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Productive Coupling of Accessible Vβ14 Segments and DJβ Complexes Determines the Frequency of Vβ14 Rearrangement[1]

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Abstract

To elucidate mechanisms that regulate Vβ rearrangement, we generated and analyzed mice with a V(D)J recombination reporter cassette of germline Dβ-Jβ segments inserted into the endogenous Vβ14 locus (*Vβ14*^{Rep}). As a control, we first generated and analyzed mice with the same Dβ-Jβ cassette targeted into the generally expressed c-*myc* locus (c-*myc*^{Rep}). Substantial c-*myc*^{Rep} recombination occurred in both T and B cells and initiated concurrently with endogenous Dβ to Jβ rearrangements in thymocytes. In contrast, Vβ14^{Rep} recombination was restricted to T cells and initiated after endogenous Dβ to Jβ rearrangements, but concurrently with endogenous Vβ14 rearrangements. Thus, the local chromatin environment imparts lineage and developmental stage-specific accessibility upon the inserted reporter. Although Vβ14 rearrangements occur on only 5% of endogenous TCRβ alleles, the Vβ14^{Rep} cassette underwent rearrangement on 80–90% of alleles, supporting the suggestion that productive coupling of accessible Vβ14 segments and DJβ complexes influence the frequency of Vβ14 rearrangements. Strikingly, Vβ14^{Rep} recombination also occurs on TCRβ alleles lacking endogenous Vβ to DJβ rearrangements, indicating that Vβ14 accessibility per se is not subject to allelic exclusion.

During lymphocyte development, TCR and Ig V region exons are assembled from germline V, D, and J segments. V(D)J recombination is initiated by the lymphocyte-specific RAG 1 and 2 proteins, which introduce DNA double strand breaks between a pair of participating gene segments and their flanking recombination signal sequences (RSs),[4] with the RAG-

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generated ends then being joined by the nonhomologous end joining pathways to complete the process (1). Chromosomal V(D)J recombination is regulated within the contexts of lineage specificity, developmental stage specificity, and allelic exclusion via modulation of accessibility of participating V, D, and J gene segments to the RAG endonuclease (2, 3). However, despite intense efforts, much remains to be learned about the molecular mechanisms that determine V(D)J recombinational accessibility and the factors that influence the choice of particular gene segments for recombination (4, 5).

TCR V region exons are assembled in a highly regulated fashion during $\alpha\beta$ T cell development (3, 6). In $CD4^-/CD8^-$"double-negative" (DN) thymocytes, TCRβ V region exons are assembled in an ordered fashion with Dβ to Jβ joining initiating in $CD44^+/CD25^+$ stage II DN thymocytes before Vβ rearrangement to a preassembled DJβ complex in $CD44^-/CD25^+$ stage III DN thymocytes (7). TCRβ locus Dβ to Jβ rearrangements occur on both alleles, while the Vβ to DJβ rearrangement step is thought to occur on one allele at a time (3, 8, 9). Following the assembly and expression of in-frame (productive) VβDJβ rearrangements on the first allele, further Vβ to DJβ rearrangements on the second allele are prevented via feedback regulation to enforce *TCR*β locus allelic exclusion (3, 8, 9). However, Vβ to DJβ rearrangements can occur on the second allele following assembly of out-of-frame (nonproductive) VβDJβ rearrangements on the first allele (3, 8, 9). Expression of productive VβDJβ rearrangements in DN thymocytes also signals differentiation into $CD4^+/CD8^+$"double-positive" (DP) thymocytes and initiation of Vα to Jα rearrangements (6). The assembly and expression of productive VαJα rearrangements leads to cell surface expression of $\alpha\beta$ TCRs that signal differentiation into $CD4^+$ or $CD8^+$"single-positive" thymocytes, which exit the thymus as $\alpha\beta$ T cells.

The molecular mechanisms that direct the assembly of endogenous TCRβ V region exons have not been elucidated. Ordered TCRβ rearrangement is likely mediated by developmental stage-specific modulation of Vβ, Dβ, and Jβ recombinational accessibility, intrinsic properties of the participating RSs, and other chromosomal factors such as distance (10–12). In addition, Vβ rearrangement likely involves factors that actively promote the physical juxtaposition of RAG-accessible Vβ/Dβ RSs separated across large chromosomal distances (4, 5). Specific replacement of the endogenous Vβ14 RS with the 3′Dβ1 RS, which possesses a 5- to 10-fold higher intrinsic ability to recombine with 5′Dβ RSs (13), led to a corresponding increase in the frequency of primary Vβ14 to DJβ rearrangements (11). These findings led to the suggestion that this particular RS replacement enhances the likelihood that juxtaposed Vβ14 segments and DJβ complexes generate productive synaptic complexes by increasing RAG binding to the Vβ14 RS and/or by increasing RAG-mediated cleavage. In the context of this interpretation, it was suggested that endogenous Vβ14 segments may actually be juxtaposed with DJβ complexes much more frequently than they rearrange (11), further implying that Vβ14 segments also may be recombinationally accessible in a much higher percentage of developing $\alpha\beta$ T cells than the frequency with which they rearrange to DJβ complexes.

[4]Abbreviations used in this paper: RSs, recombination signal sequence; DN, double negative; ES, embryonic stem; WT, wild type; RDBC, RAG2-deficient blastocys complementation.

To test the notion that Vβ14 segments become recombinationally accessible much more frequently than they rearrange, we wished to directly monitor RAG access to the endogenous *Vβ14* locus, rather than use correlative measures of V(D)J recombinational accessibility, such as active germline transcription, nuclease sensitivity, open chromatin structure, or expression of an inserted reporter gene (14–17). For this purpose, we have developed a V(D)J recombination reporter cassette and assessed its ability to rearrange when inserted in place of the endogenous Vβ14 RS.

Materials and Methods

Generation of targeting constructs and probes

The Vβ14^{Rep} targeting vector was constructed in pLNTK using a 2.3-kb *Nde*I fragment for the 5′ homology arm and a 2.4-kb *Nde*I-*Sph*I fragment containing Vβ14^{Rep} for the 3′ homology arm. Vβ14^{Rep} was created by first replacing the 236-bp *Nde*I-*Bgl*I fragment containing the Vβ14 RS with an PCR product that amplified genomic sequence between the *Nde*I site and the Vβ14 RS and also introduced *Eco*RI and *Bgl*II sites just inside the *Nde*I site and a *Cla*I site just inside of the *Bgl*I site. The 582-bp *Acc*I-*Eco*RV fragment spanning the 5′Dβ1 RS and just 3′ of Jβ1.1 was blunt-end ligated into this *Cla*I site. The 5′Vβ14 probe is a 1.4-kb *Pst*I-*Nde*I fragment. The 3′Vβ14 probe is a 0.7-kb *Sph*I-*Hin*dIII fragment. The CWΔP probe is a 1.5-kb *Hin*dIII fragment. The c-*myc*^{Rep} targeting vector was constructed in pLNTK using a 4.5-kb *Sph*I genomic fragment for the 5′ homology arm and a 3.0-kb *Sph*I genomic fragment for the 3′ homology arm. The 762-bp *Acc*I-*Eco*RV fragment containing Dβ1 and Jβ1.1 was inserted at the unique *Dra*III site in the 5′ homology arm using blunt-end ligation. The c-*myc*A probe is a 1.5-kb *Xba*I fragment. The c-*myc*B probe is a 1.6-kb *Xho*I-*Kpn*I fragment; the c-*myc*D probe is a 700-bp *Xho*I-*Bam*H1 fragment.

