Interleukin 7 Receptor Control of T Cell Receptor γ Gene Rearrangement: Role of Receptor-associated Chains and Locus Accessibility

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Summary

VDJ recombination of T cell receptor and immunoglobulin loci occurs in immature lymphoid cells. Although the molecular mechanisms of DNA cleavage and ligation have become more clear, it is not understood what controls which target loci undergo rearrangement. In interleukin 7 receptor (IL-7R)α2/2 murine thymocytes, it has been shown that rearrangement of the T cell receptor (TCR)-γ locus is virtually abrogated, whereas other rearranging loci are less severely affected. By examining different strains of mice with targeted mutations, we now observe that the signaling pathway leading from IL-7Rα to rearrangement of the TCR-γ locus requires the γc receptor chain and the γc-associated Janus kinase Jak3. Production of sterile transcripts from the TCR-γ locus, a process that generally precedes rearrangement of a locus, was greatly repressed in IL-7Rα2/2 thymocytes. The repressed transcription was not due to a lack in transcription factors since the three transcription factors known to regulate this locus were readily detected in IL-7Rα2/2 thymocytes. Instead, the TCR-γ locus was shown to be methylated in IL-7Rα2/2 thymocytes. Treatment of IL-7Rα2/2 precursor T cells with the specific histone deacetylase inhibitor trichostatin A released the block of TCR-γ gene rearrangement. This data supports the model that IL-7R promotes TCR-γ gene rearrangement by regulating accessibility of the locus via demethylation and histone acetylation of the locus.

Key words: T cell receptor • thymus • VDJ recombination • interleukin 7 • T cell receptor γ locus • γδ T cells

Signals from the IL-7 receptor are critical for early stages in development of several of the lymphoid lineages (1, 2). Thus, IL-7Rα−/− mice produce very few αβ T cells and B cells, and the γδ T cell lineage is even more severely repressed (3, 4). The IL-7Rα signal triggers at least two types of responses in lymphoid precursor cells (for review see reference 5). One signal is for survival of pro-T cells and is associated with the level of bcl-2 in the cells (6); thus, the αβ T cell deficiency in IL-7Rα−/− mice can be partly ameliorated by a bcl-2 transgene (7, 8). The second signal from IL-7 receptor promotes VDJ recombination at several loci, including TCR-β (9, 10), IgH (11, 12), and the TCR-γ locus (13, 14); of these loci, rearrangement of the TCR-γ locus is the most severely repressed in IL-7Rα−/− mice.

VDJ recombination is a stringently regulated event that is restricted (with few exceptions) to certain early stages in lymphoid development. The mechanisms of this strict regulation are not fully understood. However, several types of controls are identified or presumed. One level of control is expression of the recombinase components, RAG1 and -2, which mediate cleavage of target gene segments (15). Thus, expression of the RAG genes is restricted to early lymphoid cells. On the other hand, relocation of the target locus involves components that are not restricted to the lymphoid lineage; these include Ku, p350 kinase catalytic subunit, XRCC4, and DNA ligase (for review see references 16, 17). IL-7 has been shown to promote the expression of RAG1 and -2 in pro-T cells (9, 18). Moreover, IL-7Rα−/− mice showed suppressed expression of the RAG genes in pro-T cells, whereas the later stage (CD4+CD8+) expressed RAG
genes normally, indicating that after the pro-T cell stage expression of the Rag genes becomes IL-7R independent (10).

A second control of VDJ recombination governs whether a locus is accessible to cleavage by the Rag proteins (for review see references 5, 16). This control is necessary because the motifs recognized by the Rag proteins are similar in all rearranging loci. Since different cell types rearrange different loci, there is presumed to be a mechanism governing accessibility of the locus. For example, pro-T cells rearrange the TCR-\(\beta\), \(-\gamma\), and \(-\delta\) loci at about the same time, do not fully rearrange the immunoglobulin loci, and the TCR-\(\alpha\) locus is rearranged at a later stage. Little is understood of the process rendering a locus accessible to recombination. The enhancer of a locus, normally defined based on its ability to promote transcription, also can be involved in promoting rearrangement of a locus. This is thought to account for the observation that a given locus generally produces sterile transcripts before it undergoes rearrangement. Deletion of the respective enhancers abolishes rearrangement of the TCR-\(\gamma\) locus (19, 20), and greatly suppresses rearrangement of the Igh locus (21), the Igk locus (22), and the TCR-\(\alpha\) locus (23). Transcription of a gene is not required for its rearrangement (24), so the enhancer is presumed to play a role in remodeling chromatin structure, rendering nearby regions accessible to both recombination and transcriptional machinery.

