Chromosomal excision of TCRδ chain genes is dispensable for αβ T cell lineage commitment

Bernard Khor, Tara D. Wehrly and Barry P. Sleckman

Department of Pathology and Immunology, Washington University School of Medicine, 660 South Euclid Avenue, Campus Box 8118, St Louis, MO 63110, USA

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Abstract

TCRβ, δ and γ chain genes are assembled and expressed in double-negative thymocytes prior to αβ or γδ T cell lineage commitment. Thus, cells committed to the αβ T cell lineage can possess completely assembled TCRδ and/or TCRγ chain genes. However, these genes are not expressed. TCRγ chain gene expression may be silenced through the activity of a cis-acting silencer element. In the TCRα/δ locus, the TCRδ genes lie between the Vδ and Jδ gene segments, which rearrange by deletion. Moreover, Vα to Jα rearrangements occur on both alleles in essentially all developing αβ T cells. Consequently, both TCRδ chain genes are excised from the chromosome and placed on extrachromosomal circles in mature αβ T cells. It has been proposed that this excision process is important for silencing TCRδ gene expression and permitting αβ T cell lineage commitment. A gene-targeting Cre-loxP strategy was used to invert a 75-kb region of the TCRα/δ locus encompassing all the Jα gene segments, generating the TCRα/δ allele. Initial Vα to Jα rearrangements on the TCRα/δ allele occur by inversion, resulting in chromosomal retention of TCRδ chain genes. These TCRδ chain genes can be productively rearranged and are expressed at levels similar to TCRδ chain genes in γδ T cells. However, αβ T cell development appears unperturbed in TCRα/δΔ mice. Thus, excision of TCRδ genes from the chromosome per se is not required for commitment of developing lymphocytes to the αβ T cell lineage.

Introduction

T lymphocytes can be divided into two lineages based on expression of either αβ or γδ TCRs. These cells develop from a common thymic precursor, the CD4−/CD8− (double negative, DN) thymocyte. The molecular events that promote the differentiation of DN thymocytes along the αβ or γδ T cell lineage pathway, although incompletely defined, require signals generated through the expression of T cell antigen receptor chains. Assembly and expression of TCRβ, γ and δ chain genes occur in DN thymocytes, whereas assembly of TCRα chain genes is delayed until the CD4+/CD8+ (double positive, DP) stage of thymocyte development. Expression of a γδ TCR by DN thymocytes results in signals that promote commitment to the γδ T cell lineage (1–3). In contrast, expression of a TCRβ chain in conjunction with the pre-TCRα protein forms the pre-TCR, which generates signals resulting in commitment to the αβ T cell lineage and transition to the DP stage of thymocyte development (4–8).

As assembly of TCRβ, γ and δ chain genes occurs in DN thymocytes that are not yet committed to the αβ or γδ T cell lineage, it follows that αβ T cells can have completely assembled TCRγ and/or δ chain genes (1, 9–11). If productive and expressed, these rearrangements could lead to the expression of aberrant βδ or γδ heterodimers, or possibly a γδ TCR, in αβ T cells (12, 13). However, TCRδα and γ chain genes are not expressed in αβ T cells (10). TCRγ chain gene expression may be prevented in αβ T cells through the activity of a cis-acting silencer element (14, 15).

The genes encoding the TCRα and δ chains lie in a single locus, the TCRα/δ locus (16). In the mouse, ~100 Vα/Vδ gene segments lie in the 5’ region of the locus, followed by the Dδ and Jδ gene segments, the TCRδ constant region gene (Cδ), 61 Jα gene segments and the TCRα constant region gene (Cα) (Fig. 1A). Thus, the TCRδ chain gene is embedded between the Vα and Jα gene segments. Two transcriptional enhancers, the TCRδ (Eδ) and TCRα (Eα) enhancers, have been defined in the locus (17–20). Eδ lies in the intron between Jα2 and Cδ and is active in DN thymocytes, promoting germline transcription and rearrangement of TCRδ, but not TCRα, chain genes (21).
A modified version of the TCR \( \text{loxP} \) Cre recombinase was used to invert a 75-kb region of the intervening sequences, including the TCR \( \alpha \) gene segments, from the chromosome (Fig. 1A) (25). Although these extrachromosomal circles contain TCR \( \alpha \) chain genes and persist in naive \( \alpha \beta \) T cells, expression of these TCR \( \alpha \) chain genes is not observed (9, 10, 26, 27). It is possible that the genetic program of TCR \( \alpha \) chain gene expression in \( \alpha \beta \) T cells may rely on excision of these genes from the chromosomal retention and expression of fully assembled TCR \( \alpha \) chain genes in TCR \( \alpha ^{\pm} \) mice. These findings are discussed in the context of their importance in \( \alpha \beta \) T cell development and lineage commitment.

