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Gene-targeted deletion and replacement mutations of the T-cell receptor β-chain enhancer: The role of enhancer elements in controlling V(D)J recombination accessibility

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ABSTRACT **To assess the role of transcriptional enhancers in regulating accessibility of the T-cell receptor β-chain (TCRβ) locus, we generated embryonic stem cell lines in which a single allelic copy of the endogenous TCRβ enhancer (Eβ) was either deleted or replaced with the immunoglobulin heavy-chain intronic enhancer. We assayed the effects of these mutations on activation of the TCRβ locus in normal T- and B-lineage cells by RAG-2 (recombination-activating gene 2)-deficient blastocyst complementation. We found that Eβ is required for rearrangement and germ-line transcription of the TCRβ locus in T-lineage cells. In the absence of Eβ, the heavy-chain intronic enhancer partially supported joining region β-chain rearrangement in T- but not in B-lineage cells. However, ability of the heavy-chain intronic enhancer to induce rearrangements was blocked by linkage to an expressed neomycin-resistance gene (*neo*^{r}). These results demonstrate a critical role for Eβ in promoting accessibility of the TCRβ locus and suggest that additional negative elements may cooperate to further modulate this process.**

During the early stages of lymphocyte development, immunoglobulin and T-cell receptor (TCR) variable region (V) genes are assembled from germ-line gene segments by the variable, diversity, joining (VDJ) recombination process (1, 2). Both immunoglobulin and TCR variable region gene segments are assembled by a common V(D)J recombinase (3–5); however, V(D)J recombination is regulated with respect to both lineage and developmental stage, as well as in the context of allelic exclusion (5). Such regulation is achieved by modulating accessibility of the different germ-line antigen receptor loci to the common V(D)J recombinase (3), a process that, within V(D)J recombination substrates, has been correlated with the activity of transcriptional enhancer elements (6–11).

Transgenic experiments have clearly demonstrated that the heavy-chain intronic enhancer (Eμ) and TCRβ enhancer (Eβ) elements can promote rearrangement of V(D)J recombination substrates in normal developing lymphocytes (7–9). The function of enhancer elements in promoting endogenous immunoglobulin gene rearrangements has also been assessed by gene-targeted mutation (12–14). Specific deletion of Eμ decreased but did not eliminate endogenous immunoglobulin heavy-chain joining region (J_H) rearrangements (12). In contrast, replacement of Eμ with a neomycin-resistance gene (*neo*^{r}) led to a more substantial inhibition (13). Together, these two studies suggested that an inserted *neo*^{r} gene may interfere with recombination by inhibiting additional cis regulatory elements that act in concert with Eμ to provide full rearrangement potential.

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The minimal TCRβ enhancer is located within a 0.5-kb DNA segment that lies 7 kb 3′ of the constant region β-chain 2 (Cβ2) region. This enhancer was defined by its ability to activate a heterologous promoter in a T-cell-specific manner (15, 16). Furthermore, the minimal Eβ was shown to function similarly to Eμ in promoting accessibility of a transgenic mini-locus V(D)J recombination substrate in developing T- and B-lineage cells (8, 9). To elucidate the potential role of Eβ in controlling accessibility of the endogenous TCRβ locus, we generated enbryonic stem (ES) cells heterozygous for mutations in which this element was either specifically deleted or replaced by Eμ with or without a *neo*^{r} gene. We then used recombination-activating gene 2 (RAG-2)-deficient blastocyst complementation (17) to assay effects of these mutations on TCRβ gene rearrangement and expression.

MATERIALS AND METHODS

Targeting Vectors. A *Bam*HI–*Bgl*I DNA fragment containing Eβ was isolated from a 129vs EMBL3 genomic library and cloned into pBluescript (Stratagene). To generate the pEβKO targeting vector, *neo*^{r} driven by the phosphoglycerate kinase gene (*PGK-1*) promoter (18), flanked by the *loxp* sites (*lox–neo*^{r}) (20), was cloned between the *HpaI and Nco*I sites of the *Bam*HI–*Bgl*I genomic fragment, thereby deleting the minimal TCRβ enhancer. The final pEβKO plasmid was obtained by cloning a thymidine kinase gene (*tk*) driven by the *PGK-1* promoter (19) into the *Sal*I site located between the genomic and plasmid sequences (Fig. 1*A*). To generate the pEβR replacement vector, the *loxP–neo*^{r} cassette linked to a 700-bp *Xba*I–*Eco*RI fragment containing the Eμ enhancer (22, 29) was cloned between the *Hpa*I and *Nco*I sites of the *Bam*HI–*Bgl*I genomic fragment, thereby replacing Eβ by Eμ plus the *loxP–neo*^{r} cassette. The final pEβR plasmid was obtained by cloning the *PGK–tk* cassette into the *Sal*I site located between genomic and plasmid sequence (Fig. 1*A*).