Gene targeting and generation of embryonic stem (ES) cells

The Vβ14^{Rep} targeting vector was electroporated into Jβ1$^{\omega/\omega}$ES cells (18), while the c-*myc*^{Rep} targeting vector was electroporated into TC1 ES cells as described (19) to generate c-*myc*^{RepNeo} and Vβ14^{RepNeo} ES cells, respectively. c-*myc*^{RepNeo} clones were identified by Southern blotting using the 5′ probe on EcoR1-digested DNA, and confirmed with the 3′ probe (c-*myc*$^{+}$, 20 kb; c-*myc*^{RepNeo}, 12 kb). Vβ14^{RepNeo} clones were identified by Southern blot analysis with the Vβ14 5′ probe on *Bam*HI-digested DNA (Vβ14$^{\omega}$, 18 kb; Vβ14^{RepNeo}, 8 kb) and confirmed with the Vβ14 3′ probe on *Eco*RI-digested DNA (Vβ14$^{\omega}$, 5 kb; Vβ14^{RepNeo}, 4.24 kb) The *pgk-Neo*^{r} gene was removed from independently targeted clones by infection with AdenoCre- and Cre-deleted clones identified by Southern blot analysis of *Bgl*II-digested DNA with the CWΔP probe (Vβ14$^{\omega}$, 3.6 kb; Vβ14^{RepNeo}, 4 kb; Vβ14^{Rep}, 2 kb).

Generation of mice and lymphocytes

Vβ14$^{Rep/\omega}$ lymphocytes were generated through RAG2-deficient blastocyst complementation (RDBC) as described (20). Germline Vβ14$^{Rep/WT}$ and Vβ14$^{Rep/Rep}$ mice obtained from breeding RDBC-derived chimeric mice with 129SvEv mice. c-*myc*^{RepNeo} ES cells were injected into C57BL6 blastocyts to generate chimeras for germline transmission

of the c-*myc*^{RepNeo} allele. Chimeras were bred to 129SvEv mice to generate c-*myc*^{RepNeo} mice. c-*myc*^{RepNeo} mice were bred to E2A-Cre-transgenic mice (21) to remove the *pgk-Neo*^{r} gene. c-*myc*^{Rep} mice were identified by Southern blot analysis of *Xho*I-digested DNA and hybridization to the c-*myc*D probe (5.5-kb c-*myc*^{Rep}; 7.5-kb c-*myc*$^{RepNeo;}$ 4.7-kb c-*myc*$^{+}$). Resulting c-*myc*^{Rep} mice were further bred to 129SvEv mice to outcross the E2A-Cre transgene, which was verified by PCR using primers 5′-CCTGGAAAATGCTTCTGTC CG-3′ and 5′-CAGGGTGTTATAAGCAATCCC-3′ specific for the *Cre* gene. These studies have been reviewed and approved by the Institutional Animal Care and Use Committees of Boston Children's Hospital and the Children's Hospital of Philadelphia.

Flow cytometry analysis and cell sorting

Thymocytes and splenocytes were stained with PE-conjugated anti-CD4 and FITC-conjugated anti-CD8 or PE-conjugated anti-TCRβ and FITC-conjugated anti-Vβ14 (BD Pharmingen). FACS data acquisition and analysis was performed on a FACSCalibur flow cytometer using CellQuest software (BD Biosciences). Cell sorting of DNII and DNIII thymocytes was performed using a MoFlo cell sorter (DakoCytomation) following staining of CD4-depleted thymocytes (Miltenyi Biotec) with FITC-conjugated anti-CD8α, FITC-conjugated anti-CD4, FITC-conjugated anti-TCRβ, FITC-conjugated anti-B220, FITC-conjugated anti-TCR$\gamma\delta$, PE-conjugated anti-CD25, and CYC-conjugated anti-CD44.

PCR analysis of rearrangements

Vβ14^{Rep} Dβ to Jβ rearrangements were detected by PCR using the 5′Vβ14 5′-TGTCTTTGGTGACTTCTGACTTG-3′ and Jβ1.1 5′-CAACGTG AGTCTGGTTCCTTTACC-3′ primers and probed with the P1 primer 5′-CCTACAACTGTGAGTCTGGTTCCTTTACC-3′. Endogenous *TCR*β locus Dβ to Jβ rearrangements were detected by PCR as previously described (18). c-*myc*^{Rep} Dβ to Jβ rearrangements were detected by PCR using the 5′c-*myc* 5′-GAAGACTGCGGTGAGTCGTGATCT-3′ and Jβ1.1 primers and probed with the P1 primer. For sequence analysis, PCR products representing Vβ14^{Rep} or endogenous *TCR*β locus Dβ to Jβ1 rearrangements were cloned into the pGEM T-Easy vector and then sequenced with either the T7 or Sp6 primers. For seminested PCR analysis of Vβ14^{Rep} or endogenous *TCR*β locus Dβ to Jβ rearrangements, the second PCR were conducted with the 5′Vβ14a 5′-AAATCAAGCCCTAAC CTCTAC-3′ and the Jβ1.1 primer (Vβ14^{Rep}) or with the 5′ primer 5′-TGTCTTTGGTGACTTCTGACTTG-3′ and the P2 primer 5′-CCT GACTTCCACCCGAGGTT-3′ (endogenous), using the first PCR product as template and 32 cycles of amplification.

Southern analysis of rearrangements in hybridomas

T cell hybridoma clones were produced by fusion of Con A and IL-2-stimulated T cells with the thymoma cell line BW-1100.129.237 as described (19). B cell hybridoma clones were produced by fusion of LPS stimulated splenocytes with the myeloma cell line NS1 as described (22). TCR $\alpha\beta^{+}$ and Vβ14^{+} T cell hybridomas were selected by flow cytometry and IgM^{+} B cell hybridomas were selected by ELISA for further analysis. Genomic DNA was isolated, digested with *Eco*RI, and analyzed by PCR or Southern blotting and hybridization with the following probes: the 3′-J_H4 probe—a 1.5-kb *Hin*dIII-*Eco*R1

fragment, the 3′-Jβ1 probe—a 0.777-kb *Drd*1 fragment, the VDJβ probe—a 0.743-kb *Afl*III-*Hae*III fragment, the 5′-Vβ14 probe, the 3′-Vβ14 probe, the CWΔP probe, and the DJβ1 probe amplified with primers 5′-AATCTTAAGGGGTGAAGAGAGG-3′ and 5′-ATTCTGTCTGTCCCAAGGCCC-3′.

Results

The Dβ-Jβ cassette functions as reporter of V(D)J recombinational accessibility

Within the context of a TCRβ minilocus (TCRβ^{PF}), Dβ1 to Jβ1.1 rearrangement in developing lymphocytes is dependent upon the presence of transcriptional elements, but occurs independent of TCRβ^{PF} chromosomal integration site (10, 23–25); while, despite the presence of a TATA box in the 5′Dβ1 RS, germline Dβ1/Jβ1 transcription and Dβ1 to Jβ1 rearrangement are both absolutely dependent upon the upstream pDβ1 promoter (25–30). Thus, we reasoned that insertion of a germline Dβ1 and Jβ1.1 genomic fragment lacking the pDβ1 promoter (the Dβ-Jβ cassette) into particular genomic loci could be used as a reporter to directly monitor V(D)J recombinational accessibility of the local chromatin environment by assaying Dβ-Jβ cassette rearrangements throughout lymphocyte development. If so, recombination of the Dβ-Jβ cassette inserted into a generally transcribed locus should occur in both B and T lymphocytes and initiate concurrently with endogenous Dβ to Jβ rearrangements in DNII thymocytes when *Rag1*/*Rag2* are first expressed. The c-*myc* locus is transcribed throughout B and T cell development (31, 32). Thus, to test this notion, we used Cre-*loxP*-mediated gene targeting to generate mice with germline Dβ1 and Jβ1.1 segments and their RSs inserted into the first intron of the c-*myc* locus (the c-*myc*^{Rep} mutation) (Fig. 1*A*) on a single allele (c-*myc*$^{Rep/WT}$ mice). As expected, lymphocyte development was indistinguishable between c-*myc*$^{Rep/WT}$ and wild-type (WT) mice (data not shown).

