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Immunology

Function of the pre-T-cell receptor α chain in T-cell development and allelic exclusion at the T-cell receptor β locus

(gene targeting/T-cell differentiation/allelic exclusion)

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ABSTRACT **The pre-T-cell receptor, composed of the T-cell receptor (TCR) β chain (TCRβ), pre-Tα (pTα) chain, and CD3 molecules, has been postulated to be a transducer of signals during the early stages of T-cell development. To examine the function of the transmembrane pTα chain during thymocyte development, we generated $pT\alpha^{-/-}$ embryonic stem cells and assayed their ability to differentiate into lymphoid cells *in vivo* after injection into recombination-activating gene (RAG)-2-deficient blastocysts. Thymocytes representing all stages of T-cell differentiation were detected in the thymus of $pT\alpha^{-/-}$ chimeric mice, indicating that thymocyte development can occur without pTα. However, greatly reduced thymocyte numbers and substantially increased percentages of both $CD4^-CD8^-$ thymocytes and TCR $\gamma\delta^+$ thymocytes suggest that pTα plays a critical role in thymocyte expansion. To investigate the role of the pTα chain in allelic exclusion at the *TCRβ* locus, a functionally rearranged *TCRβ* minigene was introduced into $pT\alpha^{-/-}$ and $pT\alpha^{+/-}$ embryonic stem cells, which were subsequently assayed by RAG-2-deficient blastocyst complementation. In the absence of pTα, expression of the transgenic *TCRβ* inhibited rearrangement of the endogenous *TCRβ* locus to an extent similar to that seen in normal *TCRβ* transgenic mice, suggesting that pTα may not be required for signaling allelic exclusion at the *TCRβ* locus.**

Lymphoid precursor cells progress into mature $TCR\alpha\beta^+$ T cells through successive stages characterized by expression of distinct surface markers. These distinct developmental stages include the immature $CD4^-CD8^-$ [double-negative (DN)] stage, $CD4^+CD8^+$ [double-positive (DP)] stage, and mature $CD4^+$ or $CD8^+$ [single-positive (SP)] stage (1). Rearrangement of the *TCRβ* gene appears to be initiated at the DN stage before the rearrangement and expression of *TCRα* gene (2, 3). Accumulating evidence has suggested that the T-cell receptor (TCR) β chain, in the absence of TCRα chain, is necessary and sufficient to signal transition from DN to DP stage, as well as expansion of thymocytes (4, 5). At these early stages, the TCRβ chain complexes with the transmembrane pre-T-cell receptor α (pTα) chain and CD3 proteins to form the pre-TCR (6–8). Therefore, the pre-TCR may be responsible for transducing signals essential for early thymocyte development. Likewise, the pre-B-cell receptor, composed of membrane-bound μ heavy chain (μ_m), surrogate light chain, Igα, and Igβ, has been postulated to play critical roles during the early stages of B-cell development (9). This hypothesis has been strongly supported by the observations that targeted disruptions of the μ_m and λ_5 components of the pre-B-cell receptor in mice significantly impaired the transition from the pre-B-cell stage to the mature B-cell stage and expansion of B-cell precursors (10, 11).

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A single specificity of B and T cells is maintained through allelic exclusion, which ensures that only one of the two alleles potentially encoding immunoglobulin and TCR is expressed. The phenomenon of allelic exclusion has been extensively analyzed in transgenic mice. For B cells, high-level expression of a functional membrane-bound (μ_m), but not secreted μ heavy chain (μ_s), inhibits V(D)J rearrangement at the endogenous IgH locus (12, 13), suggesting that the μ_m may be involved in allelic exclusion at the IgH locus. This hypothesis was further supported by the finding that removal of the membrane exon of the μ_m chain not only blocks the transition from pre-B cells to mature B cells but also abolishes allelic exclusion at the IgH locus (14). Similarly, expression of a functionally rearranged *TCRβ* gene in transgenic mice inhibits V(D)J rearrangement of the endogenous *TCRβ* locus (15), suggesting that the TCRβ may play analogous roles in effecting the allelic exclusion at the *TCRβ* locus. It has been proposed that the μ_m may exert its effects on allelic exclusion through the pre-B-cell receptor (9). In analogy, the TCRβ chain may signal allelic exclusion through the pre-TCR.

