reference 27) were detected in a similar fashion. Primers specific for Vβ4 (5′-CCCTGATATGCGAAGATGATCTCAGGC-3′), Vβ6 (5′-GAAAGCCTGATGCGATCG-3′), Vβ11 (5′-TGCTGTGTCATCACCCACCATC-′), Vβ12 (5′-AGTACTCCGACCCAGCATGAGA-3′), and a region immediately downstream of the last Jβ6 segment in the Jβ2 gene cluster (5′-TGAGAGCTTGGTCTCTACTGATT-3′) were used to detect Vβ−γ−(D)β rearrangements, and primers specific for V γ4 (5′-TGCTCCCTTGCAACCCACCATC-′) and Jγ1 (5′-CCAGGGGATATGCTCCGAGGAGT-3′) were used to detect the respective rearrangements in the TCR-γ locus. Template DNA was used at three or more different concentrations in each experiment to ensure linearity of the PCR signal. As a control for the amount of template DNA, aliquots of a PCR mastermix were amplified with oligonucleotides specific for the IgM constant region (5′-CACTAGCCAACCCTATTAGC-′) and 5′-TGCTCCCTTGCAACCCACCATC-3′) and the amplification cycle consisted of 45 s at 94°C, 1 min min 30 s at 62°C (Vβ−→(D)β) or 50°C (Vγ4→Jγ4), and 2 min at 72°C. The cycle was repeated 26 times. PCR products were separated on 1.2% agarose gels, blotted with a vacuum blotter (Bio-Rad; Richmond, CA) onto Hybond N+S nylon membrane (Amersham, Arlington Heights, IL) and detected by hybridization with end-labeled Jβ2.7 (5′-TATGACACAGTCTTTGGCT-′) or Cγ2-specific (5′-CCCTGCCACCCAGCATGAGA-3′) probes, as appropriate.

Results

No Evidence for Cell Surface Expression of Two TCR-β Chains in pTα−/− Mice. Assuming sufficient time for TCR-β rearrangements on both alleles, no feedback inhibition after a functional TCR-β chain has been generated and no selective disadvantage of cells with two functional TCR-β chains, one would expect that up to 1/5 (20%) of all TCR-β+ cells express two types of β chains, indicating the complete absence of allelic exclusion (see reference 4 for calculations). Any, even partial violation of these assumptions would reduce the percentage of double-expressing cells accordingly, albeit unpredictably. Given the low frequency of cells that express a particular Vβ element, it is clear that evidence for incomplete allelic exclusion is not easy to obtain by simple cell surface staining with antibodies specific for just two particular Vβ elements.

To alleviate this problem, we used a combination of antibodies directed against Vβ6 or Vβ8.1/8.2 elements on one hand and a pool of nine antibodies recognizing TCRs that contain most of the remaining Vβ elements on the other. Vβ6- or Vβ8.1/8.2-specific antibodies were chosen as individual antibodies, because they detect the two most frequent Vβ T cell populations on the B6 background, representing ~5% and 15% of all TCR-αβ cells, respectively. To increase further the sensitivity of our analysis, we included as a third color a CD3ε-specific antibody, which allowed us to gate on CD3+ thymocytes/lymphocytes and exclude all other cells that do not express a TCR or only at low levels. Dead cells, which are known to absorb antibodies nonspecifically and which could therefore give rise to apparently double-expressing cells, were excluded not only by gating on live cells in a forward scatter/side scatter (FSC/SSC) analysis, but also by including PI staining as a fourth color and subsequent gating on PI− cells. By using the FSC pulse-width program on our flow cytometer, we could also efficiently exclude cell doublets, which otherwise would have raised the background of false TCR double-expressing cells.