To characterize developmental stage specificity of c-*myc*^{Rep} recombination, we analyzed c-*myc*^{Rep} Dβ to Jβ rearrangements and endogenous TCRβ locus Dβ to Jβ rearrangements by PCR in sort-purified DNII and DNIII thymocytes. PCR with a primer that hybridizes to c-*myc* sequences just upstream of the inserted Dβ-Jβ cassette (the 5′c-*myc* primer) and a primer that hybridizes to sequences within the Jβ1.1 segment (the Jβ1.1 primer) (Fig. 1*B*) should specifically amplify an 850-bp product from the germline c-*myc*^{Rep} allele and a 200-bp product from potential Dβ to Jβ rearranged c-*myc*^{Rep} alleles (Fig. 1*B*). Endogenous TCRβ locus Dβ1 to Jβ1.1 and Dβ1 to Jβ1.2 rearrangements can be detected by PCR using a primer that hybridize 5′ of Dβ1 and 3′ of Jβ1.2(18). PCR products corresponding to c-*myc*^{Rep} Dβ1 to Jβ1.1 rearrangements and endogenous *TCR*β locus Dβ1 to Jβ1.1 rearrangements were detectable using genomic DNA isolated from DNII and DNIII thymocytes (Fig. 1*C*). Notably, the ratio of the level of c-*myc*^{Rep} Dβ1 to Jβ1.1 rearrangements in DNII cells compared with DNIII cells (0.4) was similar to the ratio of the level of endogenous Dβ1 to Jβ1.1 rearrangements in DNII cells compared with DNIII cells (0.35). We cloned and sequenced 20 of these 200-bp PCR products and found that they were c-*myc*^{Rep} DJβ1.1 joins indistinguishable from similarly obtained endogenous TCRβ locus DJβ1.1 joins (data not shown), demonstrating that c-*myc*^{Rep} undergoes bona fide V(D)J recombination. Thus, as expected, c-*myc*^{Rep} recombination initiates concurrently with *Rag1*/*Rag2* expression in developing thymocytes.

To evaluate whether c-*myc*^{Rep} recombination occurs at a substantial level in B and T lineage cells, we conducted Southern blot analysis with the myc D probe on *Sac*I-digested genomic DNA isolated from the kidney, thymus, ConA/IL-2-stimulated T cells, and LPS-stimulated B cells of c-*myc*$^{Rep/WT}$ and c-*myc*$^{Rep/Rep}$ mice. The myc D probe hybridizes to a 1.6-kb *Sac*I fragment on the c-*myc*^{WT} allele, a 2.4-kb *Sac*I fragment on the unrearranged c-*myc*^{Rep} allele, and a 1.8 kb *Sac*I fragment on potential Dβ1 to Jβ1.1 rearranged c-*myc*^{Rep} alleles due to the deletion of sequences between Dβ1 and Jβ1.1 (Fig. 1*A*). We observed no rearrangement in c-*myc*$^{Rep/WT}$ and c-*myc*$^{Rep/Rep}$ kidneys, as indicated by the absence of the 1.8-kb band (Fig. 1*D*). However, we found c-*myc*^{Rep} Dβ to Jβ rearrangement in c-*myc*$^{Rep/WT}$ and c-*myc*$^{Rep/Rep}$ thymocytes, T cells, and B cells, as indicated by the presence of the 1.8-kb *Sac*I fragment and a lessened intensity of the 2.4-kb *Sac*I fragment. The ratios of the 1.8-kb rearranged to 2.4-kb rearranged band in c-*myc*$^{Rep/WT}$ and c-*myc*$^{Rep/Rep}$ tissues suggest that ~50% of c-*myc*^{Rep} alleles rearrange in B and T cells (Fig. 1*D*).

To precisely quantify the level of c-*myc*^{Rep} recombination that occurs in developing B and T cells, we generated clonal $\alpha\beta$ T cell and B cell hybridomas and assayed for c-*myc*^{Rep} Dβ to Jβ rearrangements by conducting PCR on their genomic DNA using the 5′myc and Jβ1.1 primers. In c-*myc*$^{Rep/WT}$ $\alpha\beta$ T cell hybridomas, c-*myc*^{Rep} Dβ to Jβ rearrangements occurred on 76 of 113 (67%) alleles, while 37 of 113 (33%) contained unrearranged c-*myc*^{Rep} alleles (Table I). The analysis of c-*myc*$^{Rep/Rep}$ $\alpha\beta$ T cell hybridomas revealed that 33 of 104 (32%) contained c-*myc*^{Rep} rearrangements on both alleles, 57 of 104 (54%) contained c-*myc*^{Rep} rearrangements on a single allele, and 14 of 104 (14%) contained two unrearranged c-*myc*^{Rep} alleles (Table II). The same analysis of c-*myc*$^{Rep/WT}$ B cell hybridomas revealed that 26 of 60 (43%) alleles contained rearranged c-*myc*^{Rep}, while 34 of 60 (56.6%) alleles remained unrearranged (Table I). In addition, the analysis of homozygous c-*myc*$^{Rep/Rep}$ B cell hybridomas demonstrated that 9 of 92 (10%) contained c-*myc*^{Rep} rearrangements on both alleles, 49 of 92 (53%) contained c-*myc*^{Rep} rearrangements on one allele, and 34 of 92 (37%) harbored no rearrangements (Table II). Although we find a lower level of rearrangement in B cell hybridomas as compared with T cell hybridomas, these data indicate that the c-*myc*^{Rep} cassette is RAG accessible in the majority of developing B and $\alpha\beta$ T cells. Notably, the rearrangement levels observed make the Dβ-Jβ cassette a readily discernable marker of a chromosomal locus that is accessible for V(D)J recombination and allow an estimate of the minimal level of recombinational accessibility.

Vβ14^{Rep} recombination mirrors endogenous Vβ14 rearrangement

After validating that the Dβ-Jβ cassette can serve as a marker of V(D)J recombinational accessibility, we proceeded to test whether Vβ14 segments become recombinationally accessible in developing $\alpha\beta$ T cells at a similar frequency to which they rearrange. For this purpose, we used Cre-*loxP*-mediated gene targeting to replace the endogenous Vβ14 RS with the Dβ-Jβ cassette (Fig. 2*A*) on a single TCRβ allele of Jβ1$^{\omega/\omega}$ ES cells to generate Vβ14$^{Rep/\omega}$ ES cells. The Jβ1$^{\omega}$ locus lacks the endogenous Dβ2-Jβ2 locus, so that all TCRβ rearrangements involve Dβ1-Jβ1 segments, but otherwise its rearrangement is indistinguishable from the WT TCRβ locus and it supports normal $\alpha\beta$ T cell development (18). The gene targeting also introduced a single *loxP* site and a unique *Eco*RI site to distinguish between Vβ14^{Rep} and endogenous Vβ14 rearrangements (Fig. 2*A*). Due to

deletion of the endogenous Vβ14 RS and the orientation of the inserted Dβ1 and Jβ1.1 RSs, Vβ14^{Rep} alleles are not capable of assembling productive Vβ14DJβ1 rearrangements. We used Vβ14$^{Rep/\omega}$ ES cells and RAG2-deficient blastocyst complementation (RDBC) (20) to generate chimeric mice with Vβ14$^{Rep/\omega}$ lymphocytes and bred these mice with 129SvEv (WT) mice to establish germline Vβ14$^{Rep/WT}$ and Vβ14$^{Rep/Rep}$ mice. Flow cytometric analysis of thymocytes and peripheral lymphocytes isolated from WT, Jβ1$^{\omega/\omega}$, Vβ14$^{Rep/\omega}$, Vβ14$^{Rep/WT}$, and Vβ14$^{Rep/Rep}$ mice demonstrated that the Vβ14^{Rep} allele had no discernable effect on gross $\alpha\beta$ T cell development (Fig. 2*B*). However, as expected, thymocytes and splenocytes isolated from Vβ14$^{Rep/Rep}$ mice completely lack cell surface expression of Vβ14 (Fig. 2*B*).

