rence of an Arctic ozone hole directly as a result of increased CO_2 could be one of the more serious consequences of climate change. Results presented here together with observations of lower stratospheric temperatures suggest that the interannual variability of ozone depletion in the Northern Hemisphere spring will increase considerably in the future and is likely to be determined largely by the meteorological behaviour of the upper troposphere. Therefore, in considering the ramifications of climate change, it will be important to predict details of the upper air circulation as well as mean surface parameters. □

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Mutations in T-cell antigen receptor genes α and β block thymocyte development at different stages

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Analysis of mice carrying mutant T-cell antigen receptor (TCR) genes indicates that TCR-β gene rearrangement or expression is critical for the differentiation of $CD4^-CD8^-$ thymocytes to $CD4^+CD8^+$ thymocytes, as well as for the expansion of the pool of $CD4^+CD8^+$ cells. TCR-α is irrelevant in these developmental processes. The development of $\gamma\delta$ T cells does not depend on either TCR-α or TCR-β.

IN the vertebrate immune system the recognition of a large variety of antigens is achieved by antigen-specific lymphocyte receptors, the immunoglobulin on B lymphocytes[1] and the T-cell antigen receptor on T lymphocytes[2]. There are two types of TCR, $\alpha\beta$ TCR and $\gamma\delta$ TCR, which are expressed on the surface of distinct subsets of T lymphocytes[3] that seem to be derived from different cell lineages[4].

In the main pathway of $\alpha\beta$ T-cell development in the adult thymus, bone marrow-derived stem cells undergo a series of intermediate stages. Among these stages are $CD4^-CD8^-IL\text{-}2R^+TCR^-$ (double negative, or DN) and $CD4^+CD8^+IL\text{-}2R^-TCR^{low+}$ (double positive, or DP) cells, both of which are important control points at which some signal or stimulus from the thymic microenvironment is suspected or known to be required for proliferation or differentiation to proceed[5]. The end stage of the pathway is represented by $CD4^+CD8^-TCR^{high+}$ or $CD4^-CD8^+TCR^{high+}$ cells (both are said to be in single positive, or SP, stages), which are mostly immediate precursors of major histocompatibility complex (MHC) class II-restricted helper T cells and MHC class I-restricted cytotoxic T cells, respectively.

It is well established that $\alpha\beta$ TCR play critical roles in the DP to SP transition by interacting with self-ligands expressed on the surface of thymic stroma cells (positive selection). But the role of the TCR genes or their products in the earlier DN to DP transition is uncertain. Because in the developing fetal thymus TCR-β genes rearrange before TCR-α genes[6,7], a distinct role can be envisaged for each of these genes or their products. Indeed, analysis of *severe combined immunodeficiency* (*scid*) mice carrying rearranged TCR transgenes indicated that TCR-β gene rearrangement or expression could be instrumental in the DN to DP transition[8,9]. But it is not known whether TCR-β gene rearrangement or expression is required for such a transition, and no information is yet available on a role for TCR-α gene rearrangement or expression.

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Here we use gene targeting to introduce a mutation in the TCR α or β genes into the germ line of mice. Initial analysis of these mice revealed that TCR-β gene rearrangement or expression is necessary for the maintenance of normal thymocyte number and the DN to DP transition. Our results show that a single rearranged TCR-β gene introduced as a transgene can quantitatively convert DN cells to DP cells from DN cells that have accumulated in mice mutant for the recombination activating gene-1 (*RAG-1*)[10] and increase the total thymocyte number to the normal level. By contrast, TCR-α gene expression seems to be irrelevant for these developmental events. Our study also indicates that $\alpha\beta$ T cells are unnecessary for the generation of apparently normal $\gamma\delta$ T cells.

Creation of mutations in TCR genes

The creation of a deletion at the TCR-β locus in embryonic stem (ES) cells has been described[11]. Briefly, two ES clones were produced carrying a genomic deletion of 15 kilobases (kb) that encompasses the TCR-β locus from Dβ1.3 to downstream of Cβ2. Germ-line chimaeras were obtained from both clones. A line was established from clone 59 and clean stocks derived by caesarean section.

To produce a targeted disruption of the unique TCR *C*α segment, we constructed targeting vector pPMKO-1 using 3.9 kb of homologous DNA sequences of BALB/c origin and carrying the pgk-*neo* selectable marker in the first exon of TCR *C*α (Fig. 1*a*). Two out of 160 G418-resistant D3 clones[12] were found to have the targeted mutation and clone 515 gave rise to germ-line chimaeras. For both the TCR-α and TCR-β mutations, intercrosses between heterozygous mice gave rise to homozygous mutant mice at the expected frequency of 25%. An example of the results from Southern analysis is shown in Fig. 1*b* for both TCR-α and TCR-β littermates.

Numbers of thymocytes

The numbers of total thymocytes were determined for TCR-α and TCR-β mutant mice, and for mice doubly homozygous for the TCR-α and TCR-β mutations (called TCR $\alpha\times\beta$ mutant mice). Whereas the thymuses of TCR-α mutant mice contained similar numbers of cells to those of normal littermates (Fig. 2*a*), the thymuses of the TCR-β mutant mice contained fewer cells, ranging from 6- to 60-fold less than those of normal littermates (Fig. 2*a*). TCR $\alpha\times\beta$ mutant mice had similar numbers of thymocytes as TCR-β mutant mice (Fig. 2*a*).

The importance of TCR-β in regulating the total number of thymocytes was also demonstrated by crossing a functionally rearranged TCR-β transgene[13,14] into the RAG-1 mutant mice. This resulted in restoration of the numbers of total thymocytes to wild-type levels (Fig. 2*a*). These results indicate that TCR-β is necessary to generate a thymus with the wild-type number of cells, and that it alone can lift the blockade imposed by the *RAG-1* mutation in this respect. In contrast, TCR-α is irrelevant to the generation of wild-type numbers of thymocytes.

