Supporting information for Akamatsu *et al.* (2003) *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.0237043100

Supporting Materials and Methods

Construction of the Core RAG2-Targeting Vector. The core RAG2-targeting vector (pYA801) was constructed in pLNTK (1). The 3' homology arm is a 2.4-kb *Kpn*I–*Not*I genomic fragment. The 5' homology arm is a 4.6-kb *Xba*I fragment consisting of the core RAG2 sequence and upstream genomic sequences. This homology arm was constructed through insertion of a 0.7-kb *Kpn*I–*Eco*RV fragment of core RAG2 into a vector containing the full-length *RAG2* gene and 3' UTR (pRAG2: GFP#2) (2) to create, in effect, a gene encoding core RAG2. The 0.7-kb *Kpn*I–*Eco*RV core RAG2 fragment was isolated from an intermediate plasmid (pHH11) created through ligation of an adaptor composed of annealed synthetic oligonucleotides HSH48 and 49, 5'-GAAGACTCAGAGATCGAATTCCTAAGTAGCTGAGCGGCCGCATAAT-3' and 5'-ATTAATGCGGCCGCTCAGCTACTTAGGAATTCGATCTCTGAGTCTTC-5' into the *Bbv*II and *Ase*I sites of plasmid LG.1 (3).

Western Blotting for Detection of RAG2 Core Protein. Whole thymus extract was prepared according to standard techniques and subjected to Western blot analysis (4) with anti-RAG2 antibody (PharMingen) and horseradish peroxidase-conjugated anti-rabbit antibody (Amersham Pharmacia) according to standard methods (4). ECL (Amersham Pharmacia) was used to visualize the reaction.

Supporting Results

Generation of Core RAG2 Mice. Targeted replacement was carried out in TC1 (129SvEv) embryonic stem (ES) cells by positive and negative selection using *neo* and TK markers in the vector (Fig. 6*B Left*) with subsequent deletion of the pGK-*neo*^{r} cassette by Cre-mediated recombination between the loxP sites (Fig. 6*B Right*). C57BL6 blastocysts were injected with +/c ES cells to generate chimeras that then were bred to 129SvEv mice, and the heterozygous (+/c) offspring bred to generate homozygous c/c mice. Chimeric mice were also bred into a RAG2-deficient (–/–) background (5) to obtain +/– and –/c mice. Furthermore, the core RAG2 mutation was bred onto a 129Sv/C57/B6 (F_1) background. The genotypes of the resulting mice were confirmed by PCR analysis (Fig. 6*C*).

Core RAG2 Expression Leads to Impaired T Cell Receptor (TCR)δ Rearrangement. The effect of core RAG2 expression on TCRδ V(D)J recombination was examined by Southern blot of thymic DNA from 129Sv/C57B6 core RAG2 and WT control animals (Fig. 7). The use of 129Sv/C57B6 mice permits the analysis of allelic TCRδ gene rearrangements through restriction fragment-length polymorphisms associated with Dδ and Jδ gene segments (2). Almost all TCRδ alleles appear to undergo at least some type of rearrangement in core RAG2 thymocytes, because Southern analysis of *Bgl*II-digested DNA with a probe that hybridizes just 3' of Jδ1 revealed a complete loss of the germ-line

TCRδ band (Fig. 7*B Left*). However, in contrast to control thymocytes, core RAG2 thymocytes displayed a predominant 4.5-kb band that represents Dδ1(Dδ2)Jδ1 rearrangements. The many species of other sizes observable in the Southern blot represent complete VδD(Dδ)Jδ joining. The extent of such species is reduced in the DNA from the core RAG2-expressing cells as compared with the WT cells, indicating that Dδ1(Dδ2)Jδ1 joins are accumulating, whereas complete VδD1(Dδ2)Jδ1 assembly is impaired.

The TCRδ locus is distinct from the other loci that contain "D" segments (TCRβ and Ig heavy chain) in that rearrangement is not ordered; incomplete DDδ, DJδ, and VDδ rearrangements have all been detected (6, 7). Because we have observed in our examination of the TCRβ and Ig heavy chain locus that V-to-DJ rearrangement is impaired, we asked whether V-to-D rearrangement is impaired at the TCRδ locus of core RAG2 mice by looking for V(D)Dδ rearrangements. Because the *Bgl*II digest used in the analysis described above does not permit detection of V(D)Dδ rearrangements, a second Southern blot of *Msp*I-digested thymic DNA was carried out (Fig. 7*B Right*). Consistent with the *Bgl*II Southern blot, the 3.9- and 4.1-kb *Msp*I bands represent Dδ1(Dδ2)Jδ1 rearrangements of the 129Sv and B6 alleles, respectively. Because the *Bgl*II Southern blots demonstrated that all Dδ2 genes have rearranged, the 2.3- and 2.6-kb bands represent alleles with Vδ(Dδ1)Dδ2 rearrangements, respectively, not germ-line alleles. Therefore, core RAG2 mice can perform an appreciable level of either Dδ1(Dδ2)Jδ1 or Vδ(Dδ1)Dδ2 rearrangement but a reduced level of complete TCRδ gene assembly. In accord with the reduced levels of γδ T thymocytes in core RAG2 mice (see Fig. 4*C*), we also observed that the total amount of hybridization to the Jδ1 probe is lower in core RAG2 mice compared with WT animals, reflecting the lower total level of germ-line or rearranged TCRδ DNA in the core RAG2 thymus. This observation is consistent with a greater proportion of the thymocytes being αβ cells that have deleted the TCRδ locus (Fig. 7*B*).

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