To investigate the function of the pTα gene and pre-TCR during T-cell development, we generated $pT\alpha^{-/-}$ embryonic stem (ES) cells that were assayed by injection into recombination-activating gene (RAG)-2-deficient blastocysts after which their progeny populate the lymphoid cell compartment (16). In $pT\alpha^{-/-}$ chimeric mice, T-lineage precursor cells could complete the differentiation program into mature SP thymocytes, but the thymocyte number was greatly reduced and the percentages of DN thymocytes and TCR $\gamma\delta^+$ thymocytes were greatly increased. These observations support the notion that the pre-TCR plays an essential role in the expansion of DP thymocytes. Furthermore, analysis of $TCR\beta^+pT\alpha^{-/-}$ chimeric mice indicated that, as in *TCRβ* transgenic mice, the expression of the transgenic *TCRβ* also inhibited the V(D)J rearrangements of the endogenous *TCRβ* locus, suggesting that disruption of the pTα chain does not interfere with allelic exclusion at the *TCRβ* locus.

While this work was in progress, Fehling *et al.* reported that disruption of the pTα gene in mice severely disrupted the differentiation of TCR $\alpha\beta^+$ T cells in the thymus (17). Our findings on T-cell differentiation in $pT\alpha^{-/-}$ chimeric mice generally agree with those reported by Fehling *et al.*

MATERIAL AND METHODS

Cloning pTα cDNA. Full-length pTα cDNA was amplified by PCR from a thymus cDNA library (Stratagene) using pTα-specific primers (ref. 8; data not shown). The two-step PCR reaction was done in a final 100-μl vol containing 500 ng of

Abbreviations: TCR, T-cell receptor; pTα, pre-T-cell receptor α; DN, double-negative; DP, double-positive; SP, single-positive; V(D)J, variable-(diversity)-joining; ES, embryonic stem; RAG, recombination-activating gene; PGK, phosphoglycerate kinase; μ_m, membrane-bound μ heavy chain; PE, phycoerythrin; FITC, fluorescein isothiocyanate.

thymus cDNA, 300 ng of each primer, 1× PCR buffer (Boehringer Mannheim), 0.2 mM dNTP, and 5 units of *Taq* polymerase (Boehringer Mannheim). The PCR reaction contained 26 cycles, each consisting of 1 min at 94°C and 2.5 min at 66°C. The final reaction step was followed with an extension at 72°C for 10 min.

The PCR product was cloned into the *Bam*HI site of pBluescript SK and verified by DNA sequencing. The pTα-specific primers are as follows: 5′-TAGGGATCCTGGCTGCAACTGGGCTCATGCTTC-3′; 5′-CAGGGATCCGGGCTCAGACGGGTGGGTAAGATC-3′.

Construction of pTα Targeting Construct. The pTα cDNA was used to screen a murine 129 genomic library (Stratagene). Positive phage clones were plaque-purified as described (18). Genomic DNA of pTα was extracted from the positive phage clones as described and cloned into the *Sal* I–*Bam*HI sites of pBluescript SK (18). The exon–intron structure of pTα was characterized by Southern blotting analysis and DNA sequencing. The targeting construct was designed to delete a 320-bp exon that initiated at nt 178 and ended at nt 498 (numbering is according to the pTα sequence deposited in GenBank, accession number U16958) and was generated by replacing a 1.8-kb *Nsi* I–*Bam*HI fragment containing the 320-bp exon with the phosphoglycerate kinase promoter (PGK)-neomycin resistance gene and subsequent insertion of the PGK–thymidine kinase in one end of pTα genomic sequence (Fig. 1 *A* and *B*). The 320-bp exon encodes most of the extracellular region of pTα, including the immunoglobulin domain (8). Furthermore, deletion of the exon will disrupt the reading frame of any truncated pTα transcripts that can potentially be generated by splicing from the exon 5′ of the deleted exon to the exons 3′ of the deleted exon. Therefore, homologous recombination between the targeting construct and endogenous loci should destroy the ability to produce pTα protein.