Blockade by TCR-α mutation

As shown in Fig. 3*a*, thymuses of TCR-α mutant mice are largely devoid of CD4 or CD8 SP cells but retain normal numbers of small DP cells without the interleukin-2 (IL-2) receptor (IL-2R$^-$ DP cells) (Fig. 2*b*). The latter cells do not express $\alpha\beta$ TCR, or only barely, as determined by staining with the pan-TCR-β monoclonal antibody H57-597 (ref. 15) (Fig. 3*a*). There is also no difference in the number of DN cells in TCR-α mutant mice as compared to wild-type littermates (Fig. 3*a*).

The overall results indicate that expression of a rearranged TCR-α chain is unnecessary either for the progression of thymocytes from the DN to DP stage or for the expansion of the

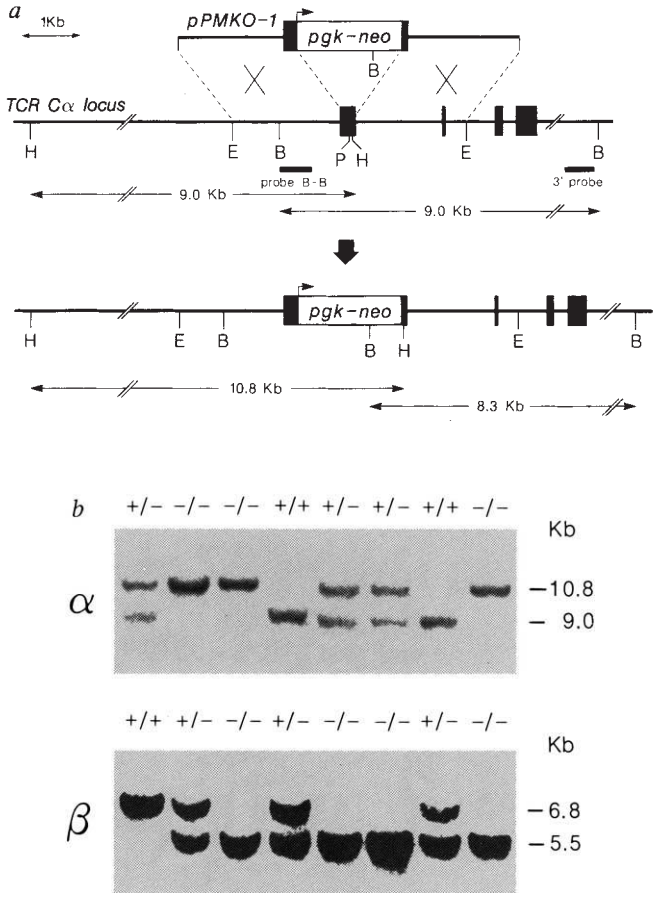

FIG. 1 Targeting of TCR *C*α and Southern analysis of TCR-α and TCR-β mutant mice. *a*, *top*, TCR-α targeting construct pPMKO-1. Crossed lines indicate hypothetical crossovers between the targeting construct and the TCR *C*α locus, *middle*, genomic structure of the TCR *C*α locus. Exon I codes for most of the extracellular domain, exon II encodes the hinge domain, exon III encodes the transmembrane and cytoplasmic domains, and exon IV contains untranslated sequences; *bottom*, structure of the disrupted TCR *C*α locus. Upon targeted integration of pPMKO-1, the pgk-*neo* gene is inserted in the first exon of TCR *C*α. Probes are probe B-B, to genotype the mice, and 3′ β probe, to screen the ES clones. Sizes of DNA fragments hybridizing to these probes are indicated within double-arrowed lines. At both left and right ends, the scale is interrupted. Abbreviations for restriction enzyme sites: H, *Hin*dIII; E, *Eco*RI; B, *Bam*HI; P, *Pfl*MI. *b*, Southern blots. *Top*, TCR-α mice. Thymocyte DNA from eight littermates was cut with *Hin*dIII and hybridized with probe B-B. The wild-type allele is at 9.0 kb and the mutant allele is at 10.8 kb. *bottom*, TCR-β mice. Tail DNA from eight littermates was cut with *Pst*I and hybridized with a 3′ Cβ probe. The wild-type allele is at 6.8 kb and the mutant allele is at 5.5 kb; +/+, wild-type; +/−, heterozygous; and −/−, homozygous mutant.
METHODS. pPMKO-1 was constructed from phage λ3.9 of BALB/c origin[23]. The 3.9-kb *Eco*RI insert of this phage was subcloned into pUC18. The pgk-*neo* selectable marker[24] was excised as a *Eco*RI–*Hin*dIII fragment from pKJ1[25], blunt-ended with Klenow DNA polymerase and inserted into the unique *Pfl*MI site in the first exon of TCR *C*α, which was blunted by treatment with T4 DNA polymerase. Before electroporation that targeting construct was released from plasmid sequences by digestion with *Eco*RI. The targeting experiment was done as described[10]. ES colonies were screened by digesting with *Bam*HI and hybridizing with the 3′ probe, a 0.5-kb *Pvu*II fragment containing the TCR-α enhancer[26]. Chimaeric mice were generated by standard protocols[27]. DNA was isolated according to ref. 28.