Generation of Targeted ES Cells. Approximately 20 μg of a given targeting construct was transfected into CCE ES cells, and homologous recombinants were selected as described (19). To delete the *PGK–neo* cassette from the targeted TCRβ locus, we transiently transfected a cytomegalovirus (CMV) promoter-driven cre expression vector (CMV-cre) (20, 21) into targeted ES cell clones under described conditions and identified specifically deleted subclones by Southern blot analyses (13).

Southern and Northern Blot Analyses. Southern and Northern blot analyses were performed as described (13).

Abbreviations: TCRβ, T-cell receptor β-chain; E_β, TCR_β enhancer; V(D)J, variable (diversity) joining; Eμ, heavy-chain intronic enhancer; ES, embryonic stem; RAG, recombination-activating gene; Cβ, constant-region beta chain.

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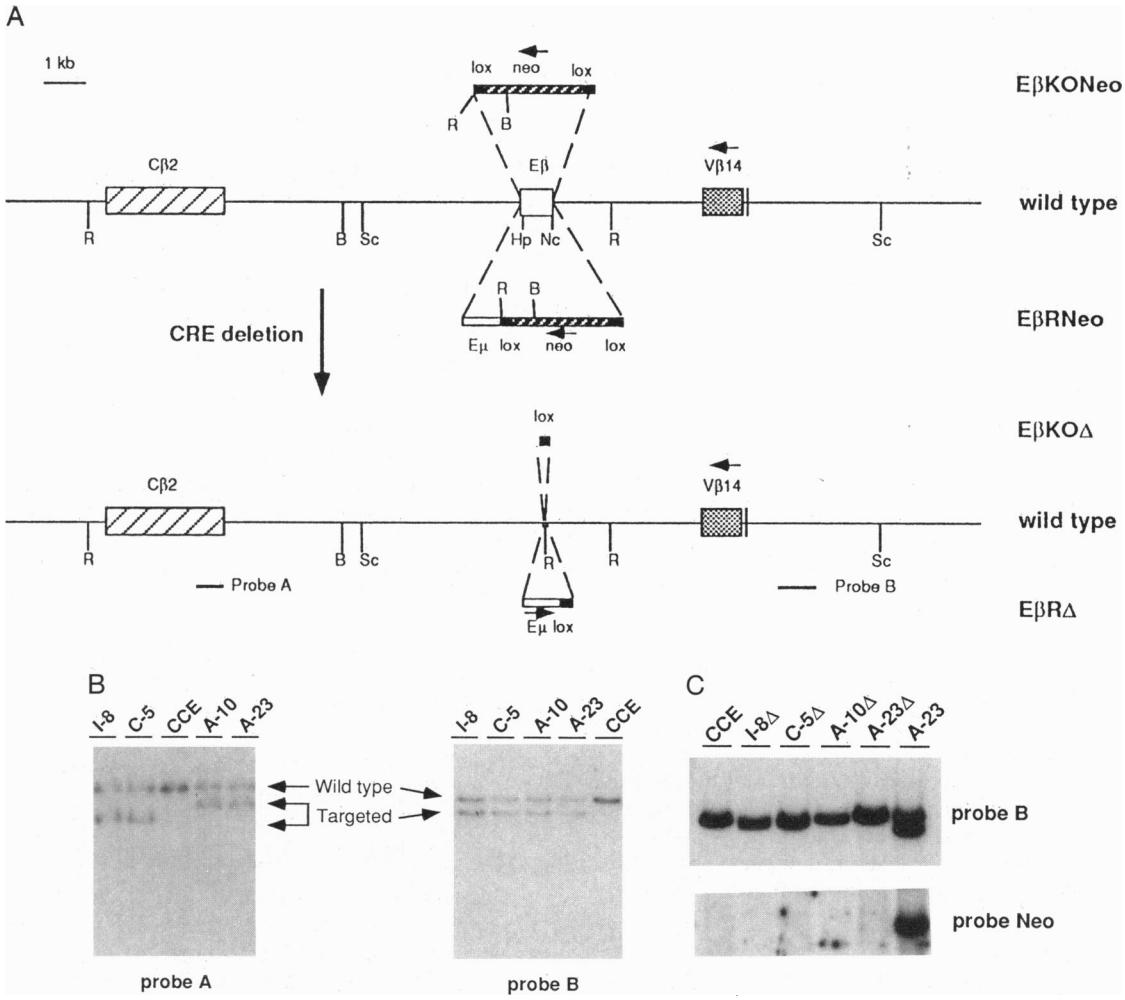