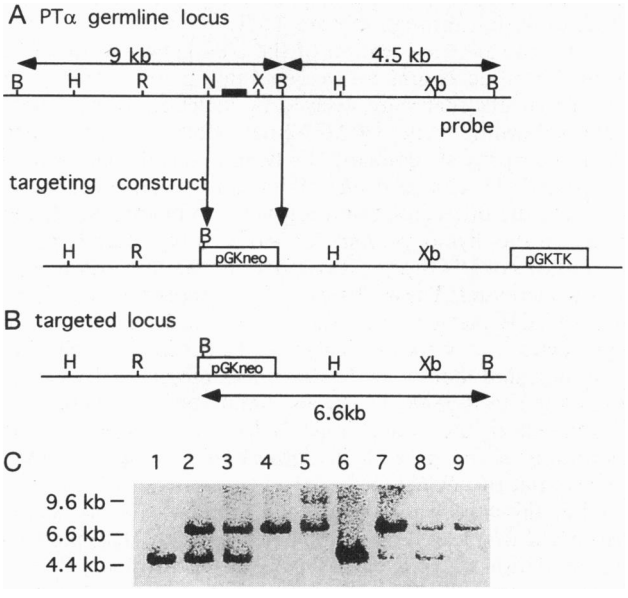

FIG. 1. Targeted disruption of the pTα gene. (*A*) Genomic configuration of the pTα locus and design of pTα targeting construct. The closed box represents the 320-bp coding exon of pTα, which was replaced with PGK-*neo*^{r} in the targeting construct. The restriction sites are as follows: B, *Bam*HI; H, *Hin*dIII; R, *Eco*RI; N, *Nsi* I; Xb, *Xba* I; X, *Xho* I. The probe is a 300- to 400-bp genomic fragment as indicated above. (*B*) The genomic configuration of the pTα locus after homologous recombination with the targeting construct. (*C*) Southern blot analysis of the targeted ES cells and the contribution of pTα$^{-/-}$ ES cell to various tissues of pTα$^{-/-}$ chimeric mice. Ten micrograms of genomic DNA was digested with *Bam*HI and probed with the probe indicated in Fig. 1*A*. Lanes: 1, normal ES cell; 2 and 3, pTα$^{+/-}$ ES cell clones pH3.6 and pH4.2; 4 and 5, pTα$^{-/-}$ ES cell clones Ta16 and Ta164; 6, thymocytes from control mouse; 7, thymocytes from pTα$^{-/-}$ chimeric mouse; 8 and 9, lung and kidney of pTα$^{-/-}$ chimeric mice.

Generation of pTα$^{+/-}$ and pTα$^{-/-}$ ES Cells. J1 ES cells were cultured and electroporated with the linearized targeting construct as described (19). The transfected cells were selected with G418 (300 μg/ml) and gancyclovior (2 μM) as described (20). Homologous recombination events were screened by Southern blotting analysis with *Bam*HI digestion and hybridization to the probe shown in Fig. 1*A*. Autoradiography was done with a phosphoimager (Molecular Dynamics). To generate pTα$^{-/-}$ ES cells, pTα$^{+/-}$ ES cells were cultured under increased G418 concentrations as described (21). ES cells surviving selection at G418 at 3.6 mg/ml were expanded and screened with Southern blot analysis. Identified pTα$^{-/-}$ ES cells were subcloned. The ES cells were injected into blastocysts from RAG-2$^{-/-}$ mice, and progeny lymphoid cells were analyzed (16).

Generation of TCRβ$^+$pTα$^{+/-}$ and TCRβ$^+$pTα$^{-/-}$ ES Cells. Approximately 40 μg of a *TCRβ* minilocus (22), which expressed a functionally rearranged Vβ8$^+$ TCRβ chain, was electroporated together with 5 μg of linearized plasmid containing PGK-hygromycin resistance gene into pTα$^{+/-}$ and pTα$^{-/-}$ ES cells. The transfectants were selected with hygromycin at 110 μg/ml, and surviving ES colonies were expanded and screened by Southern blotting for the existence of the *TCRβ* transgene (data not shown). TCRβ$^+$pTα$^{+/-}$ and TCRβ$^+$pTα$^{-/-}$ ES cells that contained four copies of the *TCRβ* transgene were subcloned and assayed by RAG-2-deficient blastocyst complementation.

Flow Cytometric Analysis. Single-cell suspensions from spleen and thymus were prepared as described (23). Half a million cells were stained with 1 μg of fluorescein (FL)-, phycoerythrin (PE)-, or biotinylated monoclonal antibodies for 30 min on ice. After being washed twice, biotin conjugates were revealed by fluorescein isothiocyanate (FITC)-, PE-conjugated streptavidin. Stained cells were analyzed with a CELLQUEST program on a FACScan (fluorescense-activated cell sorter; Becton Dickinson). Cells residing in the lymphocyte gate defined by light scatter were further analyzed (24). FITC-conjugated-anti-Ly9.1, PE-conjugated-anti-B220, anti-Vβ7, and biotinylated anti-IgM, anti-Vβ8 were from PharMingen; FITC-conjugated anti-CD8, PE-conjugated anti-CD4, anti-TCRαβ, anti-TCRγδ, and biotinylated anti-CD3, were from Southern Biotechnology Associates.