In this study, we investigated the signaling mechanism by which IL-7R promotes rearrangement of the TCR-\(\gamma\) locus. There are two ligands for the murine IL-7R: IL-7 and thymic stromal-derived lymphopoietin (TSLP) (25). IL-7 signals involving pairing of the IL-7R-\(\alpha\) chain with the \(\gamma_c\) chain (26, 27), whereas TSLP signals is thought to involve pairing of the IL-7R-\(\alpha\) chain with a different chain (2); for this reason we examined the role of \(\gamma_c\) in signaling rearrangement of the TCR-\(\gamma\) locus. There are several tyrosine kinases activated by IL-7R (28–30); we examined the role of one of these, the Janus kinase Jak3 (31, 32), which is associated with activated by IL-7R (28–30); we examined the role of one of these, the Janus kinase Jak3 (31, 32), which is associated with

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Mice. Embryonic thymus was obtained by performing timed breeding of C57BL/6 mice maintained at Animal Production (Frederick, MD). IL-7R-\(\alpha^{--}\) mice (1) and Rag2-\(--\) mice (36) were produced in the NCI facility (Frederick, MD) from breeders purchased from The Jackson Laboratory (Bar Harbor, ME). Rag2-\(--\) mice (37) were bred in the National Institutes of Health (NIH) facility (Bethesda, MD). Jak3-\(--\) mice (38) were bred at the University of Massachusetts Medical Center (Worcester, MA). Animal care was provided in accordance with the procedures outlined in the “Guide for the Care and Use of Laboratory Animals” (NIH Publication No. 86-23, 1985).