Fig. 1 shows the result of a representative analysis using the Vβ8.1/8.2-specific antibody as the individual antibody and anti-Vβ2, 3, 4, 5.1/5.2, 6, 7, 9, 10b, and 13 as pooled antibodies. In the B6 control mouse, the frequency of the CD3+ thymocytes and lymph node cells scoring positive for Vβ8.1/8.2 and one of the Vβs represented in the pool is extremely low (~0.1–0.2%). This value may reflect the low proportion of T cells that genuinely express two β chains, violating allelic exclusion, as suggested by studies of human T lymphocytes (28, 29), or it could just indicate the level of nonspecific background staining in our experiment. More important in this context, however, no significant increase in the percentage of potentially double-expressing cells could be detected in the pTα−/− mouse, despite this very sensitive staining technique. These data clearly show that T cells and thymocytes expressing intermediate to high levels of the αβ TCR are allelically excluded even in pTα−/− mice, at least at the level of cell surface expression. Equivalent results were obtained in a similar staining with Vβ6 as
the individual antibody versus a pool of antibodies including anti-Vβ8.1/8.2 (data not shown).

Lack of Cell Surface Expression of Endogenous TCR-β C chains in TCR-β-transgenic, pTα-deficient Mice. To increase further the sensitivity of our anti-Vβ staining, we included TCR-β-transgenic mice in our analysis. As transgenic line, we used mice expressing a functional Vβ8.2 TCR-β transgene, previously shown to prevent expression of endogenous TCR-β chains (2). As a consequence, essentially all mature T cells in these mice stain positive for Vβ8.2, while no other Vβ elements can be detected on the cell surface. To assess the efficiency of allelic exclusion in the absence of pTα, we crossed mice of the TCR-β-transgenic line with pTα-deficient animals and performed a four-color cytometric analysis as described above, using Vβ8.1/8.2 to detect the transgenic TCR-β chain and all other available anti-Vβ reagents in a pool to detect expression of endogenous TCR-β chains. Although the presence of the transgene allowed us to screen essentially all CD3+ cells for expression of two TCR-β chains rather than a fraction of cells expressing a particular Vβ element (i.e., Vβ8.1/8.2 or Vβ6) as above, we still found no evidence for cell surface expression of two distinct TCR-β chains, as endogenous TCR-β chains could not be detected either on thymocytes (data not shown) or on mature T cells (Fig. 2) even in the absence of pTα. These data, in conjunction with those from nontransgenic pTα-/- mice, strongly suggest that functional TCR-β chains can regulate the expression of other TCR-β genes even in the absence of an intact pre-TCR.

A TCR-β Transgene Can Mediate Effects in Early T Cell Development Despite the Absence of pTα. pTα-deficient mice exhibit a severe defect in early T cell development, which leads to a dramatic decrease in the relative proportion and absolute number of DP thymocytes and a concomitant, more than 90% reduction in total thymic cellularity (30). While analyzing Vβ surface expression in TCR-β-transgenic mice, we noticed that the presence of the TCR-β transgene on a pTα-/- background had a mild, but reproducible, effect on thymic cellularity, causing an up to threefold increase in the total number of thymocytes as compared with nontransgenic pTα-/- controls (3; data not shown). This was usually accompanied by a reversion in the DP/DN thymocyte ratio (3; compare the pTα-/- and the TCR-β-transgenic, pTα-/- panels), although the relative increase in the proportion of DP versus DN thymocytes varied considerably between individual TCR-β-transgenic, pTα-/- mice, possibly reflecting the weakness of the signal involved in this phenomenon.