Analysis of the Rearrangement of the Endogenous *TCRβ* Locus by Quantitative PCR Assay. Quantitative PCR assays were designed to detect some DJ and V(D)J rearrangements of the *TCRβ* locus. Primers specific for Vβ 6,7, Dβ2, and Jβ2.2 were used to detect rearrangements from Vβ6,7 to Jβ2 and Dβ2 to Jβ2, respectively (see Fig. 3*B*). To control for the amount of DNA in each PCR reaction, primers specific for Igκ genomic DNA (around the Cκ region) were used to amplify a 0.9-kb genomic DNA fragment. For each thymocyte DNA sample, sequentially diluted genomic DNA of 0.2, 0.05, and 0.02 μg was supplemented with genomic DNA isolated from ES cells to 0.2 μg and assayed with the PCR reactions. PCR assays were done in a final 50-μl vol containing 0.2 μg of genomic DNA, 1× PCR buffer (Boehringer Mannheim), 25 nM of each primer (Vβ6,7 and Jβ2 primers or Dβ2 and Jβ2 primers or Igκ primers), 0.2 mM dNTP, and 2 units of *Taq* polymerase (Boehringer Mannheim). The PCR reaction went for 28 cycles, each consisting of 1 min at 94°C, 1.5 min at 60°C, and 1.5 min at 72°C. The final reaction step was followed with an extension at 72°C for 10 min. One-fifth of each reaction was resolved on 1% agarose gel and assayed with Southern blotting analysis, hybridizing to a probe covering Jβ2.1 to Jβ2.2 (see Fig. 3*B*). Autoradiography and analysis of the intensity of autographic bands were done with a phosphoimager.

The primers used in these reactions were as follows: Vβ6 primer, 5′-AATTCCTGATTGGTCAGGAA-3′; Vβ7

primer, 5′-CTGATCAAAAGAATGGGAGA-3′; Dβ2 primer, 5′-ATGAGAAAGGACTTGTAACTTCTTTC-CCAC-3′; Jβ2 primer, 5′-AATCCCAGGATCCAATC-CAG-3′. Two Igκ primers are as follows: 5′-AGGGTGACT-TATTGGAGATTTCAGAAAT-3′; 5′-TCTCCTGTCTCT-TCCAAGAATACTCTGA-3′.

RESULTS

Targeted Disruption of the pTα Gene in ES Cells. A replacement targeting construct was generated and used to replace a 320-bp exon of pTα with the PGK-*neo*^{r} gene (Fig. 1 *A* and *B*). To screen for homologous recombination events, genomic DNA was digested with *Bam*HI and probed with a pTα genomic fragment that was not included in the targeting construct, giving a 4.5-kb germ-line band or a 6.6-kb mutated allele (Fig. 1 *A* and *B*). Of the ES clones screened, 25% contained one mutant allele (two of the pTα$^{+/-}$ ES clones, pH3.6 and pH4.2, are shown in lanes 2 and 3 of Fig. 1*C*).

To generate pTα$^{-/-}$ ES cells, pH3.6 and pH4.2 ES cells were grown under higher G418 concentrations. ES clones surviving the selection of G418 at 3.6 mg/ml were expanded, and their genomic DNA was analyzed (two of the pTα$^{-/-}$ ES cells, Ta16 and Ta164, are shown in lanes 4 and 5 of Fig. 1*C*). The Southern blot shown in Fig. 1*C* was stripped and probed with the 320-bp exon. While a 9-kb band was seen in lanes that contain DNA from the wild-type ES cell and pTα$^{+/-}$ ES cells, no hybridization signal can be detected in lanes containing pTα$^{-/-}$ ES cell DNA (data not shown). This result confirmed that the 320-bp exon had been deleted from the chromosome of pTα$^{-/-}$ ES cells.

Lymphoid Differentiation in pTα$^{-/-}$ Chimeric Mice. The pTα$^{-/-}$ ES cells and pTα$^{+/+}$ control ES cells were separately injected into RAG-2-deficient blastocysts, and lymphoid differentiation was analyzed in 2- to 5-week-old chimeric mice. A similar number of mature B cells were detected in the spleens of pTα$^{+/+}$ and pTα$^{-/-}$ chimeric mice, indicating that pTα was not required for the development of B-lineage cells (Fig. 2*B*). In the thymus of pTα$^{-/-}$ chimeric mice, the number of thymocytes was ≈5- to 10-fold lower than that of normal mice of the same age (Fig. 2*E*). However, immature CD4$^-$CD8$^-$, CD4$^+$CD8$^+$ and mature CD4$^+$CD8$^-$ or CD4$^-$CD8$^+$ thymocytes were all present (Fig. 2*A*). Furthermore, while the percentage of CD3$^+$TCRαβ$^+$ thymocytes in pTα$^{-/-}$ chimeric mice was similar to that of control, the percentage of CD3$^+$TCR γδ$^+$ thymocytes was increased >10-fold (Fig. 2*D*). Also, while the DN thymocytes represented only 1–2% of thymocytes in control mice, a much higher percentage of DN thymocytes was present in the thymus of pTα$^{-/-}$ chimeric mice (Fig. 2*A*).