To evaluate whether Vβ14^{Rep} recombination occurs in Vβ14$^{Rep/\omega}$ lymphocytes, we first conducted PCR on genomic DNA isolated from Vβ14$^{Rep/\omega}$ ES cells and Vβ14$^{Rep/\omega}$ thymocytes using a primer that hybridizes to TCRβ locus sequences just 5′ of the inserted Dβ-Jβ cassette (the 5′Vβ14 primer) and the Jβ1.1 primer (Fig. 3*A*). This primer pair should specifically amplify an 850-bp product from the germline Vβ14^{Rep} allele and a 200-bp product from potential Dβ to Jβ rearranged Vβ14^{Rep} alleles (Fig. 3*A*). PCR products the expected sizes for both germline and Dβ to Jβ rearranged Vβ14^{Rep} alleles were amplified from Vβ14$^{Rep/\omega}$ thymocytes (Fig. 3*B*), while only PCR products corresponding to germline Vβ14^{Rep} alleles were amplified from Vβ14$^{Rep/\omega}$ ES cell genomic DNA (Fig. 3*B*). We cloned and sequenced 18 of these 200-bp PCR products and found that they were Vβ14^{Rep} DJβ1.1 joins indistinguishable from similarly obtained endogenous TCRβ locus DJβ1.1 joins (data not shown), demonstrating that Vβ14^{Rep} also undergoes bona fide V(D)J recombination.

To determine whether Vβ14^{Rep} recombination occurs at a substantial level in T and B lineage cells, we next conducted Southern blot analysis on *EcoR*I-digested genomic DNA isolated from Vβ14$^{Rep/\omega}$ ES cells, Vβ14$^{Rep/WT}$ thymocytes, and Vβ14$^{Rep/Rep}$ thymocytes, and Vβ14$^{Rep/WT}$ B cells. The 3′Vβ14 probe hybridizes to a 5.0-kb *Eco*RI fragment from germline Jb$^{\omega}$ and WT alleles, a 4.3-kb fragment from germline Vβ14^{Rep} alleles, and a 3.6-kb *Eco*RI fragment from Dβ to Jβ rearranged Vβ14^{Rep} alleles due to deletion of the nucleotides between Vβ14^{Rep} Dβ1 and Jβ1.1 segments (Fig. 2*A*). Southern blot analysis revealed the presence of 5.0-, 4.3-, and 3.6-kb bands in *Eco*RI-digested genomic DNA isolated from Vβ14$^{Rep/WT}$ and Vβ14$^{Rep/Rep}$ thymocytes, but only 5.0-and 3.6-kb bands in *Eco*RI-digested Vβ14$^{Rep/WT}$ B cell DNA (Fig. 3*C*). The ratios of the intensities of the 4.3- and 3.6-kb bands in Vβ14$^{Rep/WT}$ and Vβ14$^{Rep/Rep}$ thymocytes demonstrates that a substantial fraction of Vβ14^{Rep} alleles recombined, indicating that the *Vβ14* locus becomes recombinationally accessible in a higher percentage of thymocytes than Vβ14 rearrangement occurs. These data also reveal that Vβ14^{Rep} recombination occurs in T, but not B, lymphocytes, mirroring the lineage-specific pattern of endogenous Vβ14 rearrangement. Critically, these data demonstrate that insertion of the Dβ-Jβ segments and their RSs into the *Vβ14* locus does not promote recombinational accessibility in B cells. Thus, local chromatin environment imparts lineage-specific recombinational accessibility upon the inserted reporter.

To determine the developmental stage in which Vβ14^{Rep} recombination initiates, we analyzed Vβ14^{Rep} Dβ to Jβ rearrangements and endogenous TCRβ locus Dβ to Jβ rearrangements by PCR in sort-purified DNII and DNIII Vβ14$^{Rep/WT}$ thymocytes. PCR products corresponding to Vβ14^{Rep} Dβ1 to Jβ1.1 rearrangements and endogenous TCRβ locus Dβ1 to Jβ1.1 rearrangements were both detectable using genomic DNA isolated from DNIII thymocytes (Fig. 3*D*). However, PCR products corresponding to Vβ14^{Rep} Dβ1 to Jβ1.1 rearrangements were barely detectable using genomic DNA isolated from DNII cells (Fig. 3*D*), while PCR products corresponding to endogenous TCRβ locus Dβ1 to Jβ1.1 rearrangements were detectable at a substantial level using DNA isolated from DNII thymocytes (Fig. 3*D*), validating the presence of DNII cell genomic DNA. Notably, the ratio of the level of Vβ14^{Rep} Dβ1 to Jβ1.1 rearrangements in DNII cells compared with DNIII cells (0.09) was significantly less than the ratio of the level of endogenous Dβ1 to Jβ1.1 rearrangements in DNII cells compared with DNIII cells (0.43). These experiments were conducted three times with similar results each time (data not shown). We also conducted seminested PCR analyses of Vβ14^{Rep} Dβ to Jβ rearrangements, endogenous TCRβ locus Dβ to Jβ rearrangements, and endogenous Vβ14 to DJβ rearrangements in sort-purified DNII and DNIII Vβ14$^{Rep/WT}$ thymocytes. PCR products corresponding to Vβ14^{Rep} Dβ1 to Jβ1.1 rearrangements were barely detectable using genomic DNA isolated from DNII cells (Fig. 3*E*); while, PCR products corresponding to endogenous TCRβ locus Dβ1 to Jβ1.1 rearrangements were detectable at a substantial level using DNA isolated from DNII thymocytes (Fig. 3*E*). Importantly, PCR products corresponding to Vβ14 to DJβ1.1 and DJβ1.2 rearrangements were detectable in DNIII, but not DNII, thymocytes (Fig. 3*F*), validating the purity of the sorted cells. In addition, a Cβ2 PCR product was detectable in both DNIII and DNIII thymocytes (Fig. 3*F*), demonstrating the presence of DNII cell genomic DNA. Thus, the developmental stage-specific initiation of Vβ14^{Rep} recombinational accessibility largely mirrors that of endogenous Vβ14 rearrangements, which are readily detectable in DNIII, but not DNII, thymocytes (33).