FIG. 1. Targeted mutations of the Eβ region. (*A*) Representation of Eβ mutations. EβKONeo, *neo*^{r} replacement; EβRNeo, Eμ plus *neo*^{r} replacement; EβKOΔ, Eβ deletion; and EβRΔ, Eμ replacement. Eβ is represented by an open box, Cβ2 and Vβ14 by hatched boxes, *loxP* sequences by bold bars, *neo*^{r} by hatched bars, and Eμ (700-bp *Xba*I–*Eco*RI fragment) by an open bar. Arrows indicate the transcriptional orientation of the *neo*^{r} gene. Restriction sites are as follows: R, *Eco*RI; B, *Bam*HI: Hp, *Hpa*I; Nc, *Nco*I; Sc, *Sac*I. Probe A is a *Xmn*I–*Sal*I [from the *Xmn*I site up to the poly(A) site] Cβ2 cDNA fragment and probe B is a *Hin*dIII–*Bgl*II genomic DNA fragment. (*B*) Southern blot analysis of the targeted ES cells. I-8 and C-5 are EβKONeo ES cell clones; A-10 and A-23 are EβRNeo ES cell clones. Control DNA was extracted from wild-type CCE ES cells. (*Left*) *Eco*RI-digested DNA was assayed for hybridization with probe A. The wild-type (10.5 kb) and targeted (EβKONeo: 8.8 kb and EβRNeo: 9.5 kb) alleles are indicated. (*Right*) DNA was digested with *Bam*HI plus *Sac*I and assayed for hybridization to probe B. The wild-type (10 kb) and targeted (9 kb) alleles are indicated. (*C*) Southern blot analysis of the cre-deleted clones. I-8Δ and C-5Δ clones were derived from I-8 and C-5; A-10Δ and A-23Δ clones were derived from A-10 and A-23. DNA was digested with *Bam*HI plus *Sac*I and assayed for hybridization with probe B (*Upper*) and a *neo*^{r} probe (*Lower*).

Generation and Analysis of Chimeric Mice. Heterozygous mutant ES cells were injected into RAG-2$^{-/-}$ blastocysts to generate chimeric mice, and chimerism was evaluated as described (17). To generate B cells, spleen cells were cultured in RPMI 1640 medium/15% fetal calf serum (Sigma)/and bacterial lipopolysaccharide (20 μg/ml) for 4 days. More than 98% of the resulting cells were B220$^+$ (data not shown). To generate T cells, spleen cells were cultured in RPMI 1640 medium/15% fetal calf serum (Sigma), ConA at 10 μg/ml (Sigma)/recombinant interleukin 2 at 100 units/ml for 5 days. More than 98% of the resulting cells were Thy-1$^+$ (data not shown).

DNA PCR Assays. For standard PCRs, 1 μg of DNA was amplified in 50 μl of 200 mM Tris·HCl, pH 8.8/100 mM $(NH_4)_2SO_4$/100 mM KCl/20 mM $MgSO_4$/1% Triton X-100/ bovine serum albumin at 0.2 mg/ml/0.5 units of *Taq* polymerase (Perkin–Elmer)/1 mM of each dNTP/100 ng of each primer for 32 cycles (1 cycle: 94°C for 30 sec, 57°C for 2.5 min, 72°C for 2.5 min), then incubated at 72°C for 10 min. For long PCRs (see Fig. 4), 1 μg of DNA was amplified as described above, except that we used 5 units of *Taq* polymerase (Perkin–Elmer) and 5 units of *Taq* extender (Stratagene) for 32 cycles (1 cycle: 94°C for 30 sec, 65°C for 3 min, 72°C for 3 min), then incubated at 72°C for 10 min. One-tenth of each PCR was run on a given gel.

RNA PCR Assays. RNA was prepared as described (23). RNA (5 μg) was incubated for 30 min at 42°C in 50 μl of 50 mM Tris·HCl, pH 8.3/75 mM KCl/10 mM $MgCl_2$/0.5 mM spermidine/10 mM dithiothreitol/4 mM sodium pyrophosphate/1 mM of each dNTP/50 units of RNasin ribonuclease inhibitor (Boehringer Mannheim)/0.5 μg of primers/50 units of avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim). Four microliters of this reaction was amplified under the same conditions as for standard DNA PCRs. One-tenth of the reaction was assayed on a given gel.