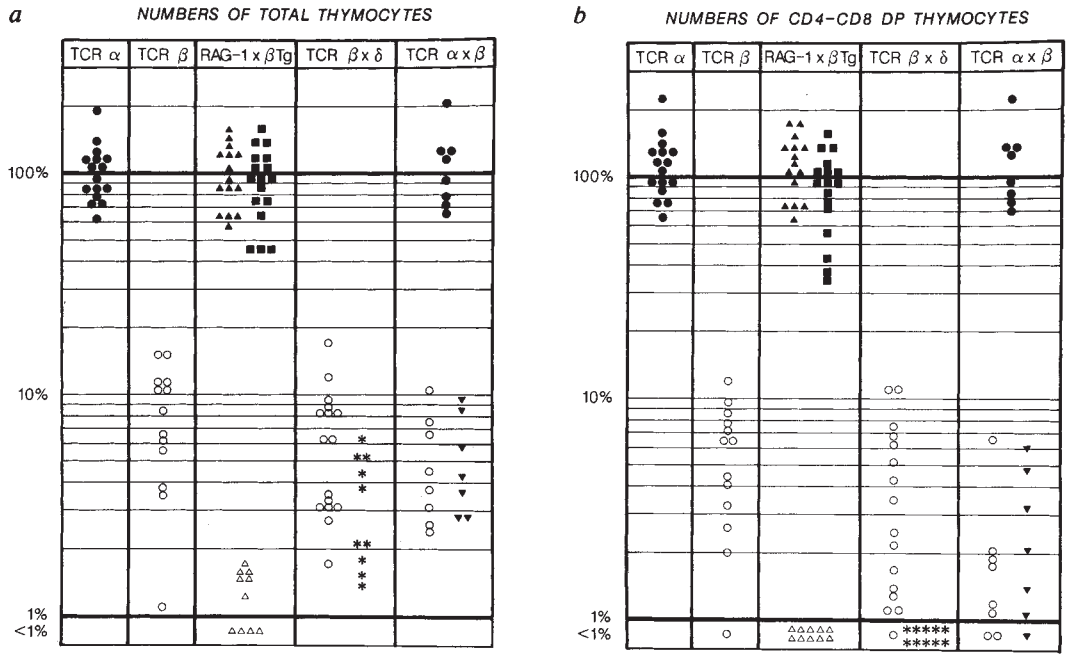

FIG. 2 Thymocyte numbers. *a*, Numbers of total thymocytes. As there is much variation in numbers of thymocytes between mice of different litters of the same age but much less between mice belonging to the same litter, the numbers of total thymocytes are shown in comparison to wild-type or heterozygous littermates in a logarithmic scale. TCR-α, TCR-α mutant mice (●): $n=16$, average 101%. TCR-β, TCR-β mutant mice (○): $n=13$, average 8.3%. RAG-1 × β Tg: RAG-1 mutant mice (△), $n=10$, average 1.4%; TCR-β transgenic mice (■), $n=17$, average 92%; TCR-β transgenic RAG-1 mutant mice (▲), $n=15$, average 102%. TCR $\beta \times \delta$: TCR-β mutant mice, $n=16$, average 6.6%; TCR $\beta \times \delta$ mutant mice (*), $n=10$, average 3.4%. TCR $\alpha \times \beta$: TCR-β mutant mice, $n=8$, average 5.2%; TCR-α mutant mice, $n=8$, average 110%; TCR $\alpha \times \beta$ mutant mice (▼), $n=7$, average 5.3%. *b*, Numbers of CD4-CD8 double-positive thymocytes. Data are shown for the same mice as in *a*. Percentage of DP thymocytes was measured by FACScan. TCR-α: $n=16$, average 112%; TCR-β: $n=13$, average 5.8%; RAG-1 × β Tg: RAG-1 mutant mice, $n=10$, average 0%; TCR-β transgenic mice, $n=17$, average 88%, TCR-β transgenic RAG-1 mutant mice $n=15$, average 115%; TCR $\beta \times \delta$: TCR-β mutant mice, $n=16$, average 4.2%; TCR $\beta \times \delta$ mutant mice, $n=10$, average 0.04%. TCR $\alpha \times \beta$: TCR-α mutant mice, $n=8$, average 118%; TCR-β mutant mice, $n=8$, average 2%; TCR $\alpha \times \beta$ mutant mice, $n=7$, average 2.7%.

METHODS. Mice were between three weeks and three months old. The background is (129/Sv × C57BL/6) for TCR-α and RAG-1 mutant mice, and (129/Ola × Balb/c) for TCR-β and TCR-δ mutant mice. We have also obtained TCR-β and TCR-δ mutant mice in a (129/Ola × C57BL/6) background and have found no effect of genetic background on thymocyte numbers and percentage of DP cells (data not shown). TCR-β transgenic mice were of founder 101 (ref. 14). The non-erythroid cells isolated from the thymuses were counted using a haemacytometer. At least one and usually two or more wild-type or heterozygous littermates were analysed in parallel with the mutant mice. The average number of total thymocytes of these littermates was given the value of 100%, and the number of total thymocytes in individual mutant mice was converted into a percentage. The percentage of DP thymocyte was measured by FACScan analysis by staining with 53-6.7 (Becton Dickinson) for CD8 and GK1.5 (Pharmingen) for CD4. The number of DP thymocytes was calculated by multiplying the number of total thymocytes with the percentage of DP thymocytes. This number was converted into a percentage using the wild-type or heterozygous littermates as 100%. In the case of TCR $\beta \times \delta$ mutant mice, TCr-δ mutant mice were included in the reference group of wild-type or heterozygous littermates, as they have normal numbers of total thymocytes and of DP thymocytes (our unpublished observations).