Vβ14^{Rep} recombination occurs in a much higher percentage of developing αβ T cells than Vβ14 rearrangement

To quantify the overall level of Vβ14 recombinational accessibility that occurs during $\alpha\beta$ T cell development, we generated Vβ14$^{Rep/\omega}$ $\alpha\beta$ T cell hybridomas and analyzed Vβ14^{Rep} and endogenous TCRβ rearrangements using a series of TCRβ locus probes on *Eco*RI-digested DNA (data not shown). Of the 76 clonal hybridomas analyzed, 47 (62%) contained Vβ14^{Rep} Dβ to Jβ rearrangements, while only 11 (14%) contained germline Vβ14^{Rep} alleles (Table III). In addition, we found that 18 (24%) contained Vβ14^{Rep} alleles with endogenous Vβ, Dβ, or Jβ rearrangements to either Vβ14^{Rep} Dβ segments or Vβ14^{Rep} DJβ complexes (Table III), the identity of which were confirmed by sequence analysis of PCR-amplified joins (data not shown). Another three (4%) contained an aberrant Dβ1 rearrangement, most likely involving recombination between the endogenous 5′Dβ1 RS and one of several cryptic RSs located just 5′ of Vβ14^{Rep} (GenBank AE000665; Table III; data not shown). Thus, although the majority of Vβ14^{Rep} recombination events involve Vβ14^{Rep} Dβ to Jβ rearrangements, Vβ14^{Rep} also can target the rearrangement of endogenous Vβ, Dβ, and Jβ segments to Vβ14^{Rep} Dβ segments or DJβ complexes. These data demonstrate that Vβ14^{Rep}

recombination occurs in a substantially higher percentage (at least 86%) of developing $\alpha\beta$ T cells than the ~7% in which primary Vβ14 rearrangements occur (11).

In addition to their productive and selected VβDJβ rearrangements, ~60% of normal $\alpha\beta$ T cell hybridomas contain DJβ rearrangements and ~40% contain out-of-frame VβDJβ rearrangements on their nonselected alleles (8, 18). Thus, to more rigorously address whether Vβ14^{Rep} recombination occurs in a substantially higher percentage of developing $\alpha\beta$ T cells than Vβ14 rearrangements occur, we also quantified Vβ14^{Rep} and endogenous Vβ14 rearrangements on nonselected TCRβ alleles in $\alpha\beta$ T cell hybridomas generated from Jβ1$^{\omega/\omega}$, Vβ14$^{Rep/\omega}$, and Vβ14$^{Rep/Rep}$ mice. Southern analysis of TCRβ rearrangements in 92 Vβ14^{+} Jβ1$^{\omega/\omega}$ hybridomas demonstrated that only 4 (5%) contained endogenous Vβ14 to DJβ rearrangements on the nonselected allele. In contrast, Southern blot analysis of 43 Vβ14^{+} Vβ14$^{Rep/\omega}$ $\alpha\beta$ T cell hybridomas revealed that 36 (84%) contained Vβ14^{Rep} recombination on the nonselected allele (Table IV). These recombination events included Vβ14^{Rep} Dβ to Jβ rearrangements and endogenous Vβ, Dβ, or Jβ rearrangements to Vβ14^{Rep} Dβ segments and DJβ complexes (data not shown). We next quantified Vβ14^{Rep} recombination in a panel of 55 Vβ14$^{Rep/Rep}$ $\alpha\beta$ T cell hybridomas. Of the 38 with endogenous DJβ rearrangement on the nonselected allele, 20 (52%) contained Vβ14^{Rep} Dβ to Jβ rearrangements on both alleles (Table V). Together, these data demonstrate unequivocally that Vβ14^{Rep} recombination occurs in a substantially higher percentage of developing $\alpha\beta$ T cells than the percentage in which Vβ14 rearrangements occur. Thus, we conclude that endogenous Vβ14 segments are recombinationally accessible in a much higher percentage of thymocytes than they rearrange to DJβ complexes and Vβ14 accessibility per se is not subject to allelic exclusion.

Discussion

We have shown here that local chromatin environment imparts lineage- and stage-specific accessibility upon an inserted Dβ-Jβ reporter cassette, allowing the cassette to function as a reporter of V(D)J recombinational accessibility of particular chromosomal loci. In developing thymocytes, the assembly of TCRβ V region exons is ordered with Dβ to Jβ rearrangements occurring before Vβ rearrangements (7, 34). Despite initiation of endogenous Dβ to Jβ rearrangement in DNII stage thymocytes, we found that Vβ14^{Rep} Dβ to Jβ rearrangement predominantly tracks with endogenous Vβ14 to DJβ rearrangement in DNIII thymocytes. Thus, the endogenous Vβ14 segment largely becomes accessible for V(D)J recombination upon differentiation of thymocytes to the DNIII stage. These findings support the notion that ordered assembly of Vβ14DJβ complexes is mediated, at least in part, through the developmental stage-specific accessibility of the Dβ and Jβ portion of the TCRβ locus in DNII thymocytes and the Vβ14 segment in DNIII cells. In this regard, a small percentage (3%) of Jβ1$^{Rep/\omega}$ $\alpha\beta$ T cell hybridomas contained rearrangement of upstream Vβ segments directly to Vβ14^{Rep} Dβ segments, but not directly to endogenous Dβ segments, suggesting that initiation of Dβ/Jβ accessibility in DNII thymocytes, before Vβ accessibility, may ensure formation of DJβ complexes before activation of Vβ rearrangement.

Endogenous Vβ to DJβ rearrangement must proceed through the physical juxtaposition of recombinationally accessible Vβ segments with DJβ complexes across large chromosomal distances (4, 5). Despite occurrence of Vβ14 to DJβ rearrangements in only 5% of $J\beta1^{\omega/\omega}$ αβ T cells, $V\beta14^{Rep}$ recombination occurred in 86% of $V\beta14^{Rep/\omega}$ αβ T cells, on 84% of alleles in Vβ14 expressing $V\beta14^{Rep/\omega}$ αβ T cells, and on both alleles in 42% of $V\beta14^{Rep/Rep}$ αβ T cells that contain endogenous Vβ to DJβ rearrangements on only one allele. Thus, endogenous Vβ14 segments are recombinationally accessible in a much higher percentage of developing thymocytes than that in which they actually undergo rearrangement. Previously, we demonstrated that specific replacement of the endogenous Vβ14 RS with the 3′Dβ1 RS resulted in an ~10-fold increase in the frequency of Vβ14 to DJβ rearrangements (11). This same RS replacement resulted in a corresponding increase in RAG-mediated cleavage of Vβ14 and Dβ segments in vitro (13). It seems unlikely that replacement of the endogenous Vβ14 RS with the 3′Dβ1 RS, either precisely or as part of the Dβ-Jβ cassette, would increase juxtaposition between Vβ14 segments and DJβ complexes. Moreover, the TATA box of the 5′Dβ1 RS is not sufficient to drive Dβ1-Jβ1 transcription or Dβ1 to Jβ1 rearrangement (25–30). Therefore, our current findings in combination with our earlier RS replacement study suggests that the frequency of Vβ14 rearrangements is determined by the productive coupling of recombinationally accessible Vβ14 segments and DJβ complexes. Finally, and most strikingly, our current observation that $V\beta14^{Rep}$ recombination occurs on TCRβ alleles lacking endogenous Vβ to DJβ rearrangements indicates that Vβ14 accessibility is not subject to allelic exclusion (discussed in detail below).

V(D)J recombination is thought to proceed via the initial assembly of the RAG proteins on one RS, followed by capture of the second RS to form a synaptic complex in which RAG-mediated cleavage occurs (35–37). RAG proteins most likely first assemble on RSs with 12-bp spacers 12-RSs) and capture RSs with 23-bp spacers 23-RSs) (36, 37). Thus, during Vβ14 to DJβ rearrangement, the RAG proteins may initially assemble on accessible 5′Dβ 12-RSs and capture Vβ14 23-RSs following structural changes in chromatin that bring Vβ14 segments in close proximity to RAG-bound DJβ complexes. In this context, Vβ14 segments could be rendered accessible for V(D)J recombination either before juxtaposition or, possibly, during synaptic complex formation through RAG2-mediated binding to, or RAG1-catalyzed ubiquitination of, histones within Vβ14 chromatin (5, 36–38). Alternatively, chromosomal factors may direct RAG assembly on accessible Vβ14 23-RSs, leading to the capture of 5′Dβ 12-RSs following juxtaposition of RAG-bound Vβ14 segments and DJβ complexes. In either scenario, the frequency of Vβ14 rearrangement would be determined either by the RAG-binding affinity or by the recombination potential of the RS sequence attached to Vβ14.