Oligonucleotides. Oligonucleotides used were as follows: 5′Dβ1, GAC AAA CCT CTC TGC CAC CTG TCT C; 5′Eβ, CCA GCT CCA GAC ACA CAC ATT GTG A; Dβ-130, TGG TTT CTT CCA GCC CTC AAG; Vβ14–510, TCT AAG CTT AAA TCA AGC CCT AAC CTC TAC; Vβ14–810, TCA GCC AGG AAT TCA GTG GC; 3′Jβ2, CAT CCT TCC TCT

GAT TAC; 5′Jβ1.1, CTC CTC ATC CTA TGG CAC; Cβ3′, GAT CTC ATA GAG GAT GGT TGC AGA C; pCβ, CCA CCA GCT CAG CTC CAC GTG GTC; 5′Act, AGA GCT ATG AGC TGC CTG ACG GCC; 3′Act, AGT AAT CTC CTT CTG CAT CCT GTC; pAct, GTC ATC ACT ATT GGC AAC GAG CGG; Lox, GGC CGC TAA TTC CGA TCA TAT TC.

RESULTS

Generation of Eβ Mutant ES cells. We used a gene-targeting approach to generate CCE ES cells in which a single copy of the endogenous Eβ was replaced with either a *neo*^{r} gene or with a *neo*^{r} gene linked downstream of the core Eμ element (Fig. 1*A*). In both cases, *neo*^{r} gene was flanked by *loxP* sites. Multiple independent targeted clones were obtained. Two independent clones that harbored the *neo*^{r} replacement (I-8 and C-5) and two that harbored the Eμ/*neo*^{r} replacement (A-23 and A-10) were selected for further analysis (Fig. 1*B*). These two types of mutant ES cells will be referred to as EβKO*neo* and EβR*neo*, respectively.

To more specifically modify the endogenous TCRβ locus, we used the cre-*loxP* system (20, 21) to delete *neo*^{r} from the mutated TCRβ locus, leaving only an inserted 82-bp sequence consisting of the *loxP* and flanking vector sequence (refs. 20 and 21; M. Cogne and F.W.A., unpublished results). For this purpose, we transiently transfected given clones with a CMV-cre expression vector, resulting in excision of the *neo*^{r} gene in 2–10% of the transfected cells (Fig. 1*C*; data not shown). By this procedure, we generated two subclones (I-8Δ and C-5Δ) in which the Eβ was specifically deleted and two subclones (A-23Δ and A-10Δ) in which the Eβ was specifically replaced with Eμ (Fig. 1*C*). The two types of targeted/*neo*^{r}-deleted subclones will be generally referred to as EβKOΔ and EβRΔ, respectively.

Inhibition of TCRβ Rearrangement by Eβ Deletion and Rescue by Eμ Replacement. EβKOΔ, EβR*neo*, and EβRΔ heterozygous mutant ES cells were independently injected into RAG-2$^{-/-}$ blastocysts, and the T- and B-cell populations of the resulting chimeric mice were assayed for effects of the mutations on expression and rearrangement of the TCRβ locus. To detect Dβ1Jβ1 and Dβ1Jβ2 rearrangements, we assayed *Hin*dIII-digested genomic DNA for hybridization to a Dβ1-specific probe (probe C; Fig. 2*A*; Fig. 2*B Upper*). A similar strategy was used to assay for Dβ2Jβ2 rearrangements (probe D; Fig. 2*A*; Fig. 2*B Lower*) and gave results completely in accord with those of the Dβ1Jβ1/Dβ1Jβ2 analyses. However, analyses of Jβ2 rearrangements were not as informative as those of Jβ1 because the Jβ2 portion of the locus is not completely rearranged in many normal T-lineage cells (Fig. 2*B Lower*).

The majority of wild-type thymocytes and splenic T cells had deleted the germ-line Dβ-hybridizing fragment, as expected, because of rearrangement of both Jβ alleles, whereas wild-type B-lineage cells retained this region in germ-line configuration (Fig. 2*B Upper*). Strikingly, thymocytes and splenic T cells from EβKOΔ chimeras retained a strong germ-line band and showed no detectable Dβ1Jβ1 or Dβ1Jβ2 rearrangements (Fig. 2*B Upper*). Quantitative PCR assays confirmed that the level of DJβ rearrangements in mature EβKOΔ T cells was 50-fold lower than in wild-type T cells (data not shown). Since most thymocytes and mature T cells had at least reached CD4$^+$/CD8$^+$ double-positive stage (data not shown), they must have undergone a functional VDJβ rearrangement on one chromosome (25, 26). Therefore, in contrast to normal T cells, which have rearranged both TCRβ alleles, most EβKOΔ T cells have a VDJβ rearrangement on one allele and no rearrangement on the other.

