The V–J Recombination of T Cell Receptor–γ Genes Is Blocked in Interleukin-7 Receptor–deficient Mice

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Summary

IL-7R–deficient mice have severely impaired expansion of early lymphocytes and lack γδ T cells. To elucidate the role of IL-7R on γδ T cell development, we analyzed the rearrangements of TCR-α, β, γ, and δ genes in the thymus of the IL-7R–deficient mice. Southern blot analysis with a Jγ1 probe revealed that more than 70% of Jγ1 and Jγ2 alleles are recombined to form distinct Vγ1.2–Jγ2 and Vγ2–Jγ1 fragments in control mice. On the contrary, no such recombination was detected in the mutant mice. The rearrangements in the TCR-α, β, and δ loci were comparably observed in control and mutant mice. PCR analysis indicated that the V–J recombination of all the Vγ genes is severely hampered in the mutant mice. The mRNA of RAG-1, RAG-2, Ku-80, and terminal deoxynucleotidyl transferase (TdT) genes was equally detected between control and mutant thymus, suggesting that the expression of common recombination machinery is not affected. These data demonstrated that the V–J recombination of the TCR γ genes is specifically blocked in the IL-7R–deficient mice and suggested the presence of highly specific regulation for TCR γ gene rearrangement.

Materials and Methods

Mice. IL-7R–deficient mice were established by replacing the exon 2 with a PGK– neo cassette as described (12). Animals heterozygous (+/−) and homozygous (−/−) for the IL-7R mu-
Southern Blot Analysis. Thymocyte genomic DNA was digested with HindIII or EcoRI restriction enzyme and electrophoresed through 0.7% agarose gel to remove contaminating genomic DNA. Oligo (dT)-primed cDNA was prepared by Molony murine leukemia virus RNase H− reverse transcriptase (GIBCO BRL, Gaithersburg, MD) at 37°C for 1 h. PCR was carried out for 25 cycles of 1 min at 94°C, 1 min at 50°C, and 1 min at 72°C. For TCR β genes, PCR was performed as described previously (20–23). The PCR products were electrophoresed in 3% agarose gel, blotted onto nylon membranes, and hybridized with 32P-labeled oligonucleotide probes. PCR primers are as follows: Vγ1.1 and Vγ1.2, 5′-CTTCCATATTTCTCCACCACAGC-3′; Jγ2, 5′-AATATGACGTTTGGCGTCTTCTTCTG-3′; Jγ4, 5′-ACTACGGAGCTTCTCCCTTTGG-3′; 5′ RAG-2, 5′-CAGTCCACAGGAGAACCTACAC-3′; 3′ RAG-2, 5′-GGTCAAGGACCTACTTCTGAG-3′. Vγ2, Vγ3, Vγ4, Vγ5, Jγ1, Vβ1, Vβ4, Vβ5, Jβ1, Vβ8.2, Dβ2, and Jβ2 primers were described previously (20–23). Oligonucleotide probes used are as follows: Jγ2, 5′-GCAATGAGGAAGTGAGTCTGGA-3′; Jγ3, 5′-GCAATGAGGAAGTGAGTCTGGA-3′; 3′ RAG-1, 5′-CTGAGAAGGTATTGACACGGA-3′; 5′ Ku-80, 5′-AGAGGACATATCCAAGGGTC-3′; 3′ Ku-80, 5′-ACAATGTTACAATCGCTGAA-3′; 5′ Tyt, 5′-ACTCCGAATCATTGCTGAA-3′; 3′ Tyt, 5′-CTTCCCTTATGTCTCCTGTCA-3′; 5′ HPRT, 5′-CCTGAAATTTGAGGATACAGG-3′; 3′ HPRT, 5′-TGCCCTATATGGCCTGAGTGC-3′. Radioactivity was analyzed using the Bio-image Analyzer.