**Methods**

**Generation of targeting constructs**

The 5' homology arm of the pTEAS'loxP targeting vector is a DNA fragment extending 3.8 kb 5' of the BglII upstream of the TE early alpha promoter (TEAp) (see Supplementary Figure 1, available at International Immunology Online). The 3' homology arm is a 7-kb BglII–NsiI fragment (see Supplementary Figure 1, available at International Immunology Online). The 5' and 3' homology arms were cloned into pLNTK to generate the pTEAS'loxP targeting vector (Supplementary Figure 1, available at International Immunology Online) (24). The 5' homology arm of the pExa'loxP targeting vector is a 5.5-kb SphI–BamHI fragment and the 3' homology arm is a 3-kb BamHI fragment (see Supplementary Figure 2, available at International Immunology Online). These 5' and 3' homology arms were introduced into the pLNTK vector to generate the pExa'loxP targeting vector (Supplementary Figure 2, available at International Immunology Online).

**Embryonic stem cells**

Cell culture and gene targeting of embryonic stem cells were carried out as previously described (24).

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**Fig. 1.** The TCR\( \alpha ^{\pm} \) allele: (A) Schematic of the wild type TCR\( \alpha ^{+/+} \) locus (TCR\( \alpha ^{+/+} \)) and the TCR\( \alpha ^{+/+} \) locus with the two \( \text{loxP} \) sites before (TEAS'loxP-\( \text{loxP} \)) and after (TCR\( \alpha ^{+/+} \)) the 75-kb Cre-mediated inversion of the intervening region. The TCR\( \alpha \) enhancer (Ex\( \alpha \)), TEAp, constant region gene (C\( \alpha \)) and J\( 1 \alpha \) gene segments are shown. The TCR\( \alpha \) constant region gene (C\( \alpha \)), enhancer (Ex\( \alpha \)), two J\( 3 \alpha \) gene segments (J\( \alpha 1 \) and J\( \alpha 2 \)) and the D\( 8 \alpha \) gene segment are also shown. The region encompassing these gene segments is drawn to scale except for the two \( \text{loxP} \) sites (open arrows). Three of the ~100 V\( \alpha \) gene segments are shown in the 5' portion of the locus. The four BglII sites (B1, B2, B3 and B4) used for analysis of the inversion are indicated. The location of the probe 1 (P1) is indicated (open rectangle) and the inset shows a Southern blot analysis of genomic DNA from DN thymocytes (22, 23). Rather, E\( \alpha \) is responsible for delaying TCR\( \alpha \) expression until the DP stage of thymocyte development (22, 24). This temporal activation of Ex\( \alpha \) is responsible for delaying TCR\( \alpha \) gene expression in \( \alpha \beta \) T cell lineage (22, 24). This temporal activation of Ex\( \alpha \) is responsible for delaying TCR\( \alpha \) gene expression in \( \alpha \beta \) T cell lineage (22, 24).