PCR Analysis. DNA was extracted (39) from adult thymi of the indicated strain or fetal C57BL/6 thymus from day 15 of gestation. In the case of fetal Rag2-\(--\) or IL-7R-\(\alpha^{--}\), several thymi were pooled, whereas all other thymi were individually treated. Approximately 1 \(\mu\)g of DNA was used for each PCR reaction in 50 \(\mu\)l reaction buffer (PE Applied Biosystems, Foster City, CA) with 0.2 mM of each NTP, 1 \(\mu\)M of each primer, and 1 U of Taq polymerase. The PCR reaction was performed with a hot start: (94°C for 3 min) 94°C for 1 min, 55°C for 1 min, 72°C for 1 min for 30 cycles (extension, 10 min at 72°C). RNA was extracted with RNAzol according to the manufacturer’s protocol and reverse transcribed as described with oligo(dT) primers (9). PCR was performed as for genomic DNA with the exception that the annealing time was 2 min at 55°C. PCR products were separated on a 1.4% agarose gel, blotted, and hybridized (37) with a probe complementary to the CDR3 sequence as previously published (40). The probes for detection of DNA gene rearrangement were derived from sequences as previously published (40). The primers for detection of sterile transcripts have been published previously (41), as have the primers for detection of GPDH mRNA (42). The following oligonucleotides were used (the bp number in parentheses indicates the precise position in the locus as reported in EMBL/GenBank/DDBJ under accession No. AF037352). No. 1, V\(\gamma_1\) sense (8,280 bp): 5'-CCAGCAGCCACTAAAATGTC-3'. No. 2, V\(\gamma_2\) sense (8,460 bp): 5’-CTGGAATATCCAACCTGGCAGATG-3’. No. 3, V\(\gamma_3\) sense (8,734 bp): 5’-GCTGTTCTTTCTCTCCTAAGAATATA-3’. No. 4, V\(\gamma_4\) sense (8,846 bp): 5’-GCTAAAGGAGATGGTGGTGC-3’. probe for V\(\gamma_4\) reverse (8,586 bp): 5’-AACAAAGTATTAGAAGTTTATTA-3’. No. 5, V\(\gamma_5\) sense (15,508 bp): 5’-CCAGCAGCCTAAAAATGTC-3’. No. 6, V\(\gamma_2\) sense (15,678 bp): 5’-GACTCCCTGGATACCTCTCGAGATCC-3’. No. 7, V\(\gamma_3\) antisense (15,947 bp): 5’-CGTTGGTCCATCGGAACAC-3’. No. 8, V\(\gamma_4\) antisense (16,059 bp): 5’-TGAGGCTATCTCTCTGGTGGTCA-3’. probe for V\(\gamma_4\) reverse (15,795 bp): 5’-CCAGCAGCCTAAAAATGTC-3’. No. 9, V\(\gamma_5\) antisense (33,661 bp): 5’-CGGATATGAGGAGAGGTCGAC-3’. No. 10, TCR-\(\gamma\) constant first exon sense (37,363 bp): 5’-AGAGTTAGTGAGCAGATCTGGTTACCGGGACCA-3’. No. 11, TCR-\(\gamma\) constant first exon antisense (37,657 bp): 5’-GATCTGCCCTCTC-3’. probe for TCR-\(\gamma\) constant (37,504 bp): 5’-GATGTGAAGCAATACATGGGAGCT-3’. Gel Mobility Shift Assay. Nuclear extracts were prepared (43) from ~10^7 fetal thymocytes (day 15 of gestation) from normal C57BL/6 mice, or from adult mice (4-6-wk-old) of Rag2-\(--\) or IL7R-\(\alpha^{--}\) strains. The binding reaction was conducted at room temperature for 1 h before addition of the \(\gamma\)-ATP 5’-end-labeled probe. After 12–16 h of hybridization at 42°C, blots were washed according to the manufacturer’s protocol (Niyomaderm, Ahrington Heights, IL) for 1 h before autoradiography. In Fig. 3 (see Results), the PCR product has not been blotted, but rather is the negative image of an original ethidium bromide-stained agarose gel. The location of primers within the TCR-\(\gamma\) locus are shown in Fig. 1 (see Results). The primers for detection of DNA gene rearrangement were derived from sequences as previously published (40). The primers for detection of sterile transcripts have been published previously (41), as have the primers for detection of GPDH mRNA (42). The following oligonucleotides were used (the bp number in parentheses indicates the precise position in the locus as reported in EMBL/GenBank/DDBJ under accession No. AF037352). No. 1, V\(\gamma_1\) sense (8,280 bp): 5’-CCAGCAGCCACTAAAATGTC-3’. No. 2, V\(\gamma_2\) sense (8,460 bp): 5’-CTGGAATATCCAACCTGGCAGATG-3’. No. 3, V\(\gamma_3\) sense (8,734 bp): 5’-GCTGTTCTTTCTCTCCTAAGAATATA-3’. No. 4, V\(\gamma_4\) sense (8,846 bp): 5’-GCTAAAGGAGATGGTGGTGC-3’. probe for V\(\gamma_4\) reverse (8,586 bp): 5’-AACAAAGTATTAGAAGTTTATTA-3’. No. 5, V\(\gamma_5\) sense (15,508 bp): 5’-CCAGCAGCCTAAAAATGTC-3’. No. 6, V\(\gamma_2\) sense (15,678 bp): 5’-GACTCCCTGGATACCTCTCGAGATCC-3’. No. 7, V\(\gamma_3\) antisense (15,947 bp): 5’-CGTTGGTCCATCGGAACAC-3’. No. 8, V\(\gamma_4\) antisense (16,059 bp): 5’-TGAGGCTATCTCTCTGGTGGTCA-3’. probe for V\(\gamma_4\) reverse (15,795 bp): 5’-CCAGCAGCCTAAAAATGTC-3’. No. 9, V\(\gamma_5\) antisense (33,661 bp): 5’-CGGATATGAGGAGAGGTCGAC-3’. No. 10, TCR-\(\gamma\) constant first exon sense (37,363 bp): 5’-AGAGTTAGTGAGCAGATCTGGTTACCGGGACCA-3’. No. 11, TCR-\(\gamma\) constant first exon antisense (37,657 bp): 5’-GATCTGCCCTCTC-3’. probe for TCR-\(\gamma\) constant (37,504 bp): 5’-GATGTGAAGCAATACATGGGAGCT-3’.
mologues of IL-7, signals through IL-7Rα together with a different chain. We therefore tested whether γc was required for rearrangement of the TCR-γ locus. Several rearrangements of this locus were examined using PCR, which generates a product if the locus is rearranged, bringing two gene segments in sufficiently close proximity to permit the polymerization reaction (Fig. 1). Using this method, thymocytes from various strains of mice were analyzed for rearrangement of Vγ3 and Vγ2, as shown in Fig. 2, and Vγ4 (data not shown), which showed the same pattern. As shown in Fig. 2, γc−/− thymocytes were as deficient as IL-7Rα−/− thymocytes in the rearrangement of this locus. Rag2−/− thymocytes are shown as a control since they are unable to initiate VDJ recombination at any locus. We conclude that both IL-7Rα and γc chains are essential parts of the receptor complex that signals the rearrangement of the TCR-γ locus.