Because the RAG-2-deficient blastocysts used to generate pTα$^{-/-}$ chimeric mice were Ly9.2$^+$, and ES cell-derived lymphocytes are Ly9.1$^+$, an anti-Ly-9.1 antibody was used to distinguish the DN thymocytes derived from pTα$^{-/-}$ ES cells and RAG-2-deficient blastocysts (25). When stained simultaneously for CD4 and Ly9.1 markers, thymocytes derived from

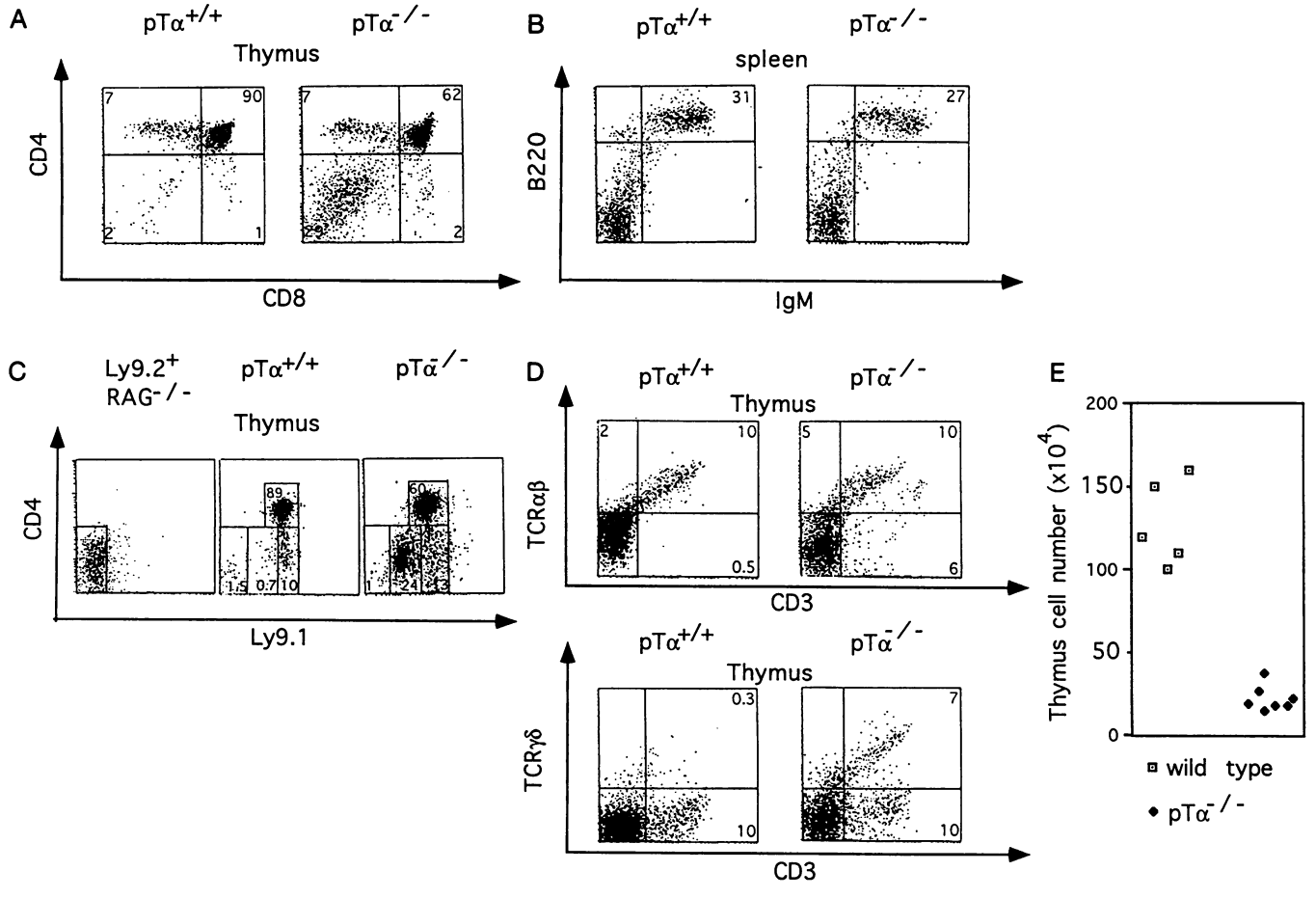

FIG. 2. Flow cytometry analysis of lymphoid cells in pTα$^{-/-}$ chimeric mice. Thymocytes from pTα$^{+/+}$ and pTα$^{-/-}$ chimeric mice were stained with FITC-conjugated anti-CD8 and PE-conjugated anti-CD4 (*A*), PE-conjugated anti-CD4 and FITC-conjugated-anti-Ly9.1 (*C*) PE-conjugated anti-TCRβ and biotinylated anti-CD3 or PE-conjugated anti-TCRγδ and biotinylated anti-CD3 (*D*). (*B*) Spleen cells from pTα$^{+/-}$ and pTα$^{-/-}$ chimeric mice were stained with PE-conjugated anti-B220 and biotinylated anti-IgM. Biotin conjugates were revealed with streptavidin–FITC. Only cells in the lymphocyte gate were analyzed. (*E*) The numbers of thymocytes derived from pTα$^{-/-}$ chimeric mice are represented as filled diamonds; wild-type mice are indicated as squares.