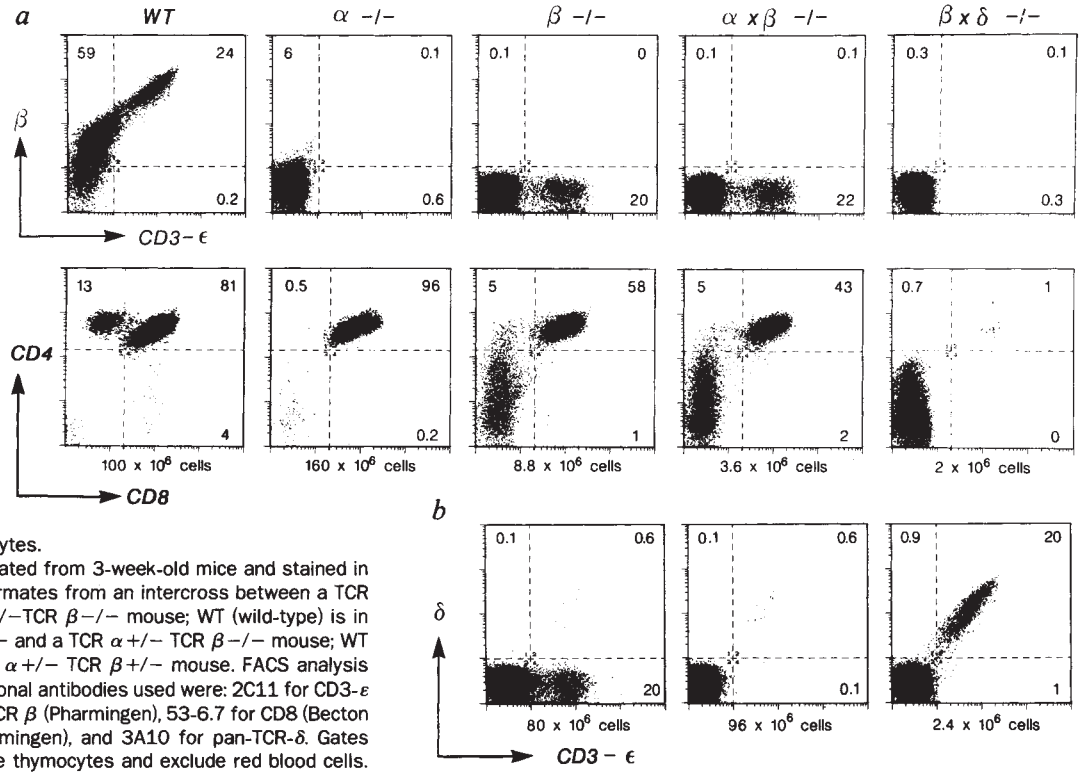

FIG. 3 FACScan analysis of thymocytes of mutant mice. *a*, *top*, CD3-ε-fluorescein isothiocyanate (FITC) (horizontally) and TCR-β-phycoerythrin (PE) (vertically); *bottom*, CD8-FITC (horizontally) and CD4-biotin-PE (vertically). WT, Wild-type; $\alpha-/-$, TCR-α mutant; $\beta-/-$, TCR-β mutant; $\alpha \times \beta-/-$: TCR $\alpha \times \beta$ mutant; $\beta \times \delta-/-$, TCR $\beta \times \delta$ mutant mouse. Percentage of cells in quadrants 1, 2 and 4 are indicated in the corners. The DN TCR-negative thymocytes in the TCR-β mutant mice are predominantly IL-2 receptor- and Sca-1 positive, and class I MHC high (data not shown). *b*, CD3-ε-FITC (horizontally) and TCR-δ-biotin-PE (vertically). Indicated below are the absolute numbers of thymocytes.

METHODS. Thymocytes were isolated from 3-week-old mice and stained in parallel. The mice in B were littermates from an intercross between a TCR $\alpha-/-$TCR $\beta+/-$ and a TCR $\alpha+/-$TCR $\beta-/-$ mouse; WT (wild-type) is in this case a TCR $\alpha-/-$ TCR $\beta+/-$ and a TCR $\alpha+/-$ TCR $\beta-/-$ mouse; WT (wild-type) is in this case a TCR $\alpha+/-$ TCR $\beta+/-$ mouse. FACS analysis was done as described[10]. Monoclonal antibodies used were: 2C11 for CD3-ε (Pharmingen), H57-597 for pan-TCR β (Pharmingen), 53-6.7 for CD8 (Becton Dickinson), GK1.5 for CD4 (Pharmingen), and 3A10 for pan-TCR-δ. Gates accomodate both small and large thymocytes and exclude red blood cells.

DP cells to the levels of wild-type mice. Thus the role of TCR-α expression seems to be confined to further differentiation to the SP stage.

Finally, as in the thymuses of wild-type littermates, about 1% of the cells bear $\gamma\delta$ TCR in the thymus of the TCR-α mutant mice, as determined by the anti-TCR-δ monoclonal antibody 3A10 (ref. 3) (Fig. 3*b*). These results suggest that TCR-α expression is unnecessary for the generation of $\gamma\delta$ thymocytes.

Blockade by TCR-β mutation

The thymuses of TCR-β mutant mice are different from those of wild-type littermates, not only in the total cell number (on average ~8% of wild type) but also in their cellular composition. First, as expected, no TCR-β-positive cells were detected by antibody H57-597 (Fig. 3*a*). Second, only about 50% of the thymocytes were double positive, although there was substantial individual variation (15–80%). This amounts to, on average, only ~6% of DP cells in the wild-type littermates (compare with Fig. 2*b*). As in wild-type littermates, these cells are small and negative for IL-2R (data not shown). Third, the proportion of large DN cells has increased to about 50% of the total cell number. As in wild-type littermates this thymocyte population includes two subpopulations of similar cell numbers: immature, IL-2R-positive, TCR-negative cells, and mature, IL-2R-negative, $\gamma\delta$ TCR-positive cells (data not shown). The increase in the proportion of DN cells in TCR-β mutant mice is due to the reduction in the numbers of DP cells: the number per thymus of each DN subpopulation is not altered by the introduction of the TCR-β mutation (Figs 2*a*, 3*a* and *b*). Finally, the TCR-β mutant thymus contains a few $CD4^+$ SP and $CD8^+$ SP cells, of which between half and two-thirds are $\gamma\delta$ T cells (data not shown). The rest are probably cells on the way to the DP stage[5].

The severe reduction of DP cells (about 6% of DP cells in the wild-type littermates) by the introduction of the TCR-β mutation indicates that TCR-β rearrangement or expression is important in the DN to DP transition in the principal differentiation pathway of $\alpha\beta$ T cells. To evaluate whether TCR-β rearrangement or expression is necessary for this transition, we introduced the TCR-β mutation to TCR-δ mutant mice (to be described elsewhere), in which we found that $\alpha\beta$ T cells develop normally and normal numbers of DN, DP and SP thymocytes are present. But introduction of the TCR-β mutation into the TCR-δ mutant mice abolishes virtually all DP cells and eliminates SP cells entirely (Fig. 3*a*), indicating that TCR-β rearrangement or expression is required for the DN to DP transition, at least in the principal differentiation pathway of $\alpha\beta$ T cells.

We investigated whether this role of TCR-β in $\alpha\beta$ thymocyte differentiation supersedes rearrangement or expression of TCR-α gene by crossing the TCR-α and TCR-β mutant mice. As shown in Figs 2*a*, *b* and 3*a*, the thymuses of the TCR $\alpha\times\beta$ mutant mice were similar to those of the TCR-β mutant mice by our criteria. The mutation in TCR-β is therefore epistatic to the mutation in TCR-α.