We could not detect rearrangements of the endogenous TCRβ locus in T- or B-lymphocytes from EβR*neo* chimeric mice (Fig. 2*B*). Strikingly, however, we detected a significant, although incomplete, level of Dβ1Jβ1, Dβ1Jβ2, and

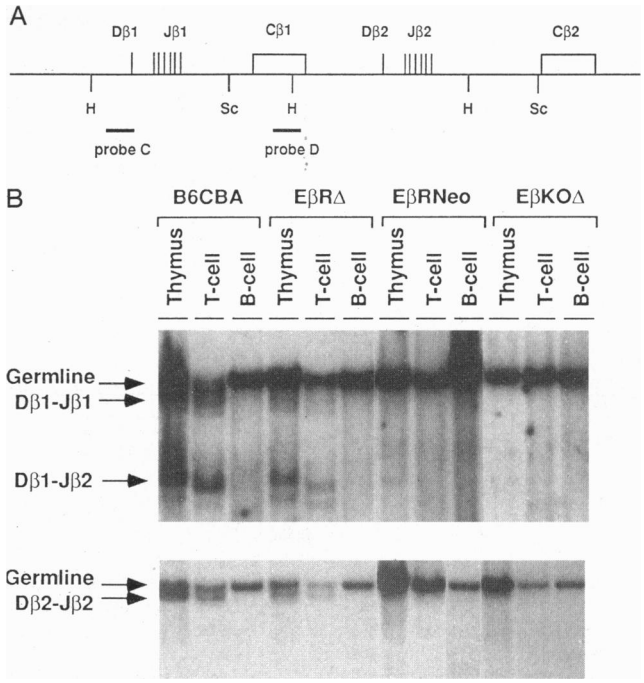

FIG. 2. Analysis of Dβ-to-Jβ rearrangements of Eβ-mutated alleles. (*A*) Representation of the TCRβ locus. The Dβ and Jβ segments are indicated by vertical bars, and constant regions are represented by open boxes. *Hin*dIII (H) and *Sac*I (Sc) sites are indicated and the locations of probe C (*Pst*I–*Bgl*II fragment; ref. 7) and probe D (Cβ1 cDNA fragment; ref. 24) are shown by thick lines. (*B*) Southern blot analysis of DNA from thymus, T cells, and B cells of B6CBA, EβRΔ, EβRNeo, and EβKOΔ chimeric mice. (*Upper*) DNA was digested with *Hin*dIII and assayed for hybridization with probe C. Bands corresponding to germ-line (9.5 kb), Dβ1–Jβ1 (8.5–7 kb), and Dβ1–Jβ2 (4.5–3.5 kb) alleles are indicated by arrows. (*Lower*) DNA was digested with *Sac*I and assayed for hybridization to probe D. The arrows show the position of bands corresponding to nonrearranged (11 kb) and Dβ2–Jβ2-rearranged (10 kb) alleles.

Dβ2Jβ2 rearrangements in thymocytes and T cells of EβRΔ chimeric mice (Fig. 2*B*). This finding indicates that after removal of the *neo*^{r} gene, the inserted Eμ can function in the absence of Eβ to stimulate endogenous TCRβ gene rearrangements. However, we did not detect DβJβ rearrangements in B cells by Southern blot analyses (Fig. 2*B*), although very low levels could be observed in DNA from EβRΔ B cells by PCR (data not shown). Thus, insertion of Eμ does not promote substantial rearrangement of the TCRβ locus in developing B cells.

Vβ14 Rearrangement Is Inhibited on the EβKO Allele and Restored on the EβRΔ Allele. To determine which allele of the mutated T cells was actually rearranged, we designed a PCR assay to detect Vβ14Dβ1Jβ rearrangements on the mutated and wild-type alleles (Fig. 3*A*). Amplification of heterozygous mutant ES cell DNA with primers Vβ-510 and 5′Eβ confirmed that both types of nonrearranged, mutated alleles and nonrearranged wild-type alleles can be detected and discriminated both by size and by differential hybridization to a *loxP* versus a Vβ14 probe (Fig. 3*B*, lanes 1 and 5). Vβ14-to-Dβ1Jβ rearrangements were detected by a PCR using primers 5′Eβ and 5′Dβ1 with ES cell DNA (Fig. 3*B*, lanes 2 and 6) and wild-type thymus DNA (Fig. 3*B*, lane 4) serving, respectively, as negative and positive controls. The PCR on EβRΔ thymus DNA generated two bands of different sizes due to the replacement of the endogenous 500-bp Eβ motif by the 700-bp Eμ fragment, with the smaller product corresponding to the wild-type allele and the other (which hybridized with both Vβ14–810 and *loxP* probes) corresponding to the mutated allele (Fig. 3*B*, lane 3).