RAG-2-deficient blastocysts were Ly9.1$^-$CD4$^-$; thymocytes from pT$\alpha^{+/+}$ chimeric mice were predominantly Ly9.1^{bright}, representing ES cell-derived DP and SP thymocytes; pT$\alpha^{-/-}$ chimeric mouse contained 24% of thymocytes that were CD4$^-$Ly9.1^{dull}, while a background level of <1% thymocytes were CD4$^-$Ly-9.1$^-$, representing DN thymocytes derived from RAG$^{-/-}$ blastocysts (Fig. 2*C*). These CD4-Ly9.1^{dull} thymocytes represent ES-cell-derived DN thymocytes because other CD4$^-$ cells, including the ES-cell-derived CD4$^-$CD8$^+$ SP thymocytes and TCR$\gamma\delta^+$ thymocytes, were CD4$^-$Ly9.1^{bright} (Fig. 2*C*; data not shown). In support of this conclusion, Southern blot analysis of the contribution of pT$\alpha^{-/-}$ ES cell to different tissues of the pT$\alpha^{-/-}$ chimeric mice revealed that the thymocytes were predominantly derived from pT$\alpha^{-/-}$ ES cells, because in the thymocyte DNA the intensity of the germ-line band of pTα was <5% that of the pTα mutant band (Fig. 1*C*, lane 7). In conclusion, the majority of DN thymocytes in pT$\alpha^{-/-}$ chimeric mice was derived from ES cells.

Allelic Exclusion. A productively rearranged TCRβ chain was integrated into the genome of ES cells to generate TCRβ^+pT$\alpha^{+/-}$ and TCRβ^+pT$\alpha^{-/-}$ ES cells, both of which were assayed by RAG-2-deficient blastocyst complementation. Because the transgene encodes a Vβ8$^+$ TCRβ chain, thymocytes from TCRβ^+pT$\alpha^{+/-}$ and TCRβ^+pT$\alpha^{-/-}$ chimeric mice were stained simultaneously with anti-CD3 and anti-Vβ8 antibodies for expression of the transgene. In normal mice, only 3% of TCR$^+$ thymocytes were Vβ8$^+$ TCR^{hi}; however, essentially all TCR^{hi} $\alpha\beta^+$ thymocytes were Vβ8$^+$ in TCRβ^+pT$\alpha^{+/-}$ and TCRβ^+pT$\alpha^{-/-}$ chimeric mice (Fig. 3*A*). Therefore, the *TCR*β transgene was expressed in most thymocytes of both TCRβ^+pT$\alpha^{+/-}$ and TCRβ^+pT$\alpha^{-/-}$ chimeric mice. About 4% of thymocytes were TCR$\gamma\delta^+$ in TCRβ^+pT$\alpha^{-/-}$ chimeric mice, probably explaining the few CD3$^+$Vβ8$^-$ cells (Fig. 3*A*; data not shown). To analyze the expression of endogenous TCRβ chain in TCRβ^+pT$\alpha^{+/-}$ and TCRβ^+pT$\alpha^{-/-}$ chimeric mice, thymocytes were simultaneously stained with anti-Vβ7 and anti-Vβ8. While 3% thymocytes from normal mouse were Vβ7$^+$, no Vβ7$^+$ thymocytes were present in TCRβ^+pT$\alpha^{+/-}$ or TCRβ^+pT$\alpha^{-/-}$ chimeric mice, suggesting that endogenous *TCR*β genes were not expressed (data not shown).