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E\( \alpha \), which lies just downstream of the C\( \alpha \) gene, is not active in DN thymocytes, promoting germine transcription. V\( \alpha \) to J\( \alpha \) rearrangement and expression of TCR\( \alpha \) chain genes in cells committed to the \( \alpha \beta \) T cell lineage (22, 24). This approach has been used to generate TCR\( \alpha \) gene deletion and T cell development (Fig. 1A) (25). Although these extrachromosomal circles contain TCR\( \alpha \) chain genes and persist in naive \( \alpha \beta \) T cells, expression of these TCR\( \alpha \) chain genes is not observed (9, 10, 26, 27). It is possible that the genetic program of TCR\( \gamma \) chain gene expression, as appears to be the case with TCR\( \gamma \) chain gene expression. Alternately, silencing TCR\( \alpha \) gene expression in \( \alpha \beta \) T cells may rely on excision of these genes from the chromosone during V\( \alpha \) to J\( \alpha \) rearrangement.

Here, a Cre–loxP approach has been used to generate a modified version of the TCR\( \alpha ^{+/+} \) locus (TCR\( \alpha ^{+/+} \)) in which initial V\( \alpha \) to J\( \alpha \) rearrangements occur by inversion. To this end, the Cre recombinase was used to invert a 75-kb \( \text{loxP} \)-flanked region of the TCR\( \alpha ^{+/+} \). This region encompasses all the J\( \alpha \) gene segments, C\( \alpha \) and Ex\( \alpha \) such that the J\( \alpha \) gene segments on the TCR\( \alpha ^{+/+} \) allele lie in the opposite transcriprional orientation as the V\( \alpha \) gene segments. Accordingly, initial V\( \alpha \) to J\( \alpha \) rearrangements on the TCR\( \alpha ^{+/+} \) allele occur by inversion, resulting in retention of TCR\( \alpha \) genes within the chromosome in mature \( \alpha \beta \) T cells. T cell development proceeds in an unperturbed fashion in TCR\( \alpha ^{+/+} \) mice despite the chromosomal retention and expression of fully assembled TCR\( \alpha \) chain genes in TCR\( \alpha ^{+/+} \) \( \alpha \beta \) T cells. These findings are discussed in the context of their importance in \( \alpha \beta \) T cell development and lineage commitment.
Southern and northern blot analysis
Southern and northern blot analysis of purified genomic DNA and RNA, respectively, was carried out as previously described (24). Previously described probes are as follows: probe 4 (P4) (9), T early alpha (TEA) (28), Cα (29), Cγ1 (10), 5′-1′ (30), 3′- (30), EαKOS′ (24), EαKO3′ (24) and glyceraldehyde-3-phosphate dehydrogenase. The recombinase activating gene-2 (RAG-2) probe is a 0.9-kb PstI–EcoRV genomic DNA fragment. The PI probe is a 0.5-kb fragment extending 3′ of the HindIII site immediately downstream of Eα. Quantitation was carried out using a Molecular Dynamics phosphomager and Imagequant software.

Flow cytometric analyses and cell sorting
Flow cytometric analyses of thymocytes, splenocytes and lymph node cells were carried out as previously described (24). The mAbs used were FITC-conjugated anti-CD8, anti-TCRα and anti-CD3ε and PE-conjugated anti-CD4, anti-TCRβ, anti-Vα2 and anti-Thy1.2 which were all obtained from Phar-mingen. Flow cytometric cell sorting was carried out using a FACSvantage.

Cell stimulation and hybridoma generation
αβ T cells were stimulated either in media containing 5 μg ml⁻¹ ConA and 50 U ml⁻¹ IL-2 for 2 days or on plates coated with 10 μg ml⁻¹ anti-TCRβ antibody (H57-597) in PBS and in media containing 50 μU ml⁻¹ IL-2 for 6 days (31). To generate αβ T cell hybridomas, activated T cells were fused to the BW-1100.129.237 thymoma and selection was carried out as previously described (24). T cell hybridomas that expressed an αβ TCR, as determined by flow cytometric analyses, were analyzed for TCRα chain gene retention and rearrangement.