In conclusion, the mutation in TCR-β blocks $\alpha\beta$ thymocyte differentiation at an earlier stage than the mutation in TCR-α: at the transition from the large, IL-2R-positive DN stage to the small, IL-2R-negative DP stage. As a result the total number of thymocytes is much reduced. In contrast, the differentiation of $\gamma\delta$ thymocytes does not seem to be affected by the TCR-β mutation.

TCR-β can induce DN–DP transition

As we reported previously, $\alpha\beta$ T-cell differentiation is blocked at a DN stage in RAG-1 mutant mice[10]. Introduction of the transgenic TCR-β gene not only restored the total number of thymocytes to the levels of wild-type mice (Fig. 2*a*), but allowed nearly complete transition of the large, IL-2R-positive, DN cells to the small, IL-2R-negative DP cells (Fig. 2*b*). These results demonstrate that a single productively rearranged TCR-β gene provides a sufficient signal for the transition of $\alpha\beta$ lineage cells from a DN stage (at least the stage reached in the RAG-1 mutant mice) to a DP stage, and for the expansion of these DP cells to the normal level.

TCR-α rearrangement

TCR-β genes are rearranged and their RNA has been detected before TCR-α gene rearrangement in the fetal thymus[6,7]. A signal may therefore be provided by TCR-β gene rearrangement or its RNA or protein product which is required for TCR-α gene rearragenement, but there is no direct evidence to support this hypothesis. We investigated rearrangement of TCR-α genes in the thymuses of the TCR-β mutant and other mice by Southern blot analysis using two probes simultaneously, a 5′Jα1 probe and a Cα probe. Typical results are shown in Fig. 4*a*. In the thymuses of the wild-type and TCR-β (as well as TCR-α) heterozygous mice, rearrangement has occurred at nearly half of the TCR-α alleles (see Fig. 4 legend for definition of the rearrangement index, RI(α)). Although somewhat reduced, the rearrangement is substantial in the thymuses of TCR-β (as well as TCR-α) mutant mice. The reproducibility of these data is shown in shown in Fig. 4 legend. In support of the Southern blot data, thymuses of TCR-α mutant mice contain, albeit at a reduced level compared to the thymuses of wild-type or other mice, TCR-α transcripts of sizes expected from the fully rearranged gene (Fig. 4*b*). Cloning and sequencing experiments confirmed that these transcripts are indeed derived from productively or non-productivity rearranged TCR-α genes (data not shown). We conclude that TCR-β rearrangement is unnecessary for TCR-α rearrangement.

TCR-β rearrangement

Using a 5′Dβ1 probe[16] we examined at TCR-β rearrangement in the different mice, including *RAG-1* mutant mice in which no TCR rearrangement occurs[10] (Fig. 4*c*). TCR-β rearrangement in the thymocytes of TCR-α mutant mice was as extensive as that in wild-type or TCR-α heterozygous mice, as indicated by the virtual disappearance of the germ-line band and the presence of several bands corresponding to *DJ* rearrangements. The levels of TCR-β transcripts corroborate these rearrangement results (Fig. 4*d*), so we confirm that TCR-α expression is not needed for TCR-β rearrangement and transcription.

In contrast to thymocyte DNA from TCR-α mutant mice, thymocyte DNA from TCR-β mutant mice gave rise to no detectable *DJ* bands and a mutant germ-line band as intense as brain DNA (negative control) from the same mutant mice. This finding is interesting because the mutant allele retains the intact Dβ1, Jβ1.1 and Jβ1.2 segments despite the 15-kb deletion[11], and therefore can potentially undergo extensive *DJ* or *VDJ* rearrangements. The lack of rearrangement may be caused by a loss of an as-yet unidentified *cis*-acting rearrangement-promoting element, as suggested by the dramatic differences in the extent of rearrangement in the wild-type and mutant alleles coexisting in the same thymocytes of TCR-β heterozygous mice (Fig. 4*c*, third lane from right).

TCR-β surface expression

Extensive rearrangement and transcription of the TCR-β locus in TCR-α mutant mice provides a potential for synthesis of TCR-β polypeptide chains and even their cell-surface expression on thymocytes. Indeed, a small fraction of thymocytes of TCR-α mutant mice seems to be stained weakly by the anti-TCR-β antibody H57-597 (Figs 3*a* and 5). Surface expression of TCR-β chains was clearly seen with thymocytes derived from TCR-α mutant mice or RAG-1 mutant mice, into which a transgenic TCR-β gene was introduced; virtually all of these thymocytes were stained with H57-597 at levels higher than the TCR-β surface-positive thymocytes from TCR-α mutant mice (Figs 3*a* and 5). The occurrence of these cells in TCR-β transgenic RAG-1 mutant mice suggests that