Although the lack of Vβ7 expression suggested that allelic exclusion was operating in the pT$\alpha^{-/-}$ chimeric mice, we also used an assay of DNA rearrangement to examine this issue. In *TCR*β transgenic mice, the V(D)J rearrangements of the endogenous *TCR*β locus are suppressed. If pTα is essential for allelic exclusion at the *TCR*β locus, there should be extensive V(D)J rearrangements at the endogenous *TCR*β loci in TCRβ^+pT$\alpha^{-/-}$ chimeric mice. To analyze the effect of expression of the TCRβ chains on V(D)J rearrangement of endogenous TCRβ locus, a quantitative PCR assay was designed to detect endogenous Vβ6/7 to Jβ2 or Dβ2 to Jβ2 rearrangements (Fig. 3*B*). It was used to analyze genomic DNA isolated from thymocytes of normal mice, *TCR*β transgenic mice, and Ly-9.1$^+$ thymocytes of TCRβ^+pT$\alpha^{+/-}$ and TCRβ^+pT$\alpha^{-/-}$ chimeric mice. Thymocyte DNA isolated from two TCRβ^+pT$\alpha^{+/-}$ chimeric mice and three TCRβ^+pT$\alpha^{-/-}$ chimeric mice were analyzed. The level of Dβ2 to Jβ2 rearrangement in the thymocyte DNA derived from *TCR*β transgenic mice was similar to that in the DNA from TCRβ^+pT$\alpha^{+/-}$ and TCRβ^+pT$\alpha^{-/-}$ chimeric mice but higher than that of normal mice (Fig. 3*C*). However, as in *TCR*β transgenic mice, the level of Vβ7 to Jβ2 rearrangement in the thymocytes of TCRβ^+pT$\alpha^{+/-}$ and TCRβ^+pT$\alpha^{-/-}$ chimeric mice was only ≈10% or less that in normal mice (Fig. 3*C*). Similar results were obtained for the Vβ6 to Jβ2 rearrangements (data not shown). Assuming that Vβ6/7(D)Jβ2 rearrangements are representative of the overall status of the *TCR*β loci, V to DJ rearrangements of the endogenous *TCR*β locus appear to be inhibited to the same extent in the thymocytes of TCRβ^+pT$\alpha^{+/-}$ and TCRβ^+pT$\alpha^{-/-}$ chimeric mice.

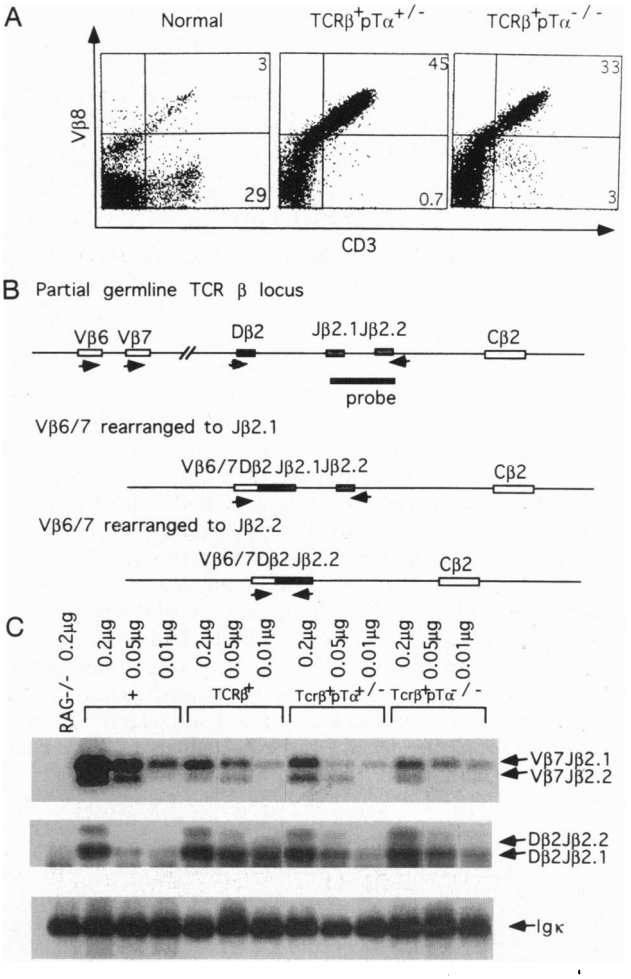

FIG. 3. Expression of the transgenic TCRβ gene inhibits the V(D)J rearrangements of the endogenous TCRβ genes in the thymocytes of TCRβ^+pT$\alpha^{+/-}$ and TCRβ^+pT$\alpha^{-/-}$ chimeric mice. (*A*) Expression of Vβ8$^+$ transgene in TCRβ^+pT$\alpha^{+/-}$ and TCRβ^+pT$\alpha^{-/-}$ chimeric mice. Thymocytes were stained with FITC-conjugated anti-CD3 and biotinylated anti-Vβ8. Biotin derivatives were revealed with PE-conjugated streptavidin. (*B*) A schematic of the quantitative PCR assay for Vβ6/7 to Jβ2 rearrangements. The location of specific primers are indicated by arrowheads. The probe covering the Jβ2.1 to Jβ2.2 region is also indicated. (*C*) Serially diluted genomic DNA isolated from thymocytes of normal mice and TCRβ transgenic mice (22) as well as from Ly9.1$^+$ thymocytes of TCRβ^+pT$\alpha^{+/-}$ and TCRβ^+pT$\alpha^{-/-}$ chimeric mice were assayed for Vβ7 to Jβ2 and Dβ2 to Jβ2 rearrangements by the PCR assay. As a control for the amount of DNA in each reaction, primers specific for Igκ genomic region were used to amplify a 0.9-kb DNA fragment from the same set of DNA samples. Southern blots of the PCR reaction products hybridized with the Jβ2 probe and the 0.9 kb Igκ probe are shown. The expected amplification products are indicated with arrowheads.

