DEMONSTRATION OF δREC-PSEUDO Jα REARRANGEMENT WITH DELETION OF THE δ LOCUS IN A HUMAN STEM-CELL LEUKEMIA

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The TCR-δ locus is located on chromosome 14, band q11.2, interspersed within the TCR-α locus (1-5). These two loci, while physically linked on one chromosome, have a precise pattern of differential expression during thymic development (3), implying an efficient regulatory mechanism that distinguishes them (6). It has recently been proposed (7) that this control might be exerted by a rearrangement that serves to delete the delta locus. A rearrangement between a δ recombining element (δRec) lying 5' of the diversity (D)δ and joining (J)δ elements, and a pseudo Jα gene (lying 3' of the δ locus) was inferred that would delete the δ locus and thus preclude TCR-δ usage. This inference was based on the cloning and sequencing of thymic extrachromosomal circular DNA excision products. This deletional event would preclude utilization of the Dδ elements in TCR-α gene rearrangement. This is supported by numerous experiments involving the cloning of rearranged TCR-α genes.

We have sequenced a direct site-specific rearrangement between the δRec and pseudo Jα genes in the human leukemic stem-cell line DU.528 (8, 9). This rearrangement resulted in the deletion of the δ locus and supports the previous predictions regarding such rearrangements.

Materials and Methods

Cell Line. The cell line DU.528 was derived from a primary leukemia with clinical and immunophenotypic features of an early T cell precursor acute lymphoblastic leukemia. The cells are CD7+, CD3-, CD4+, CD8+ and display stem-cell characteristics with the ability to differentiate into multiple lineages (8, 9).

Southern Blot Analysis. DNA was extracted from the cell line DU.528 and Southern blot analysis was performed as previously described (10, 11). The Jα75 probe was a gift of M. Minden (reference 12; “JaG”). The configuration of the Jδ1 and other δ probes have been described previously (7) and were recloned in our laboratory.

Genomic Library Preparation and Analysis. A genomic library was constructed in EMBL-3 (Promega-Biotec, Madison, WI) with partial MboI-digested DNA from DU.528. Subclones were prepared in pGEM7Zf (Promega-Biotec) and phage M13 vectors. The dideoxy chain-termination method (13) was used for DNA sequencing.

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Results

A Rearrangement Detected 75 kb 5' to Constant α. A Jα probe to the region 75 kb 5' to the constant (C)α gene detected a rearrangement in the stem-cell line DU.528. This rearrangement was cloned and sequenced. The 5' end of the sequence was identical to that previously described for the δRec element (uppercase letters, Fig. 1 C) and the 3' end (lowercase) was identical to a pseudo Ja gene (7). Between these two elements were 12 nucleotides (overlined, Fig. 1) not identified as belonging to either and consistent with "N-region" addition by the enzyme terminal deoxynucleotidyl transferase (TDT). However, the 4-bp motif CTTC found within the Dδ2 (formerly Dδ1) (7, 14) segment is also present in the 12-base stretch. Alternatively, therefore, it is possible that the 12-bp addition was formed by N-region addition of two G nucleotides between δRec and Dδ2 and N-region addition of six nucleotides TCGAGT between Dδ2 and pseudo Ja. Consistent with this latter possibility, N-region addition by TDT tends to be G rich (15).

The δRec-Pseudo Ja Rearrangement Deletes the δ Locus. A probe from between Dδ2 and Dδ3 revealed this region was deleted from both chromosomes 14 in the cell line DU.528. Southern blot analysis of germline DNA and DU.528 DNA. (A) Deletion of DNA between Dδ2 and Dδ3 from both chromosomes 14 in DU.528 detected by Probe A (Fig. 1). Restriction enzyme, Bam HI. (B) Single rearrangement in DU.528 DNA probed with Jbl probe (Fig. 1). Note absence of germline band in DU.528, consistent with deletion of the Jbl due to δRec-pseudo Ja rearrangement. Restriction enzyme, Hind III.
A Jb1 probe showed a single rearranged Jb1 band with absence of the germline configuration (Fig. 2 B). Consistent with this observation, only a single class of Jb1 rearranged clones was identified from a genomic library screened with the Jb1 probe. This rearrangement corresponded to a translocation between chromosome 1p33 and 14q11 that is the subject of a separate report. Taken together, these data show that the rearrangement between δRec and pseudo Jα deleted the Db/Jδ region on that chromosome, while events involving the translocation deleted the Dα elements on the other chromosome (16).

Discussion

We have cloned and sequenced a direct rearrangement between the δRec element and a pseudo Jα gene. There were 12 intervening nucleotides consistent with either “N-region” addition or a δRec-Dδ2-pseudo Jα rearrangement. This rearrangement deleted the δ locus.

A model of T cell ontogeny has recently been proposed in which either productive TCR-δ rearrangement or deletion of the TCR-δ locus would separate the γ/δ and α/β classes of T cells (7). The prediction regarding deletion of the delta locus in T cells undergoing TCR-α rearrangements was based on sequence analysis of extrachromosomal, circular-DNA from human thymus. This report confirms the existence of such rearrangements by analysis of a direct recombination that deletes the δ locus in a human leukemic stem-cell line. This cell is CD7+ but CD3−, CD4−, CD8− and is able to differentiate into multiple lineages. Given the phenotype of this cell, it is likely that the δRec and pseudo Jα rearrangement is an event that occurs early in T cell development and before rearrangement of the TCR-α locus.

The presence of intervening nucleotides was not predicted and could be attributed to either “N-region” sequence alone or a δRec-Dδ2-pseudo Jα rearrangement with addition of “N-region” sequences. While the extra bases could be added by non-TDT mechanisms, the presence of the intervening nucleotides might suggest that the rearrangement occurred at an early time in lymphoid ontogeny when the enzyme or enzymes involved in “N-region” addition (e.g., TDT) are active.

The demonstration of a direct rearrangement between δRec and pseudo Jα genes provides additional support for the view that this rearrangement defines an intermediate event in the process of differentiation towards the α/β lineage.

Summary

It has been hypothesized that a rearrangement between the δ recombining element (δRec) and a pseudo Jα gene serves to delete the TCR-δ locus before rearrangement of the TCR-α genes. We have now sequenced a direct, site-specific rearrangement between the δRec element and a pseudo Jα gene in a human leukemic stem-cell line. Putative “N-sequence” addition was noted at the site of recombination, suggesting that this event occurred at a time when the enzyme(s) involved in N-region addition were active in this cell. This provides support for the view that deletion of the TCR-δ locus is required before rearrangement of the TCR-α chain genes.

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References