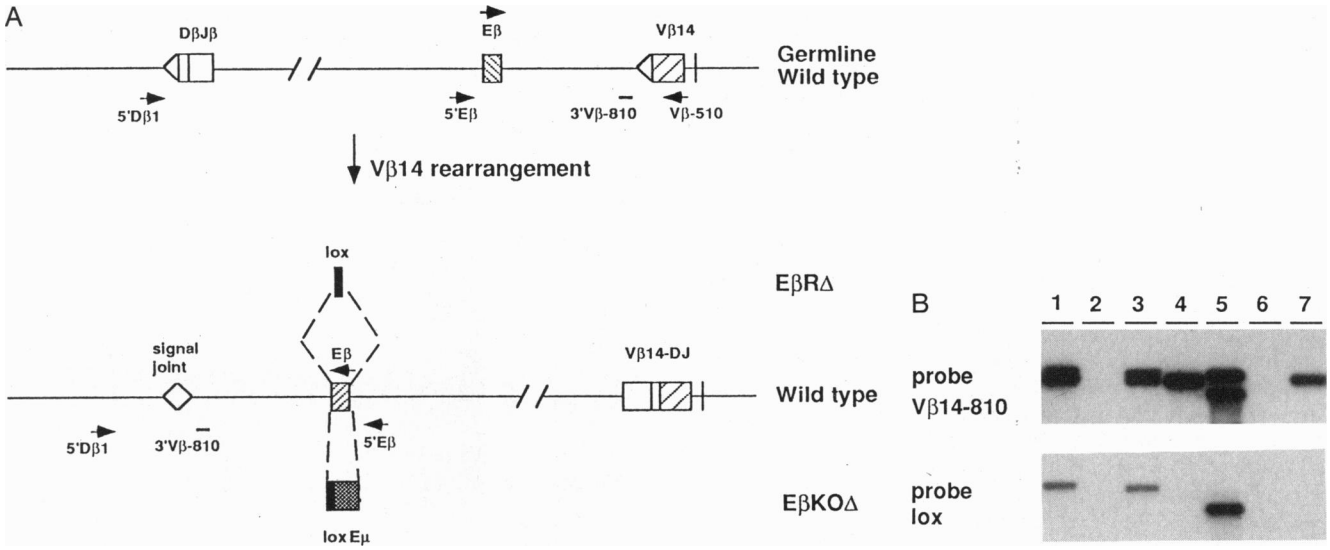

FIG. 3. PCR analysis of Vβ14-to-DβJβ rearrangements. (*A*) Representation of the TCRβ locus including the Vβ14 gene before and after recombination by inversion on wild-type, EβRΔ, or EβKOΔ alleles. Recombination sequences are represented by open triangles, Dβ and Jβ segments by open boxes, Vβ14 by hatched boxes, enhancers by filled boxes, and *loxP* sequences by bold boxes. PCR with primers 5′Eβ and Vβ-510 (arrows) amplified the nonrearranged Vβ14–Eβ region of wild-type and mutated alleles. After Vβ14 rearrangement, primers 5′Eβ and 5′Dβ1 face each other and amplify a product that includes the signal joint (diamond) and the wild-type or mutated Eβ region. Oligo-probes 3′Vβ-810 (thick line) and *loxP* were used to differentiate the products amplified from wild-type or mutated alleles. (*B*) PCR analysis of Vβ14 rearrangements. PCR products generated by amplification of EβRΔ, EβKOΔ, and B6CBA (wild-type) DNA were assayed by hybridization with oligo-probes Vβ14–810 (*Upper*) or *lox* (*Lower*). Lanes: 1, amplification of EβRΔ ES cell DNA with primers Vβ14–510 and 5′Eβ; 2, amplification of EβRΔ ES cell DNA with primers 5′Eβ and 5′Dβ1; 3, amplification of EβRΔ thymocyte DNA with primers 5′Eβ and 5′Dβ1; 4, amplification of B6CBA thymocyte DNA with primers 5′Eβ and 5′Dβ1; 5, amplification of EβKOΔ ES cell DNA with primers Vβ14–510 and 5′Eβ; 6, amplification of EβKOΔ ES cell DNA with primers 5′Eβ and 5′Dβ1; 7, amplification of EβKOΔ thymocyte DNA with primers 5′Eβ and 5′Dβ1.