PCR and sequence analysis
VDJα1 rearrangements were amplified using a primer 3′ of the Jα1 gene segment (Jα1) with the Vα-specific primers DV104S1, DV105S1, ADV7S and ADV17S2, that would amplify Vα4, Vα5, Vα6/Vα7 and Vα9 rearrangements to Jα1, respectively, on 500 ng of genomic DNA obtained either from sorted resting αβ T cells or from TCRαΔ⁹⁺ transgenic mice (Supplementary Figure 1, available at International Immunology Online). The neomycin resistance gene was deleted through Southern and northern blot analysis of purified genomic DNA and RNA, respectively, was carried out as previously described (24, 30), Eα (29) and glyceraldehyde-3-phosphate dehydrogenase. Thus, expression of the Cre recombinase in this cell resulted in deletion of the loxP-flanked neomycin resistance gene, generating the TEA5⁺loxP allele, following by inversion of the 75-kb region between the 5′ TEA and 3′ Eα loxP sites, generating the TCRαΔ⁹⁺ allele (Fig. 1A; Supplementary Figure 1, available at International Immunology Online). Fidelity of gene targetings and inversion was assessed by Southern blotting, PCR and sequence analyses (Fig. 1A; Supplementary Figures 1 and 2, available at International Immunology Online, and data not shown).

The 75-kb inverted region on the TCRαΔ⁹⁺ allele includes the TEAp, all 61 Jα gene segments, the Cα gene and Eα. Thus, the Jα gene segments on the TCRαΔ⁹⁺ allele are in the opposite transcriptional orientation with respect to the Vα gene segments (Fig. 1). As a result, initial Vα to Jα rearrangements on this allele will occur by inversion (Fig. 1B). Importantly, subsequent Vα to Jα rearrangements, which could occur by deletion or inversion, will not lead to deletion of TCRα genes from the chromosome (Fig. 1B). Chimeric mice were generated using ES cells harboring the TCRαΔ⁹⁺ allele and germline transmission of the TCRαΔ⁹⁺ allele was achieved.

Temporal activation of germline TCRα and α transcripts on the TCRαΔ⁹⁺ allele
Germline transcription and accessibility of TCRα chain genes is normally activated in DN thymocytes, whereas activation of TCRα chain gene germline transcription and accessibility is delayed until the DP stage of thymocyte development. To determine whether this temporal activation of germline transcription is intact on the TCRαΔ⁹⁺ allele, mice homozygous for the TCRαΔ⁹⁺ allele (TCRαΔ⁹⁺) on a RAG-2⁻/⁻ background without (TCRαΔ⁹⁺: RAG-2⁻/⁻) or with (TCRαΔ⁹⁺: RAG-2⁻/⁻: DO11β) the DO11 TCRβ transgene were generated. Thymocytes from TCRαΔ⁹⁺: RAG-2⁻/⁻ mice are blocked at the DN stage of development with the TCR in the germline configuration due to the absence of the RAG-2 protein (33). The presence of the DO11 TCRβ transgene in the TCRαΔ⁹⁺: RAG-2⁻/⁻: DO11β mice causes thymocyte developmental progression to the DP stage (8). However, the TCR genes remain in the germline configuration in these DP thymocytes due to the deficiency of RAG-2.

The level of germline TCRα transcripts from the TCRαΔ⁹⁺ and TCRα⁺⁺ alleles in DN thymocytes was similar, as evidenced by northern blot analysis of RNA isolated from TCRαΔ⁹⁺: RAG-2⁻/⁻ and TCRαΔ⁹⁺: RAG-2⁻/⁻ thymocytes, respectively (Fig. 2A). Importantly, germline TCRα gene transcripts were not detected in DN thymocytes from either the TCRαΔ⁹⁺ or the TCRα⁺⁺ alleles (Fig. 2B). This was evidenced by the lack of hybridization of RNA from these cells to either a Cα probe or a probe to the TEA exon, which lies immediately downstream of the TEAp (Figs 1A and 2B, data not shown) (28, 34). However,
of thymocytes with comparable fractions of DN, DP and CD4+ cells (Table 1, data not shown). Flow cytometric analyses revealed normal levels of TCR expression by TCRαβ T cells, as evidenced by cell surface TCRβ and Vα2 expression (Fig. 3B). Furthermore, purified TCRαβ and TCRαδ T cells have similar levels of mature TCRα transcripts (Fig. 3C). TCRα/δ++ and TCRαδ mice have similar numbers of thymic and splenic γδ T cells (Table 1, data not shown). Flow cytometric analyses revealed that TCRα expression by TCRαδ γδ T cells was similar to that of wild type γδ T cells (Fig. 3B). Together, these findings demonstrate that the assembly and expression of TCRα and TCRα chain genes on the TCRαδ allele are intact, permitting the efficient generation of αβ and γδ T cells in TCRαδ mice.