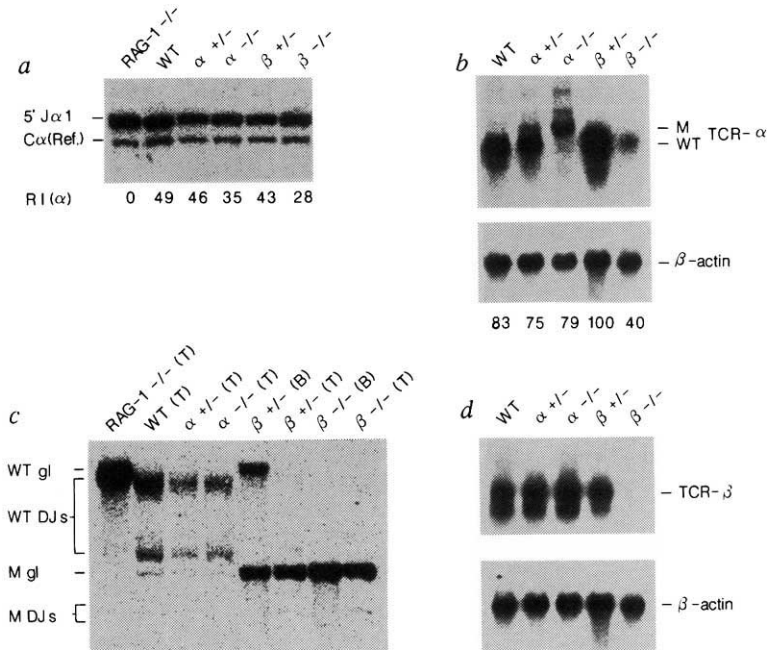

FIG. 4 *a*, Southern blot analysis of TCR-α rearrangements. Thymocyte DNAs from 3–8 weeks old mice were cut with *Sac*I and *Hin*dIII and were hybridized simultaneously with a 5′Jα1 probe and a TCR Cα probe. Lane 1, RAG-1 mutant; lane 2, wild-type; lane 3, TCR-α heterozygous; lane 4, TCR-α homozygous mutant; lane 5, TCR-β heterozygous, and lane 6; TCR-β homozygous mutant. As all *VJ* rearrangements will delete the 5′Jα1 sequence but retain the Cα sequence, the ratio of the intensity of the 5′Jα1 band to that of the Cα band is inversely proportional to the extent of rearrangements in the TCR-α locus. The rearrangement index for the TCR-α locus, RI(α), is defined by (1 − (5′Jα1/Cα ratio of sample)/(5′Jα1/Cα ratio of RAG-1 sample))) × 100. A value of zero means no *VαJα* rearrangement. The RI(α) for the TCR-β mutant thymocyte sample is 28, meaning that 28% of the chromosomes have undergone a *VαJα* rearrangement. 5′Jα1/Cα ratios (shown in table below) are given for different DNA samples. Three different RAG-1 mutant and seven different TCR-β mutant samples are analysed, prepared independently from pools of 3–10 mice each. The TCR-β heterozygous samples were derived from individual mice. Some samples are analysed in more than one experiment. Ratios vary between different experiments, depending on the specific activity of the two radioactively labelled probes. *b*, Northern blot analysis of TCR Cα transcripts in thymocytes. *Top*, hybridization with a TCR Cα probe. *Bottom*, hybridization with a β-actin probe as internal control. Lane 1, C57BL/6: lane 2, TCR-α heterozygous; lane 3, TCR-α mutant; lane 4, TCR-β heterozygous; lane 5, TCR-β mutant mice. Transcripts hybridizing to the TCR Cα probe in the TCR-α mutant mouse are longer than those in the other mice, presumably because of insertion of the 1.8-kb *neo*-selectable marker in the first exon of TCR Cα. WT and M designate transcripts from rearranged wild-type (1.8 kb) and mutant TCR-α alleles, respectively. Numbers at the bottom indicate the ratio of the intensity of the TCR Cα band to the β-actin band, normalized to 100 for the sample of the TCR-β heterozygous thymocytes. *c*, Southern blot analysis of TCR-β rearrangements. Thymocyte (T) DNAs from 3–8-week old mice cut with *Hin*dIII were hybridized with a 5′Dβ1 probe. Lane 1, RAG-1 mutant; lane 2, C57BL/6; lane 3, TCR-α heterozygous; lane 4, TCR-α mutant; lane 6, TCR-β heterozygous; lane 8, TCR-β mutant mice. For negative controls, brain (B) DNA was isolated from: lane 5, TCR-β heterozygous; lane 7, TCR-β mutant mice. 'WT' and 'M' mark the germ-line (unrearranged) wild-type and mutant bands, respectively. 'Gl', germ-line, and 'DJs', D–J rearrangements. The 5′Dβ1 probe[16] detects *DJ* rearrangements involving the Dβ1 segment by the appearance of bands of known restriction fragment sizes, and *VDJ* rearrangement by a reduction in the intensity of the band representing unrearranged alleles. Rearrangement is extensive in thymocytes of wild-type compared to RAG-1 mutant mice (negative control), as indicated by the virtual disappearance of the germ-line band and the presence of several bands corresponding to *DJ* rearrangements. TCR-β rearrangement in thymocytes of TCR-α mutant mice is as extensive as that in wild-type mice. Upon prolonged exposure, two faint bands expected from Dβ1–Jβ1.1 or Dβ1–Jβ1.2 rearrangements of the mutant allele were occasionally observed. *d*, Northern blot analysis of TCR Cβ2 transcripts in thymocytes. *Top*, hybridization with TCR Cβ2 probe. *Bottom*, hybridization with a β-actin probe as internal control. Same RNA samples as in *b*. TCR Cβ2 transcripts were present at normal levels in TCR-α mutant mice, but were absent in TCR-β mutant mice, as the TCR Cβ2 gene segment is included in the 15-kb deletion[11].

METHODS. For Southern blot analysis, thymocyte and brain DNA was isolated according to ref. 28. The 5′Dβ1 probe has been described before[10,16]; the 5′Jα1 probe is a 1·2-kb *Kpn*I-*Pst*I fragment isolated from a 4-kb *Hin*dIII genomic clone (gift from A. Winoto). This probe is immediately upstream of the first Jα segment (Jα1) and downstream of ψJα. It hybridizes to a 1.5-kb *Sac*I-*Hin*dIII fragment[29]. Note that mδRec-ψJα rearrangements do not affect the outcome. The Cα probe is a 0.7 kb *Xba*I-*Eco*RI fragment encompassing the second exon of TCR Cα and hybridizes to 1.3-kb *Sac*I-*Hin*dIII fragment. The mutation in TCR Cα does not affect the results. The intensity of the radioactivity was measured using a Fujix BAS2000 Bio-image Analyzer within the linear range of intensities. Total RNA was prepared for northern blot analysis. The TCR Cα probe was a 550-bp *Nco*I fragment isolated from cDNA 2C1B2α4 (gift from Y. Takagaki), which spans the insertion site of pgk-*neo*. The TCR Cβ2 probe was a 800-bp genomic *Sac*I fragment within the TCR Cβ2 region, isolated from cosmid 2.3W7 (ref. 16).