Our previous RS replacement study which suggested that Vβ14 segments may be recombinationally accessible in a much higher percentage of thymocytes than the frequency with which they rearrange to DJβ complexes was conducted on a single allele in cells that contained an inactivated TCRβ locus on the other allele (11). Thus, we were unable to ascertain whether the paucity of αβ T cells with Vβ14 to DJβ rearrangements on both alleles was determined by the distinct modulation of Vβ14 accessibility on each allele or by the differential coupling of recombinationally accessible Vβ14 segments and DJβ complexes on

each allele. Our current observation that Vβ14^{Rep} recombination occurred on approximately half of TCRβ alleles that lack edogenous Vβ to DJβ rearrangements in Vβ14$^{Rep/\omega}$ and Vβ14$^{Rep/Rep}$ $\alpha\beta$ T cell hybridomas indicates that Vβ14^{Rep} recombination is not subject to allelic exclusion. This finding demonstrates unequivocally that the assembly of a nonproductive (out-of-frame) VβDJβ rearrangement on the first TCRβ allele is not necessary to activate Vβ14 accessibility on the second allele. By FACS, we did not detect surface expression of Vβ14, or any Vβs, other than Vβ8 on T lineage cells of mice expressing a Vβ8DJβ transgene (A. C. Carpenter and C. H. Bassing, unpublished observations,), suggesting that, similar to the other Vβs (39), Vβ14 is subject to transgene feedback regulation and potentially to allelic exclusion. If so, our finding that Vβ14^{Rep} recombination is not subject to allelic exclusion suggests that feedback regulation of Vβ14 rearrangement may be enforced by preventing the productive coupling of recombinationally accessible Vβ14 segments and DJβ complexes, rather than through inhibition of Vβ14 accessibility. Consistent with this notion, although Vβ14 remains recombinationally accessible in DP thymocytes (40), Vβ14 is not expressed on the cell surface with other Vβs, indicating that Vβ14 allelic exclusion may be maintained through a unique mechanism, such as the induction of apoptosis in DP cells undergoing rare Vβ14 to DJβ rearrangements (40). In contrast, transgenic overexpression of TCRβ chains may inhibit endogenous rearrangements, at least to some degree, by accelerated development and not normal feedback mechanisms (41). In this context, sequence analyses of limited numbers of VβDJβ joins in WT $\alpha\beta$ T cells and direct Vβ14 to Jβ rearrangements in $\alpha\beta$ T cells with specific TCRβ RS replacements revealed two in-frame rearrangements in 5–10% of cells (33, 42, 43), indicating that normal TCRβ expression may not inhibit Vβ to DJβ rearrangement and lead to allelic exclusion in all developing $\alpha\beta$ T cells. Accordingly, our observation that Vβ14^{Rep} recombination occurs on both alleles in a substantial percentage of $\alpha\beta$ T cells may simply reflect that endogenous Vβ14 to DJβ rearrangements are not completely inhibited by feedback regulation. However, our current data cannot exclude the possibility that Vβ14^{Rep} rearranges efficiently and on both alleles in DNIII thymocytes before the assembly and expression of TCRβ chains that signal inhibition of Vβ14 RAG accessibility. Perhaps the analysis of Vβ14^{Rep} recombination and Vβ14 expression in thymocytes expressing VβDJβ transgenes or preassembled endogenous VβDJβ rearrangements may distinguish among these possibilities.

The Vβ14 segment is unique among Vβ segments due to its proximity to Dβ/Jβ segments (44), its rearrangement through inversion (44), and its continued accessibility in DP thymocytes (45). Therefore, the generation and analysis of mice containing replacement of additional Vβ RSs with inserted Dβ-Jβ cassettes will be required to determine whether the rearrangement of other Vβ segments is directed by similar mechanisms to those we have uncovered for Vβ14 in this study.

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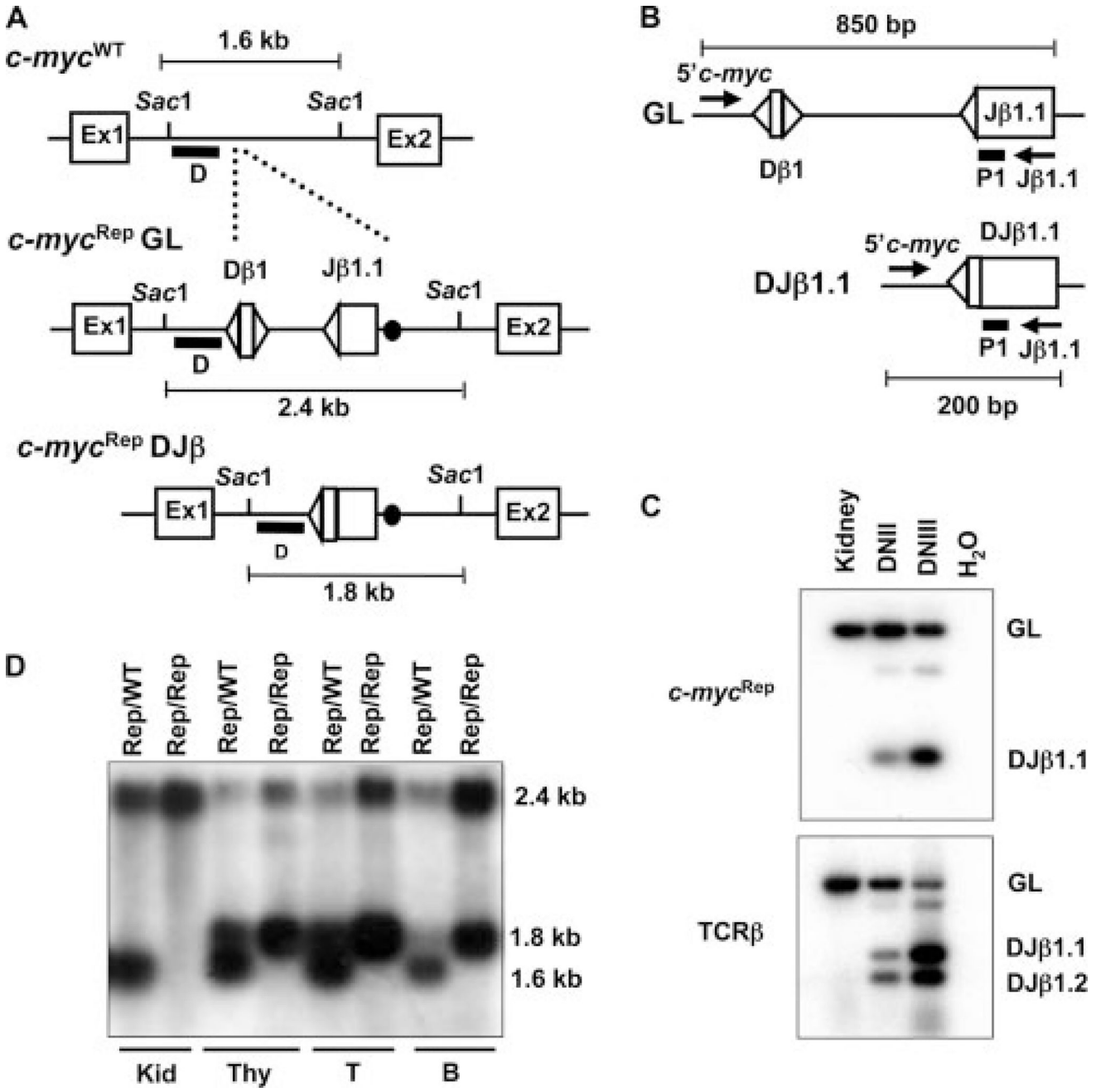

FIGURE 1.