TCRα chain genes are diversely rearranged and retained in the chromosome in TCRαδ αβ T cells

TCRα gene rearrangements were assayed in purified peripheral TCRαδ/δ δδ and TCRαδ/αδ αδ T cells and in clonal TCRαδ/δ αβ T cell hybridomas (Fig. 4). Genomic DNA isolated from mature TCRαδ Tδ T cells was digested with BglII and subjected to Southern blot analysis using P4 to detect rearrangements to the Jα1 gene segment (Figs 1A and 4A). The TCRα genes are extensively and diversely rearranged in TCRαδ αβ T cells, as evidenced by loss of the germline size and generation of multiple non-germline size P4-hybridizing bands. TCRαδ αβ T cells exhibited a similar pattern of diverse TCRα chain gene rearrangements (Fig. 4A). Analysis of TCRα chain gene rearrangements in TCRαδ αβ T cell hybridomas revealed that each had a single P4-hybridizing band, demonstrating that a single TCRα chain gene was retained in the chromosome in each of these cells (Fig. 4B). Furthermore, these TCRα chain genes were heterogeneously rearranged as evidenced by the different size P4-hybridizing bands (Fig. 4B).

To determine whether the rearranged TCRα chain genes are retained in the chromosome of TCRαδ αβ T cells, Southern blot analyses were carried out using the Cα probe on Stul-digested genomic DNA isolated from resting αβ T cells or αβ T cells that had undergone proliferative expansion (Figs 1A and 4C). TCRα genes on extrachromosomal circles should be lost upon cellular expansion if they are incapable of replicating. As compared with genomic DNA isolated from kidney, the Cα-hybridizing Stul fragment was 40% retained in genomic DNA isolated from resting TCRαδ/δ αβ T cells (Fig. 4C). However, there was essentially no detectable Cα-hybridizing band in genomic DNA isolated from TCRαδ/δ αβ T cells that had undergone proliferative expansion (Fig. 4C). In striking contrast, resting and expanded TCRαδ/δ αβ T cells both exhibit essentially 100% retention of the Cα-hybridizing band (Fig. 4C). Together, these data demonstrate that essentially all the TCRα genes in TCRαδ/δ αβ T cells exist on extrachromosomal circles, whereas those in TCRαδ/δ αβ T cells are retained in the chromosome.

TCRδ chain genes are productively and expressed in TCRαδ αδ T cells

Many of the TCRδ gene rearrangements in TCRαδ αδ αβ T cells were complete as evidenced by Southern blot and PCR analyses (data not shown). To determine the fraction that were in-frame, complete VDJδ rearrangements from resting...
TCR\(\alpha\) and TCR\(\beta\) \(\alpha\beta\) T cells were PCR amplified, cloned and sequenced. These analyses revealed that a similar fraction of complete TCR\(\alpha\) gene rearrangements were in-frame in TCR\(\alpha\)/\(\delta\) (28%) and TCR\(\alpha\)/\(\delta\) (32%) \(\alpha\beta\) T cells (Table 2). Together, these analyses reveal that TCR\(\alpha\) genes in TCR\(\alpha\)/\(\delta\) \(\alpha\beta\) T cells are diversely rearranged. Many of these rearrangements are complete and productive as is the case with TCR\(\alpha\) gene rearrangements in TCR\(\beta\)/\(\delta\) \(\alpha\beta\) T cells.