Amplification of EβKO thymus DNA resulted in only one band similar in size to the one detected in the wild-type control. Moreover, this PCR product hybridized with the Vβ14–810 probe and not with the *loxP* probe, demonstrating that detected rearrangements occurred on the wild-type allele (Fig. 3*B*, lane 7). For both mutations, the same results were obtained when rearrangements of Vβ14 to Dβ2Jβ were assayed (data not shown). These results confirm that EβKO T cells did not rearrange the Vβ14 segment on the mutated allele and that VDJβ recombination is, at least partially, restored on the targeted allele of EβRΔ T cells.

Transcription of the Nonrearranged TCRβ Allele in EβKOΔ T-Lineage Cells. To assay for transcription of the germ-line TCRβ locus, we designed a quantitative reverse transcriptase-PCR assay using primers located 5′ of Jβ1 and in the Cβ regions (Fig. 4*A*). No transcripts were found in RNA from nonlymphoid cells (Fig. 4, lane 1) or B cells (Fig. 4, lane 7). In accord with previous findings (30), RAG2$^{-/-}$ thymocytes (which are CD4$^-$/CD8$^-$ with germ-line TCRβ alleles) have readily detectable levels of germ-line TCRβ transcripts (Fig. 4, lane 2), whereas wild-type thymocytes do not (Fig. 4, lane 1) because they generally have rearranged both Jβ alleles. Thymuses from RAG-2$^{-/-}$ mice complemented with a TCRβ transgene contain large numbers of CD4$^+$/CD8$^+$ thymocytes with germ-line TCRβ loci (25, 26) and generate substantial levels of germ-line transcripts (Fig. 4*B*; lanes 3–6 represent successive 10-fold dilutions). However, the level of germ-line transcripts in EβKO thymocytes (which are predominantly CD4$^+$/CD8$^+$ cells) was at least 1000-fold lower (Fig. 4*B*; compare lanes 6 and 8). Assuming that the EβKO CD4$^+$/CD8$^+$ thymocytes have a productive V(D)J rearrangement of their wild-type allele and no rearrangement of the mutant allele, these findings indicate that the Eβ enhancer is required for efficient transcription of nonrearranged TCRβ alleles.

The TCR Locus Is Transcribed in EβRΔ-Derived B Cells. The Eμ element can activate transcription and rearrangement of the Dβ and Jβ segments of a transgenic mini-locus recombination substrate in both B- and T-lineage cells (7). To determine whether Eμ could similarly activate transcription of the endogenous TCRβ locus in B-lineage cells, we assayed total RNA from EβRΔ splenic B cells for hybridization to a constant-region β chain (Cβ) probe (Fig. 5). Strikingly, an approximately 1-kb Cβ-hybridizing transcript was readily detectable in RNA from EβRΔ B cells (Fig. 5, lane 2) and RAG-2$^{-/-}$ thymocytes (Fig. 5, lane 3) but was not detectable in that from wild-type B cells (Fig. 5, lane 1). This finding indicates that replacement of Eβ with Eμ can activate germline transcription of the TCRβ locus in B lymphocytes.

DISCUSSION

Gene targeting provides a powerful tool for analyzing enhancer function in the regulation of V(D)J recombination (12–14). In this context, a "hit and run" strategy was used to delete Eμ without leaving exogenous DNA in the mutated locus (12). Such an approach is important because insertion of expressed genes, such as *neo*^{r}, may influence expression and rearrangement of targeted loci (12, 13). In the present study, we have used the cre-*loxP* system to excise the inserted *neo*^{r} gene from the targeted locus, leaving only a small, presumably inert, inserted sequence. We have used this approach to analyze effects of both Eβ deletion and replacement of Eβ with Eμ on expression and rearrangement of the TCRβ locus.

We found that Eβ is essential for efficient TCRβ gene rearrangement, as its specific deletion reduced D-to-Jβ rearrangements on the targeted allele by >95%. This dramatic inhibition contrasts with the effects of targeted deletion of the Eμ element, which only reduced J_H rearrangement by 20–50% (12). The different effects of Eμ and Eβ deletions on their respective loci suggest that additional elements act with Eμ to promote heavy-chain joining region rearrangements, whereas Eβ is the primary element involved in activation of Jβ rearrangement. Although we did observe low-frequency D-to-Jβ joinings in the absence of Eβ, we did