TCR-α rearrangements in thymocytes

Experiment	RAG-1−/−	TCR-β+/−	TCR-β−/−	Experiment	RAG-1−/−	TCR-β+/−	TCR-β−/−
(1) Ratio 5′Jα1/Cα	7.59	4.35	5.48	(3) Ratio 5′Jα1/Cα	3.90; 4.03		2.34; 3.26; 3.42
RI (α)	0	43	28	Average ratio	3.97		3.01
(2) Ratio 5′Jα1/Cα	3.44; 3.58	1.90; 2.14; 2.17; 2.35	2.41; 2.67; 2.97; 2.98	RI (α)	0		24
Average ratio	3.51	2.14	2.76	(4) Ratio 5′Jα1/Cα	8.49; 8.69	5.00	5.35; 5.53; 5.97; 6.40; 6.62
RI (α)	0	39	21	Average ratio	8.59	5.00	5.97
				RI (α)	0	42	30

at least some of the surface TCR-β chains are not associated with any of the other known complete TCR polypeptide chains.

A few CD4-positive lymphocytes became increasingly detectable with age in the peripheral lymphoid organs and in the blood of TCR-α mutant mice but not in TCR-β mutant mice, which stained weakly with antibodies against TCR-β and CD3-ε. As an example, staining patterns of lymphocytes from mesenteric lymph nodes of a 9-month-old TCR-α mutant mouse and 8-month-old TCR-β mutant mouse are shown in Fig. 6*a*. The TCR-β and CD4-positive cells were not stained by two different anti-TCR Cδ antibodies (3A10 and GL3) and are therefore unlikely to bear mixed TCR $\beta\delta$ heterodimers[17] (data not shown). We observed an equally small population of CD4-positive dull TCR-β-positive cells in the periphery of TCR-β transgenic TCRα mutant mice (data not shown).

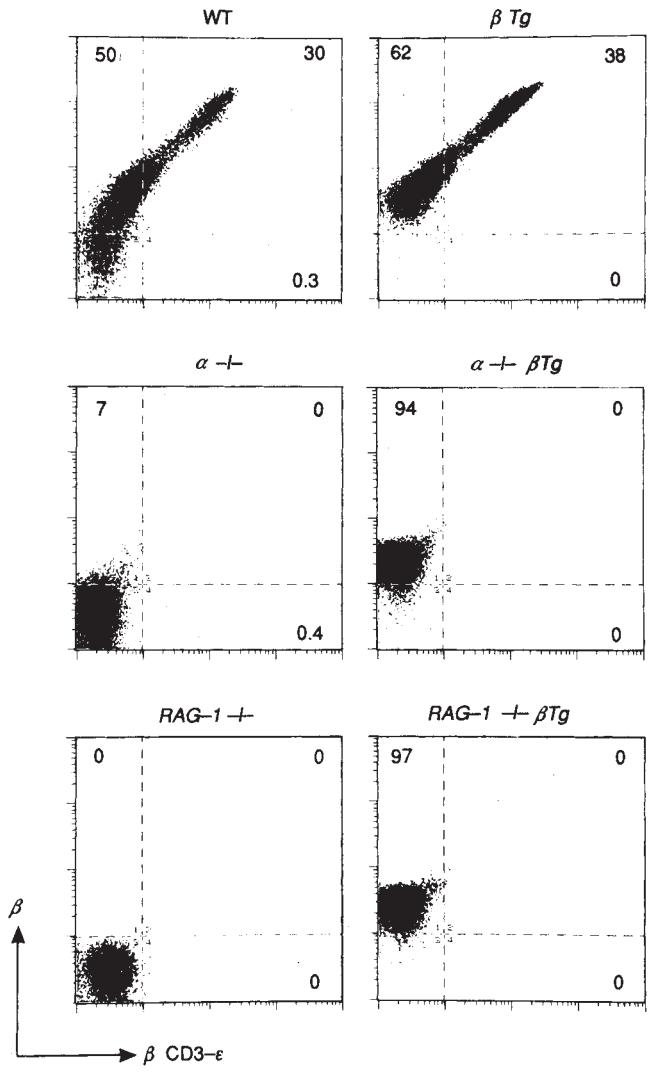

FIG. 5 FACScan of TCR-β transgenic mutant thymocytes; CD3-ε-FITC (horizontally) and β-PE (vertically). WT, wild-type; β Tg, TCR-β transgenic; $\alpha-/-$, TCR-α mutant; RAG-1$-/-$, RAG-1 mutant.
METHODS. Thymocytes were isolated from 4-week-old mice and stained as described for Fig. 3*a*.