Northern blot analysis was performed on RNA isolated from resting TCR\(\alpha\)/\(\delta\) and TCR\(\alpha\)/\(\delta\) \(\alpha\beta\) T cells. Both TCR\(\alpha\)/\(\delta\) and TCR\(\alpha\)/\(\delta\) \(\alpha\beta\) T cells have silenced TCR\(\gamma\) chain gene expression as evidenced by the lack of Cy1-hybridizing transcripts (Fig. 5). In contrast, there are robust levels of TCR\(\alpha\) transcripts in TCR\(\alpha\)/\(\delta\), but not TCR\(\alpha\)/\(\delta\) \(\alpha\beta\) T cells (Fig. 5). Remarkably, the level of TCR\(\delta\) transcripts in TCR\(\alpha\)/\(\delta\) \(\alpha\beta\) T cells is similar to that observed in \(\gamma\delta\) T cells (Fig. 5). Thus, TCR\(\delta\) chain genes are expressed in \(\alpha\beta\) T cells when retained within the chromosomal context (TCR\(\alpha\)/\(\delta\)) but not when they are placed on extrachromosomal circles (TCR\(\alpha\)/\(\delta\)).

**Discussion**

We have generated and analyzed mice with a modified version of the TCR\(\alpha\)/\(\delta\) locus (TCR\(\alpha\)/\(\delta\)) in which initial V\(\alpha\) to J\(\alpha\) rearrangements occur by inversion, resulting in the intervening sequence, including the TCR\(\delta\) genes, being retained within the context of the chromosome. The 75-kb inversion on the TCR\(\delta\) allele includes the TEAp, the J\(\alpha\) gene segments, C\(\alpha\) and E\(\alpha\) (Fig. 1). Importantly, the function of cis-acting elements appears intact on the TCR\(\delta\) allele. Germline TCR\(\alpha\) transcripts from the TCR\(\alpha\)/\(\delta\) allele are observed in DN thymocytes, whereas activation of TCR\(\alpha\) germline transcripts is delayed until the DP stage, as is observed for the TCR\(\alpha\)/\(\delta\) allele (Fig. 2). Furthermore, complete TCR\(\delta\) and TCR\(\delta\) chains encoded by the TCR\(\alpha\)/\(\delta\) allele are expressed at normal levels in mature \(\alpha\beta\) and \(\gamma\delta\) T cells, respectively (Fig. 3). However, unlike TCR\(\alpha\)/\(\delta\), \(\alpha\beta\) T cells, TCR\(\delta\) chain genes are robustly expressed in TCR\(\alpha\)/\(\delta\) \(\alpha\beta\) T cells (Fig. 5).

It has been suggested that excision of TCR\(\delta\) chain genes from the chromosome may be important for \(\alpha\beta\) T cell development and lineage commitment (25). This would occur through initial rearrangements of V\(\alpha\) gene segments, or the delta Rec elements, to J\(\alpha\) gene segments (25, 35–37). Our findings demonstrate that excision of TCR\(\delta\) chain genes from the chromosome per se is not required for \(\alpha\beta\) T cell development and lineage commitment. In this regard, development of \(\alpha\beta\) T cells is generally unperturbed in TCR\(\alpha\)/\(\delta\) mice despite the fact that all \(\alpha\beta\) T cells in these mice maintain both TCR\(\alpha\) chain genes in the chromosome (Figs 3 and 4). Furthermore, many of these TCR\(\delta\) chain genes are completely, and productively, rearranged (Table 2).

We find that the frequency of productive TCR\(\delta\) rearrangements in TCR\(\alpha\)/\(\delta\) \(\alpha\beta\) T cells is similar to that observed in TCR\(\alpha\)/\(\delta\) \(\alpha\beta\) T cells even though the TCR\(\delta\) chain genes in the latter

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**Table 1.** Thymocyte and mature T cell numbers in TCR\(\alpha\)/\(\delta\)\(\alpha\beta\) and TCR\(\alpha\)/\(\delta\)\(\gamma\delta\) mice