Role of Jak3 Kinase. Several kinases are activated by IL-7R cross-linking, including those of the src (28, 30) and Janus families (29). One of the Janus kinases, Jak3, was examined for a possible role in signaling TCR-γ locus rearrangement because Jak3 is physically associated with γc and has been shown to be essential for normal lymphoid development (38, 44, 45). As shown in Fig. 3, a deficiency in TCR-γ rearrangement was noted in these Jak3−/− mice that was comparable to the deficiency observed in thymocytes deficient in either IL-7Rα or γc chains. Thus, Jak3 is an essential component of the signaling pathway leading from IL-7R cross-linking to rearrangement of the TCR-γ locus.

Results

Role of the γc Chain. It was previously shown that IL-7Rα−/− thymocytes from two independent lines were defective in rearrangement of the TCR-γ locus (13, 14). A second chain, γc, serves as part of the receptor for IL-7 (26, 27), whereas it has been speculated that TSLP (25), a ho-
Role of Expression of the Rag Genes. Expression of Rag genes was previously shown to be sustained by IL-7 (9, 18, 46, 47) and it has been observed previously that transcripts for RAG1 and -2 are deficient in IL-7Rα-/- thymocytes (10). We examined IL-7Rα-/- thymocytes for expression of RAG1 and -2 and observed considerable variability (data not shown): different batches of thymocytes (pooled from five individuals) showed levels ranging from barely detectable to normal levels for both RAG1 and -2. Some IL-7Rα-/- mice have been shown to display a “leaky” phenotype in that they develop small numbers of CD4+CD8+ cells (2); this cell population has been shown to express Rag messages in IL-7Rα-/- thymocytes (10), whereas the same study observed suppressed Rag expression in the pro-T cells from the same mice. However, in our IL-7Rα-/- mouse colony, some pools of thymocytes from “non-leaky” mice (with minimal numbers of CD4+CD8+ cells) also expressed high levels of Rag messages, so we presume that even among pro-T1 cells (CD44+CD25-) there is variability among individual mice. Thus, the deficiency in rearrangement of the TCR-γ locus, which is observed in all our IL-7R-/- mice, could not be explained purely by a deficiency in expression of Rag genes. Moreover, rearrangement of the TCR-γ locus is more repressed than the other loci that undergo VDJ recombination (14), strongly suggesting that locus-specific influences are signaled by the IL-7Rα (5). We therefore sought additional mechanisms that could be involved in the severe repression of rearrangement of the TCR-γ locus in these mice.

Production of Sterile transcripts. A mechanism that is thought to control VDJ recombination is whether or not the locus is accessible to the RAG proteins (for review see reference 16). One indicator that the chromatin is open around a rearranging gene is the production of sterile transcripts emanating from that locus before its rearrangement, a phenomenon that has been reported for most of the loci that undergo VDJ recombination (48, 49), including the TCR-γ locus (50). Sterile transcripts from the TCR-γ locus were greatly reduced in IL-7Rα-/- thymocytes as shown in Fig. 4. Transcription from both constant and variable regions were affected, and several different regions of the locus were repressed to a similar degree. Positive controls for production of sterile transcripts are day 15 embryonic thymus and RAG2-/- thymus, both of which contain a high proportion of pro-T cells. The deficiency in sterile transcripts in IL-7Rα-/- thymocytes suggests that signals from the IL-7R may control the accessibility of the TCR-γ locus, which would in turn affect its ability to be cleaved by the RAG proteins. However, an alternative explanation is that the locus is open before the IL-7R signal, which then induces its transcription, for example by inducing transcription factors that bind the enhancer and upregulate expression of the gene. We therefore performed additional assays to distinguish whether the transcriptional defect in IL-7Rα-/- thymocytes was due to a lack of transcription factors or to inaccessibility of chromatin to those transcription factors.