The observation that a TCR-β transgene could influence thymic cellularity even in the absence of pTα, prompted us to analyze the effect of the β transgene on early T cell development in pTα-deficient mice. Early T cell development within the CD4-/8- population can be subdivided into four discrete stages that are defined by the differential expression of CD44 and CD25 surface markers (31). In normal mice, TCR-β rearrangements take place at or shortly before the CD25+/44-/low stage and only those thymocytes that manage to express a functional TCR-β chain can form a complete pre-TCR, which is required to trigger expansion and progression to the next developmental stage, defined as CD25+/CD44+/low. In pTα-deficient mice, which cannot assemble an intact pre-TCR, this transition is severely impaired and relatively few CD25+/CD44+/low cells are generated (Fig. 3B; compare the two left panels). On the other hand, in pTα+ mice expressing a TCRβ transgene, essentially all CD25+ thymocytes can form a pre-TCR, resulting in a strong increase in the proportion of CD25+/CD44+/low cells and a concomitant reduction in the percentage of CD25+/CD44-/low thymocytes (Fig. 3B,
The effects of TCR-β transgenes on various aspects of thymic T cell development in the absence of pTα (the right most panel in A, B, and C). A TCR-β-transgenic mouse expressing functional pTα (TCR-β-transgenic, pTα+), a wild-type C57BL6 (WT) and a non-transgenic pTα-deficient (pTα–) mouse are included as controls. (A) CD4/CD8 profiles. The figures on top of each panel give the total number of thymocytes found in the particular mouse analyzed in this experiment. These values are very typical for mice with the respective genotype as seen in many similar experiments. (B) Analysis of triple-negative (CD3–CD4–CD8–) thymocytes for differential expression of the developmental markers CD25 and CD44. Thymocytes from three mice of the same genotype were pooled. CD4– and CD8-expressing cells (CD25 and CD44) were excluded from the analysis by electronic gating (data not shown). The cells shown in the four panels are thus highly enriched for immature triple-negative thymocytes. (C) The generation of γδ-expressing cells is suppressed in TCR-β-transgenic mice, even in the absence of pTα. Thymocytes were stained with a PE-conjugated TCR-γδ-specific antibody and CD3-biotin, in a second step with streptavidin-TRICOLOR. Only thymocytes that are positive for both CD3 and TCR-γδ were considered as genuine γδ-expressing cells (as defined by the rectangular gate). The data shown in A and C were obtained in the same experiment with the same mouse.

Figure 3. The effects of TCR-β transgenes on various aspects of thymic T cell development in the absence of pTα (the right most panel in A, B, and C). A TCR-β-transgenic mouse expressing functional pTα (TCR-β-transgenic, pTα+), a wild-type C57BL6 (WT) and a non-transgenic pTα-deficient (pTα–) mouse are included as controls. (A) CD4/CD8 profiles. The figures on top of each panel give the total number of thymocytes found in the particular mouse analyzed in this experiment. These values are very typical for mice with the respective genotype as seen in many similar experiments. (B) Analysis of triple-negative (CD3–CD4–CD8–) thymocytes for differential expression of the developmental markers CD25 and CD44. Thymocytes from three mice of the same genotype were pooled. CD4– and CD8-expressing cells (CD25 and CD44) were excluded from the analysis by electronic gating (data not shown). The cells shown in the four panels are thus highly enriched for immature triple-negative thymocytes. (C) The generation of γδ-expressing cells is suppressed in TCR-β-transgenic mice, even in the absence of pTα. Thymocytes were stained with a PE-conjugated TCR-γδ-specific antibody and CD3-biotin, in a second step with streptavidin-TRICOLOR. Only thymocytes that are positive for both CD3 and TCR-γδ were considered as genuine γδ-expressing cells (as defined by the rectangular gate). The data shown in A and C were obtained in the same experiment with the same mouse.

third panel from the left). Interestingly, analysis of TCR-β-transgenic mice lacking pTα revealed a marked down regulation of CD25 in comparison with nontransgenic pTα–/– mice and the appearance of a significant population of CD25–44–/low cells (compare second and fourth panel in Fig. 3 B). However, this effect of the TCR-β transgene was not as prominent as in the pTα–/– background. These data indicate that the TCR-β transgene can actually influence the CD25+ compartment of CD4–8– thymocytes in the absence of pTα in a similar way as in pTα–/– mice, although to a much lesser extent.