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<tr>
<th></th>
<th>Thymus</th>
<th></th>
<th>Spleen</th>
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<tr>
<td></td>
<td>DN</td>
<td>DP</td>
<td>CD4SP</td>
</tr>
<tr>
<td>+/-</td>
<td>29.5 ± 9.4</td>
<td>255.3 ± 33.8</td>
<td>27.2 ± 8.3</td>
</tr>
<tr>
<td>+/I</td>
<td>21.9 ± 4.6</td>
<td>286.4 ± 29.3</td>
<td>23.0 ± 4.5</td>
</tr>
<tr>
<td>I/I</td>
<td>23.8 ± 3.6</td>
<td>282.1 ± 57.7</td>
<td>19.7 ± 3.6</td>
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Mean ± SD of thymocyte subpopulations and splenic \(\alpha\beta\) and \(\gamma\delta\) T cells from TCR\(\alpha\)/\(\delta\)\(\alpha\beta\) and TCR\(\alpha\)/\(\delta\)\(\gamma\delta\) mice. All mice were ~3 weeks old and a minimum of three mice of each genotype were analyzed. Cell numbers are \(\times 10^6\). CD4SP, CD4 single positive; CD8SP, CD8 single positive.
exist on extrachromosomal circles and are not expressed (Figs 4 and 5, Table 2). Although it is possible that a small fraction of TCRδ chain gene rearrangements are selected against in developing TCRα/δI/Iαβ T cells, these findings demonstrate that there is no general selection bias against the expression of productive TCRδ chain genes in these cells. It is possible that additional mechanisms may prevent TCRδ chain expression in αβ T cells that express TCRδ chain genes. For example unpaired TCRδ chains may be rapidly degraded, as is observed for unpaired TCRα chains (38–40). In this regard, flow cytometric analyses using two pan-TCRδ chain mAbs and an anti-Vδ4 mAb failed to reveal any cell surface or intracellular TCRδ chains in TCRα/δI/Iαβ T cells (data not shown). This could be due to the inability of these antibodies to recognize TCRδ chains outside the context of a γδ heterodimer or due to the instability of TCRδ chains in these cells.

Our findings demonstrate that the genetic program of developing αβ T cells is permissive for the efficient assembly and expression of a diverse array of complete TCRδ chain genes. What then is the mechanistic basis for silencing of TCRδ chain gene expression in αβ T cells? It is possible that TCRδ chain gene expression in αβ T cells is normally extinguished by a cis-acting silencer element whose function has somehow been disrupted on the TCRα/δI allele. However, robust levels of germline TCRδ transcripts are found in DP thymocytes from TCRα/δI/Iαβ:RAG-2−/−:DO11.10β mice, where the TCRδ gene remains in the chromosome (41). Furthermore, completely assembled TCRδ chain genes can be expressed in αβ TCR transgenic T cells that have not undergone Vα to Jα rearrangements on both TCRα alleles (10). Together with our findings these data suggest that TCRδ gene expression in αβ T cells is not silenced through the activity of a cis-acting silencer element.

Silencing of TCRδ gene expression could be due to the loss of TCRδ genes on extrachromosomal circles during cell division. However, these extrachromosomal circles are generated through Vα to Jα rearrangement in DP thymocytes, which do not divide until they become mature T cells and are activated by antigenic stimulation. Thus, extrachromosomal circles containing complete TCRδ chain genes can, and do, persist.