Results

V-J Recombination of TCR γ Genes Is Blocked in IL-7R-deficient Mice. To examine whether the signal from IL-7R affects the V-J recombination, we compared the rearrangement of the TCR γ genes between the thymocytes of IL-7R−/− and −/− mice. The thymocyte DNA from 4-wk-old mice was digested with HindIII or EcoRI, and a Southern blot was hybridized with the Jγ1 probe (Fig. 1 A, left). The Jγ1 probe allows the analysis of DNA rearrangements involving not only Jγ1 but also Jγ2 and Jγ3 gene segments (15). The ES cell DNA showed a 6.6-kb Jγ1, a 9.0-kb Jγ3, and a 11.7-kb Jγ2 germline fragment. The thymocyte DNA from IL-7R−/− mice showed decreased intensity of Jγ1 and Jγ2 germline fragments compared with embryonic stem (ES) cell DNA. Furthermore, a 3.6-kb Vγ1.2–Jγ2 and a 1.4-kb Vγ2–Jγ1 fragment was clearly detected. The percentage of rearranged alleles was calculated using a Bio-image Analyzer. The position of HindIII or EcoRI digestion sites was shown on the right. (A) Thymocyte DNA was digested with HindIII. A Southern blot was sequentially hybridized with the Jγ1 (left), the Jβ2 (middle), and the RAG-2 (right) probes. (B) Thymocyte DNA was digested with EcoRI. A Southern blot was sequentially hybridized with the Jβ1 (left), the Jγ1 (middle), and the RAG-2 (right) probes.

Figure 1. TCR gene rearrangements in the thymus of IL-7R-deficient mice. Lane 1, thymocytes from IL-7R−/− mice; lane 2, thymocytes from IL-7R−/− mice; lane 3, E14.1 ES cells. The position of HindIII-digested phage λ DNA fragments was shown on the right. (A) Thymocyte DNA was digested with HindIII. A Southern blot was sequentially hybridized with the Jγ1 (left), the Jβ2 (middle), and the RAG-2 (right) probes. (B) Thymocyte DNA was digested with EcoRI. A Southern blot was sequentially hybridized with the Jβ1 (left), the Jγ1 (middle), and the RAG-2 (right) probes.
ected in IL-7R +/− mice. Quantification of the radioactivity revealed that 71% and 74% of Jγ1 and Jγ2 alleles, respectively, were rearranged in thymocytes. Because γδ T cells are only 0.3% of total thymocytes (12), the majority of the Vγ1.2–Jγ2 and Vγ2–Jγ1 rearranged fragments are derived from αβ T cells or precursor cells. On the other hand, no fragment derived from Vγ1.2–Jγ2 or Vγ2–Jγ1 recombination was detected in IL-7R −/− mice (Fig. 1 A). This result demonstrates that Vγ1.2–Jγ2 and Vγ2–Jγ1 rearrangements are almost completely blocked in αβ T cells in IL-7R−/− mice.

We next examined adult and fetal thymus DNA by PCR with Vγ1.1+1.2, Vγ2, Vγ3, Vγ4, Vγ5, Jγ1, Jγ2, and Jγ4 primers. Thymus DNA revealed large amounts of PCR products with all the Vγ–Jγ primer pairs in IL-7R +/− mice. On the other hand, V-J rearrangement was greatly reduced in all the TCR γ genes in IL-7R −/− thymus; the signal of Vγ5–Jγ1 product was 150-fold reduced relative to IL-7R +/− mice, and those of Vγ1.1–Jγ4, Vγ1.2–Jγ2, Vγ2–Jγ1, Vγ3–Jγ1, and Vγ4–Jγ1 products were undetectable in IL-7R −/− mice (Fig. 2 A). Amplification with RAG-2 primers produced roughly the equal amount of PCR products in both IL-7R +/− and −/− thymus, suggesting that approximately the same amount of DNA was used in this analysis. These results support the data of Southern blot analysis and suggest that the V-J recombination is almost completely blocked in IL-7R−/− deficient mice not only in Vγ1.2 and Vγ2 genes but also in all the other Vγ genes.