DISCUSSION

The pTα gene is expressed predominantly during the early stages of thymocyte development, and its product forms a receptor complex with TCRβ and CD3 molecules on the surface of pre-T cells before expression of the TCRα chain (8). On the basis of the dramatic decrease of thymocytes in TCR$\beta^{-/-}$ mice but not in TCR$\alpha^{-/-}$ mice, and the ability of a functional *TCR*β transgene in RAG-2$^{-/-}$ mice to restore the number of thymocytes to a normal level, it has been suggested that the pre-TCR transduces signals that lead to the expansion of immature thymocytes (4, 5). Consistent with this hypothesis, our work and a recent report by Fehling *et al.* (17) show that disruption of the pTα gene also leads to a dramatic reduction of the number of thymocytes in mice (17). This result dem-

onstrates directly that the pTα chain is functionally essential for T-cell development. In this context, it may be relevant that the pTα chain has an unusually long cytoplasmic tail compared with that of the TCRα chain, suggesting that the cytoplasmic tail of the pTα chain may be directly involved in a signal-transduction process. However, because CD3 proteins have been shown to transduce signals that lead to the transition from the DN stage to the DP stage and the expansion of thymocytes (26, 27), the intrinsic ability of pTα to transduce signals must be addressed in the future.

The μ_m protein complexes with $\lambda 5$, $V_{pre\text{-}B}$ proteins, and Igα/β to form the pre-B-cell receptor complex (9). Targeted disruption of the $\lambda 5$ gene led to an impairment of the pre-B to mature B-cell transition and a diminished precursor B-cell pool (10). Similarly, disruption of the pTα gene leads to a reduced number of immature DP thymocytes in the pT$\alpha^{-/-}$ chimeric mice, although immature thymocytes can differentiate into mature SP thymocytes. While a higher percentage than normal of thymocytes in pT$\alpha^{-/-}$ chimeric mice was DN, the absolute number of DN thymocytes in pT$\alpha^{-/-}$ chimeric mice remained similar to that of normal mice. Therefore, the higher percentage of DN thymocytes in pT$\alpha^{-/-}$ chimeric mice is likely due to the defective expansion of TCR$\alpha\beta^+$ DP thymocytes.

Consistent with the recent report by Fehling *et al.* (17), disruption of the pTα chain does not seem to affect the development of TCR$\gamma\delta^+$ lineage T cells. It was only due to a reduced number of DP thymocytes in pT$\alpha^{-/-}$ chimeric mice that the percentage of the TCR$\gamma\delta^+$ thymocytes in the thymus of pT$\alpha^{-/-}$ chimeric mice was increased relative to that of normal mice.

The expression of μ_m has been implicated in the control of allelic exclusion at the IgH locus (12–14), leading to the notion that the pre-B-cell receptor signals allelic exclusion at the IgH locus. In *TCRβ* transgenic mice, expression of the transgenic *TCRβ* chain inhibits the V to DJ rearrangement of the endogenous *TCRβ* loci, suggesting a role of the TCRβ chain, and by extension, the pre-TCR in the allelic exclusion of the *TCRβ* locus. However, in pT$\alpha^{-/-}$ thymocytes, expression of a functional TCRβ chain inhibited the V to DJ rearrangements of the endogenous *TCRβ* genes to the same extent as in *TCRβ* transgenic mice. Therefore, the lack of pTα does not seem to affect allelic exclusion at the *TCRβ* locus. Because TCRα chain is rearranged and expressed in pT$\alpha^{-/-}$ chimeric mice, we were unable to determine whether TCRβ chain could complex with other proteins on the surface of the immature pT$\alpha^{-/-}$ thymocytes in the absence of TCRα chain. However, assuming that no other gene products can compensate for the loss of pTα, it appears that the pre-TCR may not be involved in the signaling of allelic exclusion at *TCRβ* locus.

On the basis of recent findings that pre-TCR mediates rapid clonal expansion of early thymocytes and RAG-2 protein is degraded through phosphorylation by CDC2 before entry into S phase (28, 29), it has been postulated that allelic exclusion at the *TCRβ* locus could be achieved if RAG-2 is not fully reactivated until the accessibility of the *TCRβ* locus for rearrangement is switched off and accessibility of the *TCRα* locus is switched on. If this hypothesis is true, allelic exclusion would be a by-product of cell-cycle regulation during early thymocyte expansion (29). Our data argue against this hypothesis because defective expansion of the immature thymocytes in pT$\alpha^{-/-}$ chimeric mice does not appear to affect allelic exclusion.

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