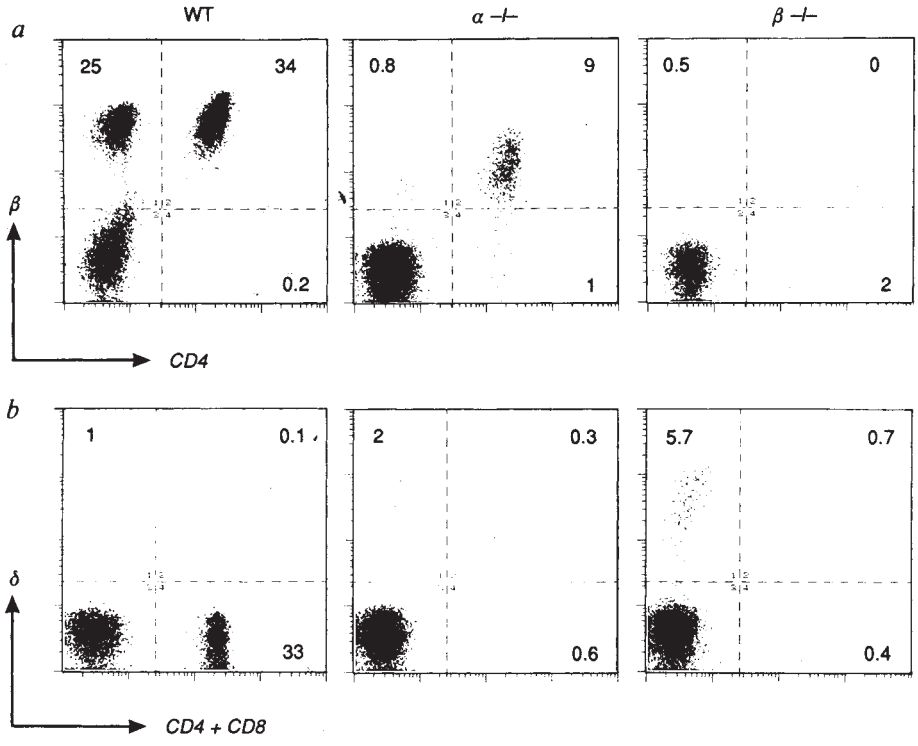

FIG. 6 *a*, Dull TCR-β-positive cells in the periphery of the TCR-α mutant mouse. Lymphocytes are from mesenteric lymph nodes of a 9-month-old wild-type mouse (*left*), a TCR-α mutant littermate (*middle*), and an 8-month-old TCR-β mutant mouse (*right*). CD3-ε-FITC (horizontally) and TCR-β-PE (vertically) the total numbers of lymphoid cells were 5×10^6, 22×10^6 and 9×10^6, respectively. *b*, $\gamma\delta$ T cells in the periphery of young TCR-α and TCR-β mutant mice. The number of $\gamma\delta$ T cells in the mutant mice is similar or slightly increased compared to the number in wild-type younger littermates (less than 2–3 months old), and most $\gamma\delta$ T cells in the mutant mice are CD4-CD8 double negative. Splenocytes were from a 5-week-old (*left*) TCR-α heterozygous, (*middle*) TCR-α mutant, and (*right*) TCR-β mutant mouse. Staining was with CD8-FITC + CD4-FITC (horizontally) and TCR-δ-PE (vertically). The total number of cells was respectively 44×10^6, 60×10^6 and 40×10^6 lymphoid cells.
METHODS. The mice in each panel were stained in parallel using antibodies described for Fig. 3*a*. The gates were put on the small, lymphoid-like cells. For both *a* and *b*, the control mouse is a littermate of the TCR-α mutant mouse.

γδ T-cell development

In both the TCR-α and TCR-β mutant mice, γδ T-cell development in the thymus appears to be unaltered; the number of γδ thymocytes is the same as in wild-type mice (Fig. 3*b*). The numbers of γδ T cells in the spleen, lymph nodes and gut epithelium are similar or slightly increased in young mutant mice as compared to wild-type littermates (an example for the spleen is shown in Fig. 6*b*).

Discussion

We have shown that TCR-β rearrangement or, more likely, expression of a functionally rearranged TCR-β gene, is not only necessary but also sufficient for driving most of the immature IL-2R-positive DN thymocytes to the DP stage and for expanding the pool of thymocytes. We have also shown that TCR-α expression is irrelevant to these processes, but together with TCR-β rearrangement and expression it allows further differentiation into mature, CD4 or CD8 SP thymocytes expressing high levels of αβ TCR and CD3.

The conclusion that TCR-β is sufficient is based on the observations that both the severe drop in total thymocyte numbers (to about 1% of the wild-type level) and the blockade at a DN stage in the RAG-1 mutant mice are fully restored upon introduction of a productively rearranged TCR-β transgene. These observations differ from earlier findings in which *scid* mice[18] were host for the same TCR-β transgene[8,9]. In these TCR-β transgenic *scid* mice the number of double-positive cells reaches only about 2% of wild-type level and the total number of thymocytes is about 3% of wild-type[8]. The most likely explanation for the difference is that the *scid* mutation affects the physiology of the thymocyte in a more general way rather than just preventing correct $V(D)J$ recombination[18].

The origin of the small number (about 5% of the wild-type level) of DP cells remaining in the thymus of TCR-β mutant mice is unclear. They may belong to a minor αβ T-cell pathway in which DN → DP transition occurs independently of TCR-β rearrangement or expression. Alternatively, these cells may belong to the γδ T-cell lineage because they are virtually abolished when TCR-δ mutation is introduced into the TCR-β mutant mice. No role for DP cells as intermediates in the differentiation of γδ T cells has been demonstrated. But TCR-δ-positive DP cells have been detected in the late embryonic thymus[3] and about 1% of TCR-δ-positive cells in the postnatal TCR-β mutant thymus are DP (data not shown).

The hypothesis that TCR-β rearrangement provides a signal for TCR-α rearrangement is challenged by our findings of TCR-α rearrangements and full-size TCR-α transcripts in the TCR-β mutant mice. These findings are analogous to those made recently in the B-cell system: Ig-κ rearrangements can occur in the absence of an Ig-μ product in Ig-Cμ mutant mice[19]. However, rearrangements may occur sequentially at the TCR-β and TCR-α loci, but with a causal relationship.

Surface expression of TCR-β without TCR-α (α-less TCR) has been found in DN thymocytes of TCR-β transgenic mice[20], in DN and DP thymocytes of TCR-β transgenic *scid* mice[8,9], and in a DP thymocyte line[21]. We have now extended these observations to DP thymocytes in TCR-β transgenic TCR-α mutant, TCR-β transgenic RAG-1 mutant and probably to TCR-α mutant mice. The α-less TCR could be an artefact of the mutant or TCR-β transgenic mutant mice. But cells expressing such TCR may be normal intermediates of αβ thymocyte differentiation, in analogy to the pre-B cells that expresses Ig-μ without any light chains on the surface. Surface expression rather than only cytoplasmic expression of the TCR-β chain may be essential for transduction of the signal for the induction of CD4 and CD8 and/or proliferation of DP cells. This hypothesis can be tested by crossing variously altered TCR-β transgenes[14] into the RAG-1 or TCR-β mutant mice.

The presence of CD4-positive dull TCR-β positive cells in the periphery of the TCR-α mutant and TCR-β transgenic TCR-α mutant mice, suggests that some thymocytes bearing TCR-β without TCR-α may develop into mature T cells and emigrate to the periphery in normal mice, although such T cells could be present only in the genetically manipulated mice. Recently another strain of TCR-α mutant mice has been reported[22] but no T cells with α-less TCR were observed; the reason for this discrepancy is not clear, although these cells may have been overlooked.

Finally, our studies indicate that the TCR-α or TCR-β mutation does not block γδ T-cell development. The αβ T-cell-deficient mice should be useful for analysing the *in vivo* functions of γδ T cells. □

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