Generation and analysis of c-*myc*^{Rep} mice. *A*, Schematic diagrams of the WT c-*myc* locus (WT), the c-*myc* locus with the inserted germline Dβ1 and Jβ1.1 segments (c-*myc*^{Rep}). The RSs are depicted as triangles and the *loxP* site as a black circle. Open boxes depict the relative locations of the three c-*myc* exons. The relative location of the *Sac*I sites and the sizes of the *Sac*I-digested genomic fragments for WT, c-*myc*^{Rep} germline (GL), and c-*myc*^{Rep} DJβ alleles are indicated. ■, The location of the 5′Vβ14, DJβ1, and 3′Vβ14 probes. *B*, Schematic diagrams of the GL and Dβ to Jβ rearranged (DJβ) c-*myc*^{Rep} alleles. The relative location of the 5′c-*myc* and Jβ1.1 primers are indicated with arrows. ■, The

location of the P1 probe. The sizes of the PCR products for c-*myc*^{Rep} GL and c-*myc*^{Rep} DJβ alleles are indicated. *C*, PCR analysis of c-*myc*^{Rep} Dβ to Jβ rearrangements, endogenous Dβ to Jβ rearrangements, and endogenous Vβ14 to DJβ rearrangements using the 5′c-*myc*/Jβ1.1. 5′Dβ1/P2, and Vβ14/P2 primer sets on genomic DNA isolated from Vβ14$^{Rep/\omega}$ kidneys and sort-purified DNII or DNIII thymocytes. Products corresponding GL and rearranged (DJβ1.1) c-*myc*^{Rep} alleles, as well as endogenous TCRβ rearrangements, are indicated. *D*, Southern blot analysis of c-*myc*^{Rep} Dβ to Jβ rearrangements using probe D on *Sac*I digested genomic DNA isolated from c-*myc*$^{Rep/WT}$ or c-*myc*$^{Rep/Rep}$ kidney, thymus, T cells, or B cells. Restriction fragments representing GL and rearranged (DJβ1.1) c-*myc*^{Rep} alleles, as well as endogenous c-*myc* alleles, as indicated.

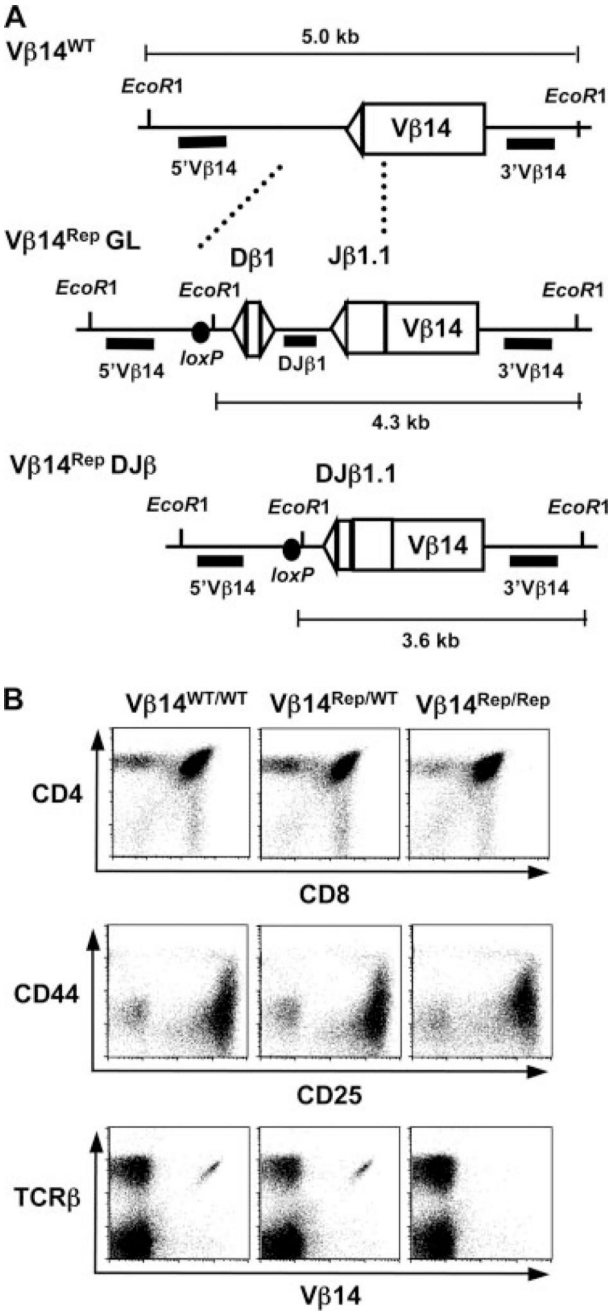

FIGURE 2.

Generation and characterization of Vβ14$^{Rep/\omega}$, Vβ14$^{Rep/WT}$, and Vβ14$^{Rep/Rep}$ mice. *A*, Schematic diagrams of the WT Vβ14 locus (WT), the Vβ14 locus with the inserted germline Dβ1 and Jβ1.1 segments (Vβ14^{Rep}), and the Dβ to Jβ rearranged Vβ14^{Rep} locus (DJβ). The RSs are depicted as triangles and the *loxP* site as a black circle. The relative location of the *Eco*RI sites and the sizes of the *Eco*RI-digested genomic fragments for WT, Vβ14^{Rep}, and DJβ alleles are indicated. Solid black bar shows the location of the 5′Vβ14, DJβ1, and 3′Vβ14 probes. *B*, Flow cytometric analysis of thymocytes and splenocytes from 4- to 6-wk-old WT, Vβ14$^{Rep/WT}$, and Vβ14$^{Rep/Rep}$ mice. Shown are representative anti- CD4-PE/-

CD8-FITC and anti-CD44-PE/-CD25-FITC stains of thymocytes and anti-TCRβ-PE/-Vβ14-FITC stains of splenocytes.