Previous experiments in normal (pTα+) mice have shown that a TCR-β transgene inhibits TCR-γ rearrangements and the generation of γδ-expressing cells (32). Interestingly, the TCR-β transgene was able to suppress γ rearrangements, as measured by PCR with primers specific for Vγ4Jγ1, even in the absence of pTα (Fig. 4), although somewhat less efficiently than in the presence of pTα. The TCR-β transgene also mediated a strong reduction in the number of γδ cells in pTα-deficient mice (Fig. 3 C). This effect appears particularly striking, when taking into account that the percentage and absolute number of γδ cells is strongly augmented in normal (nontransgenic) pTα–/– mice in comparison to wild-type littermates (30) (Fig. 3 C). The inhibition of TCR-γ rearrangements and the strong suppression of γδ T cell development illustrates once more that the TCR-β transgene is not innocuous despite the absence of pTα.

Introduction of a functionally rearranged TCR-β transgene into mice lacking one of the recombination activating genes (RAG-1 or RAG-2) leads to the induction of DP cells and the generation of essentially normal numbers of thymocytes (33), a result thought to reflect the physiological function of the pre-TCR. In this context, it was of interest to determine the potential effects of a TCR-β transgene on thymocyte development in RAG–/– mice lacking pTα. To this end, we crossed RAG–/– mice carrying the TCR-β transgene with mice deficient for both RAG-2 and pTα, and analyzed thymi from littermates of the F2 intercross. Fig. 5 shows the result of a representative staining experiment. There was no difference between nontransgenic RAG–/–, pTα–/– and RAG–/–, pTα–/– mice with respect to the total number of thymocytes (~1–4 × 106) and the CD4/CD8 profiles (virtually complete ab-
which normally accompanies the selection of DN CD25
promoting the differentiation of DN thymocytes into DP
transgenic TCR-β reported above. Taken together, the data indicate that a
Introduction of a TCR-β transgene in RAG-2-deficient mice
resulted in the generation of a significant number of DP
thymocytes, as reported previously (33). Surprisingly, expres-
sion of immature thymocytes. CD4 and CD8 staining of thymocytes iso-
lated from a TCR-β-transgenic, RAG-deficient mouse (pTα+) and from
a TCR-β-transgenic mouse that is RAG-deficient and in addition lacks
pTα (pTα-/-). A wild-type C57BL6 (WT [B6]) and a RAG-deficient mouse are included as controls. The figures on top of each panel refer to
the total number of thymocytes found in the respective animal. Similar
results were obtained in four independent experiments. No differences were found between nontransgenic RAG-2/- × pTα-/- and RAG-2/- ×
pTα+ mice (data not shown).

Figure 5. Expression of TCR-β transgenes in RAG-2-deficient mice
in the absence of pTα induce differentiation, but no substantial prolifera-
tion of immature thymocytes. CD4 and CD8 staining of thymocytes iso-
lated from a TCR-β-transgenic, RAG-deficient mouse (pTα+) and from
a TCR-β-transgenic mouse that is RAG-deficient and in addition lacks
pTα (pTα-/-). A wild-type C57BL6 (WT [B6]) and a RAG-deficient mouse are included as controls. The figures on top of each panel refer to
the total number of thymocytes found in the respective animal. Similar
results were obtained in four independent experiments. No differences were found between nontransgenic RAG-2/- × pTα-/- and RAG-2/- ×
pTα+ mice (data not shown).

Discussion
The data reported here demonstrate that expression of a
functional TCR-β chain efficiently prevents the expression of a second functional chain on the cell surface of thymocytes and lymph node T cells in both nontransgenic and
TCR-β-transgenic mice even in the absence of pTα. At first glance, this result seems to suggest that an intact pre-TCR is not required for mediating allelic exclusion. However, impaired cell surface expression of two functional TCR-β chains in pTα−/− mice does not automatically exclude a role for the pre-TCR in implementing allelic exclusion in normal mice. Recent data indicate that TCR-α chains can, at least in part, substitute for pre-TCR-α chains, because they induce the generation of almost normal numbers of thymocytes in pTα-deficient mice, when expressed at a relatively early developmental stage (34, 35). Moreover, a recent analysis of pTα−/− × TCR-α−/− and pTα−/− × TCR-β−/− mice has provided strong evidence that a significant proportion of the αβ thymocytes that develop in pTα−/− mice are actually derived from precursors that have been selected for further development based on the presence of a functional TCR-β chain and early expression of a conventional TCR-α chain (35). The underlying assumption that some Vα−→αβ rearrangements and TCR-α expression can occur already in the CD25+ DN subpopulation is supported by a PCR-based analysis of TCR-α rearrangements in sorted CD25+ thymocytes from normal mice (36). If early expression of TCR-α chains in a few CD25+ pre-T cells was responsible for the generation of most mature αβ T cells in pTα−/− mice, the early formation of an αβ TCR in these cells might well be accountable for the allelic exclusion of TCR-β chains that is observed despite the absence of pTα.