Role of Transcription Factors. To test for defects in transcription factors in IL-7Rα-/- thymocytes, we examined nuclear proteins that bind to the enhancer regions which are located 3' to three of the four constant regions of the TCR-γ locus. The enhancer confers lymphoid-specific transcription if coupled to a heterologous promoter (51, 52). Within the enhancer, three sites (NF-γ2, 3, and 4) have been identified based on footprinting studies. The NF-γ2 site resembles the consensus motif for binding of STAT5, which has been previously shown to be activated by IL-7 (29, 53). The proteins binding the NF-γ3 site were shown to contain c myb and core-binding factor (54). The components of the complex that bind the NF-γ4 site have not been identified. In Fig. 5, nuclear extracts from IL-7Rα-/- thymocytes are compared with those from RAG2-/- thymocytes, since thymic development in both of these strains is arrested at pro-T cell stages. The IL-7Rα-/- thymocytes did not show a deficiency of complexes that bound any of...
conclusion, we found no evidence that IL-7R

The sequence 5′-CpG-3′ is often associated with silencing of genes (for review see reference 55). This silencing is thought to be based on the binding of proteins to these methylated sites, thereby altering the access of transcriptional machinery (see below). Methylation is also able to block VDJ recombination of minichromosomes after replication (56). We previously noted that during T cell development, the TCR-γ locus was demethylated just before undergoing VDJ recombination (M. K., unpublished data). We therefore tested whether IL-7R signals controlled the methylation of the TCR-γ locus. This was indeed the case as shown by Southern blot analysis in Fig. 6. This assay is based on the properties of two restriction enzymes, MspI and HpaI, both of which cleave at the same restriction site, whereas methylation of the cytosine in that site interferes with cleavage by HpaI but not MspI. As shown, HpaI was unable to cleave sites in the TCR-γ locus in thymocytes from IL-7R−/− or Rag2−/− mice whereas thymocytes from Rag2−/− or SCID mice were cleaved by HpaI. Fig. 6 also illustrates the position of the methylation-sensitive cleavage sites within TCR-γ clusters 1 and 2 (the probe used for hybridization is 100% identical with cluster 1 and 95% identical with cluster 2). One site in cluster 1 generating the 7.7-kb fragment is located between the joining region and the constant region, whereas the other site is located within the first enhancer. This indicates that signals from the IL-7R induce demethylation of the TCR-γ locus, which may in turn keep the locus “open” for either transcription (production of sterile transcripts) or rearrangement. We have also examined the methylation status of the TCR-β locus (data not shown) and found that, in distinction to the TCR-γ locus, it appears to be equally demethylated in IL-7R−/− and Rag2−/− thymocytes. Thus, the requirement for IL-7R signals in rearrangement of the TCR-γ locus is consistent with an effect on accessibility.

Figure 5. Nuclear proteins from IL-7R−/− thymocytes binding to regions of the TCR-γ enhancer. Thymocyte cell suspensions were prepared from normal C57BL/6 embryos (day 15 of gestation), IL-7R−/−, or Rag2−/− mice. Nuclear extracts were prepared. Gel mobility shift assays were performed using labeled oligonucleotides from three sites in the enhancer region of the TCR-γ locus. To assess specificity of binding, unlabeled competitors were added in 10-fold excess to the labeled oligonucleotide (specific denotes the same sequence used in the labeled probe; non-specific is an unrelated sequence of the same size). Antibodies were added to determine whether the NF-κB-binding protein complex contained STAT5a or -b proteins.