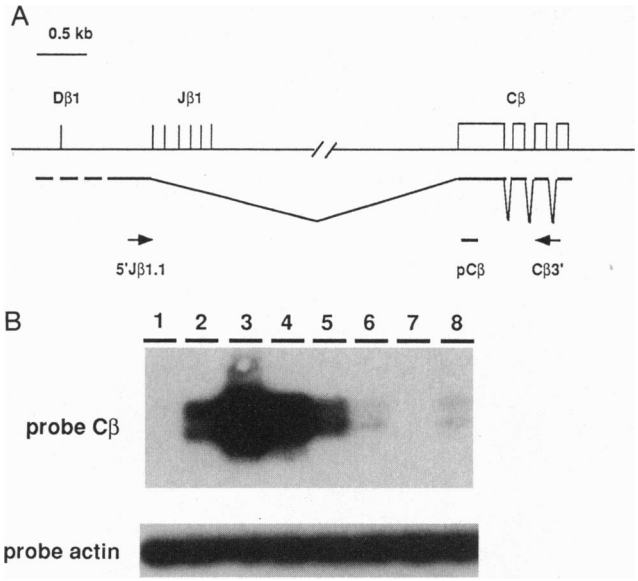

FIG. 4. Reverse transcriptase-PCR analysis of TCRβ locus germline transcription. (*A*) Representation of the reverse transcriptase-PCR assay used to detect germ-line JβCβ transcripts. Dβ and Jβ segments are represented by thin bars and Cβ exons are indicated by open boxes. The uncharacterized start site is shown by a dotted line; the actual transcript is shown by a thick line. Positions of the oligonucleotides are indicated by arrows (PCR primers) or by short lines (oligonucleotide probe). The amount of cDNA was standardized by amplification of actin transcripts. (*B*) Analysis of PCR products. RNA from the following sources were reverse-transcribed and amplified. Lanes: 1, wild-type B6CBA thymocyte cDNA; 2, RAG-$2^{-/-}$ thymocyte cDNA; 3, RAG-$2^{-/-}$ transgenic β-chain thymocyte cDNA; 4–6, 10^{-1}, 10^{-2}, and 10^{-3} dilutions of RAG$2^{-/-}$ transgenic β-chain cDNA into B-cell cDNA, respectively; 7, B-cell cDNA; 8, EβKOΔ thymocyte cDNA. The membrane was first hybridized with the ^{32}P-labeled pCβ oligonucleotide (*Upper*) and then stripped and hybridized with the labeled pAct oligonucleotide (*Lower*).

not detect VβDJβ rearrangements on the Eβ-deleted allele, which suggests either that Eβ is required for Vβ-to-DJβ rearrangement, that the D-to-Jβ rearrangement step is rate limiting (or both).

Previous analyses of transgenic recombination substrates implicated transcriptional enhancers in the tissue-specific control of V(D)J recombination (7–11). Our current studies

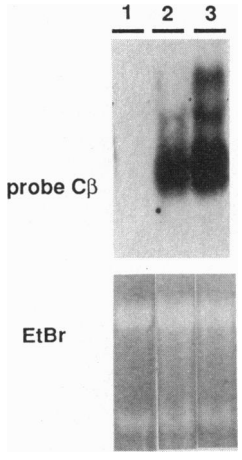

FIG. 5. Northern blot analysis of TCRβ transcripts. Approximately 10 μg each of total RNA from wild-type B6CBA B cells (lane 1), EβRΔ B cells (lane 2), and RAG-2-deficient thymocytes (lane 3) was fractionated on a 1% formaldehyde–agarose gel and assayed for hybridization with a Cβ cDNA probe (24). The amount of RNA was estimated by ethidium bromide staining (*Lower*).

showed that in T-lineage cells, Eμ (in the absence of Eβ) maintained germ-line transcription of the unrearranged Jβ locus and activated Dβ-to-Jβ and Vβ-to-DJβ rearrangements in cis. These results indicate that Eβ and Eμ share elements that can activate the endogenous TCRβ locus. We also found that the ability of Eμ to promote rearrangement of the endogenous TCRβ locus was inhibited by inclusion of a linked *neo*^{r} gene. By analogy to other systems, the promoter of the inserted *neo*^{r} gene may exert an inhibitory effect by competing for the activity of the enhancer (27, 28).

It is notable that replacement of Eβ by Eμ activates constant-region β chain (Cβ) transcription but does not activate Dβ-to-Jβ rearrangement in B-lineage cells. While it remains to be determined whether transcription is activated in progenitor B cells at the point where rearrangement occurs, this result is reminiscent of the finding that enhancer-promoted activation of transcription does not activate Vβ-to-DJβ rearrangement in a transgenic recombination mini-locus (8, 9). However, the result contrasts with those obtained with transgenic TCR VβDβJβ mini-locus recombination substrates in which Eμ and Eβ both promoted D-to-Jβ rearrangement in B and T cells (7–9). One possible explanation for this difference is that additional negative elements, which could modulate rearrangement activity in B cells, may be present in the endogenous TCRβ locus but not in the mini-locus. Identification of such putative elements should further elucidate mechanisms that regulate V(D)J recombinational accessibility.

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