A priori, it would also be possible that neither the TCR-α nor the pTα chain are involved in mediating allelic exclusion and that TCR-β chains can signal independently, even in the absence of these two partner chains. Our analysis of TCR-β-transgenic, pTα−/− mice clearly demonstrates that TCR-β chains can signal without being associated with a pTα chain, resulting in marked effects on early T cell development; for instance, a small increase in the number of DP thymocytes, partial downregulation of CD25 in the pre-T cell population, significant inhibition of Vγ−→γ and Vβ−→ββ rearrangements, suppression of the generation of γδ-expressing cells and, most striking, induction of DP thymocyte formation in RAG−/− × pTα−/− mice. These effects of a transgenic TCR-β chain in the absence of pTα could be due to the association of TCR-β chains with some other proteins, like TCR-α, TCR-δ (37), or the hypothetical VpreT subunit (20), giving rise to a signaling complex that can assume part but not all of pre-TCR function. The idea that the transgenic TCR-β chain mediates its effects in the absence of pTα via a signal-trans-
The question arises whether the observed effects of the transgenic TCR-β chain in the absence of pTα have some physiological relevance or whether they are solely due to a peculiarity of the transgenic system; for instance, expression of the TCR-β transgene at inappropriately high levels. In this context, it should be mentioned that the TCR-β chains in our TCR-β-transgenic mice can be expressed on thymocytes in gpi-linked form, a feature not found in non-transgenic animals (38). If the significant inhibition of endogeneous Vβ→(D)Jβ rearrangements in pTα-deficient mice was due to a transgenic artifact, one might anticipate a variation in the degree of inhibition when analyzing different TCR-β-transgenic lines. This may explain, why Xu et al. (23) have seen an equally strong inhibition of endogeneous TCR-β rearrangements in both pTα+ and pTα−/− thymocytes, while in our experiments the potentially artifactual, transgene-specific effects of the TCR-β chain may be fortuitously less pronounced, allowing us to detect significant differences in the absence and presence of pTα. Moreover, the use of a stable line of transgenic mice allowed us to compare endogeneous rearrangements within the same transgenic background, which was not possible in the chimeric mice of Xu et al. (23), because they were generated from pTα+ and pTα−/− ES cell clones that had been transfected individually with the respective TCR-β transgene, almost certainly giving rise to pTα+ and pTα−/− animals with distinct transgene copy numbers and/or insertion sites. Whatever the reason for the discrepant results may be, our observation that the inhibition of endogeneous rearrangements at the TCR-β locus is less profound in the absence of pTα, despite the capacity of the transgenic β chain to signal in part independently of pTα, clearly indicates a role of pTα and the pre-TCR in the regulation of Vβ→(D)Jβ rearrangements. The importance of an intact pre-TCR for allelic exclusion at the level of TCR gene rearrangements therefore may be even more obvious in normal (nontransgenic) mice, in which a newly formed, functional TCR-β chain is by definition expressed at physiological levels and therefore possibly less prone to pTα-independent signal transduction. This will be assessed by comparing the rearrangement status at the TCR-β locus in a statistically significant number of sorted DN CD25− thymocytes from pTα-deficient mice and wild-type littermates at the single cell level.

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