The three enhancer sites. In some nuclear extract preparations from IL-7R−/− thymocytes, an additional band was observed that migrated faster than the band in the control RAG2−/− extract; we do not know whether this smaller complex is functionally significant, but suspect it represents a degradation product. In any case, we observed no clear deficiency in any complex in IL-7R−/− thymocytes. Since STAT5 is activated by IL-7, and since the NF-κB site resembles a STAT5 consensus site (see Materials and Methods for sequence), we tested whether the complex binding to that site contained STAT5a or -b using antibodies directed against these proteins. No effect of these antibodies was observed on the complexes, nor was complex formation blocked, nor was there an increase in mass observed (as a positive control for the antisera, they were shown to supershift the STAT5 complex induced in the YT line by IL-2 stimulation; data not shown). Hence, the complex that binds the NF-κB site does not appear to contain STAT5. In conclusion, we found no evidence that IL-7R−/− thymocytes lacked any of the nuclear proteins previously implicated in activating the TCR-γ enhancer. However, some transcription factors can bind their motif in an “inactive” state, then phosphorylation allows them to interact with the transcriptional machinery (AP-1 is an example of such a transcription factor). Thus, it is possible that one of these transcriptional complexes, although it binds DNA, is inactive in these thymocytes. It is also possible, since these sites were identified in lines of mature T cells, that pro-T cells use different sites to regulate transcription.

Methylation Status of the TCR-γ Locus. To determine whether signals from the IL-7R could control chromatin structure of the TCR-γ locus, we examined the methylation of this gene. Methylation of the cytosine residues in the sequence 5′-CpG-3′ are often associated with silencing of the TCR-γ locus (data not shown) and found that, in distinction to the TCR-γ locus, it appears to be equally demethylated in IL-7R−/− and Rag2−/− thymocytes. Thus, the requirement for IL-7R signals in rearrangement of the TCR-γ locus is consistent with an effect on accessibility.
Discussion

IL-7R α−/− mice show a severe deficiency in rearrangement of the TCR-γ locus. In this study we examine the mechanism by which IL-7R α signals VDJ recombination of the TCR-γ locus. Based on studies in knockout mice, we observed that the γc component of the receptor and the γc-associated kinase Jak3 are required to deliver the signal from IL-7R α to the TCR-γ locus. The production of sterile transcripts from the TCR-γ locus was greatly impaired in IL-7R α−/− thymocytes. This transcriptional defect could either reflect a deficiency in transcription factors or an inaccessibility of the TCR-γ locus. The latter hypothesis is favored by three observations in IL-7R α−/− thymocytes: (a) we readily detected the transcription factors binding to regions that have been shown to be important for expression of the TCR-γ genes; (b) the TCR-γ locus was methylated, which may obstruct its accessibility to both transcriptional and recombinational proteins; and (c) the need for IL-7R α signals could be overcome in vitro by the use of the specific histone deacetylase inhibitor TSA.

The IL-7R α effect on rearrangement of the TCR-γ locus is unlikely to be simply the result of the trophic effect of promoting the survival of cells undergoing the rearrangement. First, the TCR-γ locus rearranges in pro-T cells at about the same time as do the TCR-α and β loci. Thus, death of the pro-T cell would be expected to equally repress rearrangement of all three loci, yet the latter two loci show detectable rearrangements in IL-7R α−/− mice, whereas rearrangement of the TCR-γ locus is almost abrogated. Even though pro-T2 cells are virtually absent in these mice, and this is the stage in which TCR gene rearrangements normally begin, only rearrangement of the TCR-γ locus is eliminated. Second, a bcl-2 transgene was shown to promote development of the αβ lineage but not the γδ lineage (7, 8), so although the IL-7R α signal also provides a trophic effect, there is no evidence that this is sufficient to sustain TCR-γ locus rearrangement. On the other hand, introducing a rearranged TCR-γ transgene only partially restored γδ T cell development in γc−/− mice (59), reflecting the requirement for a second trophic signal from the IL-7 receptor.