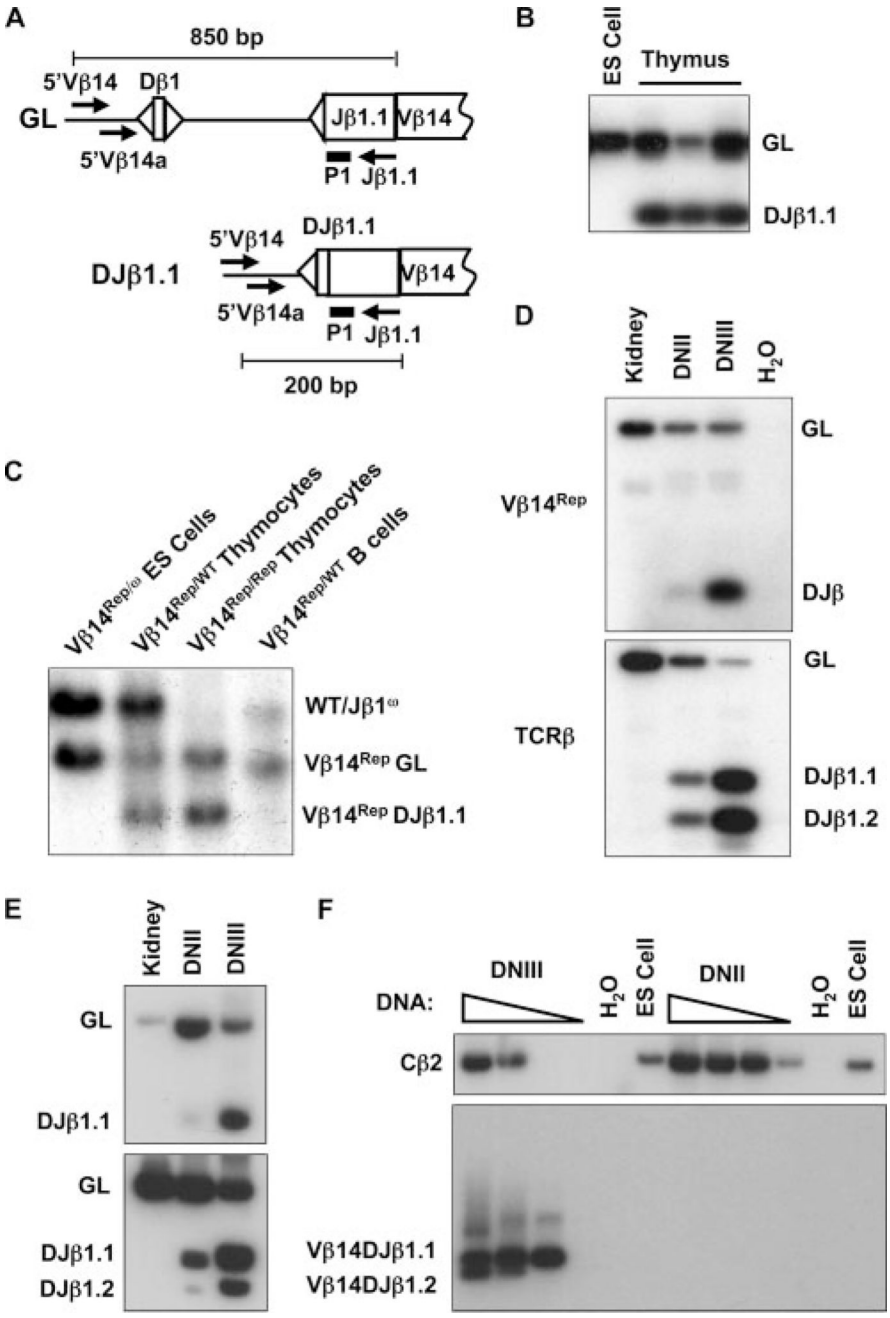

FIGURE 3.

Vβ14^{Rep} recombination mirrors endogenous Vβ14 rearrangement. *A*, Schematic diagrams of the germline (GL) and Dβ to Jβ rearranged (DJβ1.1) Vβ14^{Rep} alleles. The relative location of the 5′Vβ14, 5′Vβ14a, and Jβ1.1 primers are indicated with arrows. A solid black bar shows the location of the P1 probe. The sizes of the PCR products for Vβ14^{Rep} GL and Vβ14^{Rep} DJβ alleles are indicated. *B*, PCR analysis of Vβ14^{Rep} recombination using the 5′Vβ14 and Jβ1.1 primers on genomic DNA isolated from Vβ14$^{Rep/\omega}$ ES cells, thymocytes, and purified B cells. Products corresponding to GL and rearranged (DJβ1.1) Vβ14^{Rep} alleles are indicated. *C*, Southern blot analysis of Vβ14^{Rep} recombination using the

3′Vβ14 probe on *EcoR*I-digested genomic DNA isolated from Vβ14$^{Rep/\omega}$ ES cells, Vβ14$^{Rep/WT}$ thymocytes, Vβ14$^{Rep/Rep}$ thymocytes, Vβ14$^{Rep/WT}$ purified B cells. Bands corresponding to WT, Jβ1$^{\omega}$ (ω), GL Vβ14^{Rep} (Vβ14^{Rep} GL), and Dβ to Jβ rearranged Vβ14^{Rep} (Vβ14^{Rep} DJβ1.1) alleles are indicated. *D–F*, Analysis of Vβ14^{Rep} Dβ to Jβ rearrangements, endogenous Dβ to Jβ rearrangements, and endogenous Vβ14 to DJβ rearrangements in developing thymocytes. Products corresponding GL and rearranged (DJβ1.1) Vβ14^{Rep} alleles, as well as endogenous TCRβ rearrangements, are indicated. *D*, PCR using the 5′Vβ14/Jβ1.1 and 5′Dβ1/P2 primer sets on genomic DNA isolated from Vβ14$^{Rep/WT}$ kidneys and sort-purified DNII or DNIII Vβ14$^{Rep/WT}$ thymocytes. *E*, Seminested PCR analysis of Vβ14^{Rep} and endogenous Dβ to Jβ rearrangements on genomic DNA isolated from Vβ14$^{Rep/\omega}$ kidneys and sort-purified DNII or DNIII Vβ14$^{Rep/\omega}$ thymocytes. *F*, Seminested PCR analysis of endogenous Vβ14 to DJβ rearrangements on genomic DNA isolated from Vβ14$^{Rep/WT}$ ES cells and sort-purified DNII or DNIII Vβ14$^{Rep/WT}$ thymocytes. Also shown are PCR amplifications of Cβ2 to demonstrate the presence of DNA in the DNII and ES cell reactions.

Table I

c-myc^{Rep} rearrangements in c-myc$^{Rep/WT}$ hybridomas

Cell Type	Total # Cells	*c-myc*^{Rep} Status	# Cells (%)
$\alpha\beta$ T cells	113	Germline	37 (33)
		DJβ	76 (67)
IgM$^+$ B cells	60	Germline	34 (57)
		DJβ	26 (43)

Table II

c-myc^{Rep} rearrangements in c-myc$^{Rep/Rep}$ hybridomas

Cell Type	Total # Cells	*c-myc*^{Rep} Status	# Cells (%)
$\alpha\beta$ T	104	Germline/Germline	14 (14)
		Germline/DJβ	57 (54)
		DJβ/DJβ	33 (32)
IgM$^+$ B	92	Germline/Germline	34 (37)
		Germline/DJβ	49 (53)
		DJβ/DJβ	9 (10)

Table III

Vβ14^{Rep} rearrangements in Vβ14$^{Rep/Rep}$ hybridomas

Cell Type	Total #	Vβ14^{Rep} Status	# Cells (%)
		Vβ14^{Rep} germline	11 (14)
		Vβ14^{Rep} DJβ	47 (62)
$\alpha\beta$ T cells	76	5'Dβ1 RS to Vβ14^{Rep} 3'Dβ RS	3 (4)
		DJβ1-Jβ1 RS to Vβ14^{Rep} 3'Dβ RS	3 (4)
		Vβ to Vβ14^{Rep} DJβ	3 (4)
		Vβ to Vβ14^{Rep} Dβ	2 (3)
		VDJβ1-Jβ1 RS to Vβ14^{Rep} 3'Dβ RS	4 (5)
		5'Dβ1 to cRS	3 (4)
IgM^{+} B cells	37	Vβ14^{Rep} germline	37 (100)

Table IV

Vβ14 locus rearrangements on non-selected alleles in Vβ14^{+} T cell hybridomas

Genotype	Total #	Vβ14 or Vβ14^{Rep} Status	# Cells (%)
Jβ1$^{\omega/\omega}$	92	Vβ14 germline	88 (95)
		Vβ14 rearranged	4 (5)
Vβ14$^{Rep/\omega}$	43	Vβ14^{Rep} germline	7 (16)
		Vβ14^{Rep} rearranged	36 (84)

Table V

Vβ14^{Rep} rearrangements in VβDJβ/DJβ Vβ14$^{Rep/Rep}$ T cell hybridomas

Total # Cells	Allele 1	Allele 2	# Cells (%)
38	Germline	Germline	9 (24)
	Germline	DJβ	9 (24)
	DJβ	DJβ	20 (52)