Rearrangements of TCR α, β, and δ Genes Take Place Normally in IL-7R-Deficient Mice. We next analyzed the rearrangement of other TCR genes by Southern blot analysis. First, the Southern blot was hybridized with Jβ2 probe (see Fig. 1 A, middle). The ES cell DNA showed a 4.8-kb germline Jβ2 fragment. Thymocyte DNA showed decreased intensity of the Jβ2 germline fragment and smear patterns of Jβ2 recombined fragments in both IL-7R +/− and −/− mice. Quantification revealed that 81% and 69% of Jβ2 alleles are rearranged in IL-7R +/− and −/− thymus, respectively. Thus, the frequency of Jβ2 rearrangement is greatly reduced in all the TCR genes but also in all the TCR δ genes but not in all the TCR α and β genes (Fig. 2 B). These results demonstrate that IL-7 is not essential for both Dβ–Jβ and Vβ–Dβ–Jβ recombinations. It is recently reported that IL-7 supported Dβ to Jβ rearrangements but not Vβ to Dβ–Jβ rearrangement in fetal thymic organ culture of fetal liver precursor cells (26). However, our results do not support the notion that IL-7 may play some specific role on Dβ–Jβ recombination. All
these results suggested that the rearrangements of TCR α, β, and δ genes take place normally in IL-7R-deficient mice.

Expression of Common Recombination Machinery in IL-7R-deficient Mice. RAG-1 and RAG-2 are indispensable for V–D–J recombination, and several other gene products such as TdT, Ku p70/80 and DNA-dependent protein kinase catalytic subunit are also involved in V–D–J recombination (27). To examine whether the signal from IL-7R affects the expression of these genes, we amplified cDNA prepared from adult thymocytes of IL-7R-deficient mice. cDNA was amplified by PCR using RAG-1, RAG-2, TdT, Ku-80, and HPRT primers, and the Southern blots of PCR products were hybridized with each probe.

Discussion

TCR γ genes are frequently recombined in αβ T cells (28). More than 70% of Vγ1.2 and Vγ2 alleles are recombined in total thymocytes. In this study, we used this phenomenon to examine whether TCR γ recombination is blocked in αβ T cell precursors of IL-7R-deficient mice. IL-7R-deficient mice had no detectable TCR γ recombination by Southern blot analysis. Furthermore, the recombination of all the Vγ genes was undetectable in fetal and adult thymi by PCR analysis. Thus, we demonstrated that the signal from IL-7R is indispensable for the V–J recombination of TCR γ genes in αβ T cell precursors. And it is highly possible that the TCR γ recombination is also blocked in γδ T cell precursors as well as in αβ T cell precursors. This would be certainly one reason why IL-7R-deficient mice lack γδ T cells.

There are three significant features in our observation.

First, this blockade is specific for TCR γ genes. The recombination of TCR α, β, and δ genes are not affected. In addition, the recombination of IgH and L chain genes is probably not hampered by the mutation, because the IL-7R-deficient mice have decreased but certain numbers of surface IgM+ B cells in the periphery (12). Second, the recombination of all the Vγ genes is blocked. In a previous report, IL-7 induced the rearrangement of Vγ2 and Vγ4, but not Vγ3 or Vγ5 genes in fetal thymic organ culture of fetal liver precursors (2). In contrast, not only Vγ2 and Vγ4 but also all the other Vγ genes in the TCR γ1, γ2, and γ4 clusters are hampered to recombine in the mutant mice. Third, TCR γ gene recombination is blocked not only in γδ but also in αβ T cell precursors. These features suggest the presence of highly specific regulation for TCR γ gene rearrangement.

To explain the specific inhibition of TCR γ recombination in the IL-7R-deficient mice, one possibility can be considered. It is to suppose that the TCR γ locus may contain a specific cis-control element(s). One possible candidate for this element is TCR γ enhancers. Recently, it was reported that IL-7 induces the phosphorylation and DNA binding activity of Stat5 protein in T cells (29). Because each TCR γ enhancer contains a Stat5 binding sequence (30, 31), Stat5 may play a role on the regulation of TCR γ recombination. It is also possible that some unknown factor(s) other than Stat5 may specifically regulate the recombination of TCR γ locus.

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