The IL-7R α chain is reported to be a component of the receptor for TSLP, a homologue of IL-7. Knockout of IL-7 (60, 61) has a less severe phenotype than knockout of IL-7R α, in that more αβ T cells develop and γδ T cells are at least detectable. This has led to the suggestion that TSLP can partly compensate for the absence of IL-7 in IL-7 knockout mice, and implies that TSLP and IL-7 have similar activities. There has also been some speculation that the receptor for TSLP does not use the γc chain (2). Thus, if TSLP were capable of inducing T cell development, it might be independent of γc and Jak3, which could explain why knockout mice for γc and Jak3 have a less severe phenotype.
more like IL-7- than IL-7R α-deficient mice. In any case, our data show that both IL-7R α and γ chains are required for the signal to rearrange the TCR-γ locus. To test whether receptors from another cytokine family could induce rearrangement of the TCR-γ locus, we have injected IL-7R −/− mice with oncostatin M. However, we have not detected rearrangement of the locus as of the writing of this paper.

To determine whether the TCR-γ enhancer is the target of IL-7R stimulation, we examined nuclear proteins of IL-7R stimulation, we examined nuclear proteins against the STAT5 pathway being involved in rearrangement of the TCR-γ locus. We have observed that the binding of a transcription factor to DNA does not necessarily indicate that it is transcriptionally active. Cross-linking of the IL-7R has been shown to activate the transcription factor STAT5, inducing its translocation to the nucleus (29), and it has been shown that there is a deficiency in nuclear STAT5 in IL-7R −/− thymocytes (62). We have confirmed the latter finding that IL-7R thymocytes were deficient in nuclear proteins that bind a STAT5 motif (data not shown). STAT5 seemed a likely candidate for the relevant IL-7–induced transcription factor, since one of the enhancer sites resembles a consensus binding site for STAT5. However, we observed that the complex that actually binds this enhancer site in vivo does not contain STAT5a or b-based on a failure of specific antibodies to block binding in vitro. These findings argue against the STAT5 pathway being involved in rearrangement of the TCR-γ locus, which is also consistent with the observation of the relatively normal T cell development that occurs in STAT5a−/−b−/− mice (63).

It has recently been suggested that the IL-7 effect on rearrangement of the IgH locus may be via the transcription factor Pax5 (12) based on several findings: (a) Pax5 binds a site in the IgH locus; (b) Pax5−/− mice resemble IL-7R α−/− mice in that the IgH locus successfully rearranges D to J, but fails to normally rearrange V to D; (c) IL-7R α−/− B cells showed reduced expression of Pax5. However, we detected normal levels of Pax5 transcripts in pro-T cells from IL-7R α−/− mice (data not shown), suggesting that its regulation is dependent on IL-7R in the B but not the T lineage, and arguing against it mediating the signal to rearrange the TCR-γ locus. That study parallels ours in noting that IL-7R α−/− mice were defective in producing sterile transcripts from the IgH locus and proposing that IL-7R regulated accessibility of that locus. Since thymocytes normally perform D to J, but not V to D, rearrangements, we tested whether TSA treatment would overcome the latter block. TSA did not induce V to D rearrangements in normal thymocytes when used under similar conditions to those in Fig. 7 (data not shown), indicating that histone deacetylation is not sufficient to account for the suppression of this rearrangement in the T cell lineage.

Our data favor the hypothesis that IL-7R signals an opening of the TCR-γ locus accompanied by locus-specific demethylation, with one site being in the enhancer, which could be a major controlling region. This idea is supported by the observations that in cell lines, methylated minichromosomes show repressed VDJ recombination (56) and that in transgenic mice, methylation of the transgene represses its rearrangement (64). A number of molecular mechanisms have been suggested to participate in transcriptional repression by DNA methylation (for review see reference 55). Methylated DNA binds proteins such as MeCP2 (which is essential for embryogenesis), which can silence chromatin over a considerable distance; for example, MeCP2 separated by 1,774 bp from a transcriptional start site reduced transcription >70% (65). MeCP2 is thought to mediate this repression by attracting histone deacetylase to the region (57). Our data support the idea that IL-7 controls the acetylation status of histones resulting in “active” chromatin. To determine how rapidly IL-7 induced demethylation of the TCR-γ locus, it would be useful to treat IL-7−/− mice with IL-7, then monitor the methylation status of the locus. The alteration of chromatin by IL-7R signals may then allow access for transcription as well as cleavage by the Rag proteins. It remains to be determined which cis-acting elements in the TCR-γ locus respond to the IL-7 receptor signal, how they induce demethylation, whether this can cause specific histone acetylation of the TCR-γ locus, and whether this causes a direct increase of locus accessibility.
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