Fig. 4. TCRδ gene rearrangement on the TCRα/δI allele. (A) Southern blot analysis of genomic DNA isolated from purified resting TCRα/δI/Iαβ T cells or kidney from wild type mice (K) was digested with BglII and probed with P4 (Fig. 1A). (B) Southern blot analysis of genomic DNA isolated from nine (one through nine) TCRα/δI/Iαβ T cell hybridomas or a single TCRα/δI/Iαβ or γδ T cell hybridoma. The DNA was digested with BglII and probed with P4 (Fig. 1A). The expected size of a germline-sized band is indicated (arrow) as are the molecular weight markers. (C) Southern blot analysis of genomic DNA isolated from kidney (K), naïve resting αβ T cells (−S) or αβ T cells that had undergone proliferative expansion (+S) from TCRα/δI/Iαβ (+/+) or TCRα/δI/Iαβ (I/I) mice. DNA was digested with StuI and probed with the Cδ probe or a probe to the RAG-2 gene (R2) as a DNA loading control. The percent retention of the Cδ probe-hybridizing bands, normalized to the intensity of the RAG-2-hybridizing band, is shown with the intensity of the Cδ-hybridizing band from the kidney sample set at 100%.
Fig. 5. Expression of CÎ³- but not CÎ²-hybridizing transcripts in TCRÎ³Î³ Î¨ T cells. RNA isolated from purified resting TCRÎ³Î³ (+/+ and TCRÎ³Î³ Î²Î² ÌŒ) Î¨ T cells, TCRÎ³Î³ â¬γ T cells (â¬γ) and RAG-2â¬γ splenocytes (R2â¬γ) was hybridized with probes to TCRÎ³ (CÎ³) and TCRÎ±(CÎ±) constant region genes and to glyceraldehyde-3-phosphate dehydrogenase (G).

Table 2. Frequency of productive TCRÎ± rearrangements in TCRÎ³Î³â¬½ and TCRÎ³Î³â¬½ Î¨ T cells

<table>
<thead>
<tr>
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<th>â¬½/Î²</th>
<th>Î²/Î²</th>
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<tbody>
<tr>
<td>DV104</td>
<td>9/16 (56)</td>
<td>6/18 (33)</td>
</tr>
<tr>
<td>DV105</td>
<td>6/27 (22)</td>
<td>7/23 (30)</td>
</tr>
<tr>
<td>ADV7</td>
<td>6/22 (27)</td>
<td>6/28 (21)</td>
</tr>
<tr>
<td>ADV17</td>
<td>7/21 (33)</td>
<td>9/30 (30)</td>
</tr>
<tr>
<td>Total</td>
<td>28/86 (32)</td>
<td>28/89 (28)</td>
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VDÎ³Î± rearrangements using four different Î³ genes (DV104, DV105, ADV7 and ADV17) were PCR amplified from resting TCRÎ³Î³ (+/+ or TCRÎ³Î³ Î²Î² ÌŒ) Î¨ T cells, cloned and sequenced. The number of in-frame rearrangements is shown as a fraction of the total number sequenced. The percentage of in-frame rearrangements is shown in brackets.

at significant levels in naive Î¨ Î¨ T cells (Fig. 4) (9, 10, 26, 27). Although simply placing TCRÎ± chain genes on extrachromosomal circles may prohibit expression, robust gene expression occurs on plasmid-based circular extrachromosomal substrates that contain appropriate cis-acting elements. In this regard, excision circles generated by VÎ± to JÎ± rearrangement contain entire TCRÎ± chain genes with their promoters and the TCRÎ± enhancer. However, optimal TCRÎ± chain gene expression in â¬γ T cells requires Ex and not EÎ± (21, 24). Furthermore, optimal germline TCRÎ± chain gene transcription in DP thymocytes also relies on Ex (41). VÎ± to JÎ± rearrangement on the wild type TCRÎ³Î³ allele place Ex, which remains in the chromosome, in trans with TCRÎ± promoters on excised extrachromosomal circles. In contrast, Ex and TCRÎ± promoters remain in cis after VÎ± to JÎ± rearrangement on the TCRÎ³Î³ allele. Thus, silencing of TCRÎ± gene expression in Î¨ Î¨ T cells likely occurs because requisite cis-acting elements, such as Ex, are placed in trans with TCRÎ± promoters on extrachromosomal circles.

Supplementary data

Supplementary data are available at International Immunology Online.

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Abbreviations

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<th>Abbreviation</th>
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<tr>
<td>DN</td>
<td>double negative</td>
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<tr>
<td>DP</td>
<td>double positive</td>
</tr>
<tr>
<td>ES</td>
<td>embryonic stem</td>
</tr>
<tr>
<td>P1</td>
<td>probe 1</td>
</tr>
<tr>
<td>RAG</td>
<td>recombinase activating gene</td>
</tr>
<tr>
<td>TEA</td>
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References


