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Immunology

Abnormal recombination products result from aberrant DNA rearrangement of the human T-cell antigen receptor β-chain gene

(DNA inversion/T-cell tumor line CEM)

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ABSTRACT **Two unusual rearrangements of the T-cell antigen receptor β-chain gene have occurred in the human T-cell tumor line CEM. The β chain of the T-cell antigen receptor is encoded in germ-line DNA by immunoglobulin-like gene segments that rearrange during the somatic development of T cells to form active genes. Structural analysis of rearranged immunoglobulin genes has already revealed a great deal about the mechanisms by which these genes rearrange. To further characterize the mechanism by which β-chain genes rearrange, we have determined the organization of the rearranged β-chain gene segments in the human T-cell tumor line CEM. Three rearranged joining (J) or diversity (D) segments of the β-chain gene are found in CEM. One of these segments rearranged during the formation of a normal rearranged β-chain gene that comprises a variable (V_β), D_β, and J_β gene segment associated with a constant region gene segment. Two abnormal recombination products are found at the other rearranged β-chain locus. One product has the structure, J_β-D_β-J_β, with the J_β gene segments joined in a head-to-head fashion, while the other one consists of a V_β-D_β recombined segment not associated with a J_β gene segment. We propose that the J_β-D_β-J_β structure was formed by an inversion of 6 kilobases of DNA and subsequently, a V_β-D_β rearrangement occurred. The presence of these products in CEM has important implications for our understanding of the mechanism by which somatic rearrangements of β-chain gene segments occur.**

The ability of T cells and B cells to specifically recognize the many foreign antigens presented to them is dependent on their capacity to generate a diverse set of cell surface receptors. Studies have demonstrated that the α and β subunits of the T-cell antigen receptor are very similar in structural organization to the immunoglobulin heavy (H) and light chains (reviewed in ref. 1). The complete human β-chain gene locus consists of two closely linked constant (C_β) segments, $C_{\beta 1}$ and $C_{\beta 2}$, each of which is preceded by a cluster of functional joining (J_β) segments and at least one diversity (D_β) segment, and an unknown number of variable (V_β) segments (see Fig. 1*A* and refs. 2–6). During T-cell development, one of each of the V_β, D_β, and J_β segments is brought together by DNA rearrangement to generate a rearranged β-chain gene. The number of possible different β chains encoded by these segments is large, because their combinatorial assortment appears to be random, and the recombination joints are imprecise and occasionally contain additional nucleotides (*N* gene segments; ref. 7). Understanding the mechanisms by which these gene segments rearrange is critical to our understanding of T-cell function and chromosomal rearrangement.

Several different lines of evidence have contributed to our current understanding of the mechanisms by which immunoglobulin genes rearrange. Analysis of a number of immunoglobulin germ-line gene segments and their rearranged products has suggested that sequences flanking the germ-line segments play a critical role in directing somatic DNA recombination (reviewed in refs. 8 and 9). These flanking sequences consist of three elements—a conserved heptamer, a nonconserved spacer sequence of either 12 or 23 base pairs (bp), and a conserved nonamer. DNA recombination can only occur between one segment with a 12-bp recognition sequence and another segment with a 23-bp recognition sequence (the 12/23 rule). These sequences appear to determine only the approximate location of the rearrangement event with the result that out-of-frame gene products frequently occur. Furthermore, a variety of aberrantly rearranged immunoglobulin genes have been identified in which the rearrangement mechanism appears to have misinterpreted these signals and generated unusual gene products that cannot produce functional immunoglobulin polypeptides (10–12). Similarly, a large portion of the estimated 99% of pre-T cells that die in the thymus (reviewed in ref. 13) probably have nonproductive rearrangements of their α- or β-chain genes of the T-cell antigen receptor.

Three general mechanisms have been proposed to explain the process by which gene segments undergo DNA rearrangement—looping out/deletion, sister chromatid exchange, and inversion. Evidence for each of these mechanisms has derived from the identification of both productively and aberrantly rearranged genes in B and T cells (reviewed in refs. 1, 8, and 9). The human T-cell tumor line CEM was identified during a search for T cells that utilized mechanisms of β-chain gene rearrangement, other than looping out/deletion. One β-chain locus of CEM contains two aberrant recombination products. One product, a $J_{\beta 1}$-D_β-$J_{\beta 2}$ structure, was created by a chromosomal inversion, and the other product was formed by a V_β-D_β rearrangement without D_β-J_β rearrangement. The second β-chain locus of CEM has undergone a normal V_β-D_β-J_β recombination. The finding of these aberrant products has implications for our understanding of the recognition signals for DNA rearrangement, the mechanisms involved, and the relative timing of D_β-J_β and V_β-D_β rearrangements.

MATERIALS AND METHODS

Southern Blots. Genomic DNA was prepared from human B cells and T cells, digested with restriction enzymes, electrophoresed on agarose gels, and transferred to nitrocellulose by the method of Southern (14). Hybridization, washing, and autoradiography were as described (15).

Construction and Screening of CEM Subgenomic and Genomic Libraries. *Eco*RI and *Hin*dIII DNA fragments containing the rearranged β-chain genes of CEM were partially purified by agarose gel electrophoresis and ligated into

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Abbreviations: V, variable; D, diversity; J, joining; C, constant; H, heavy; kb, kilobase(s); bp, base pair(s).

pBR322. Transformation of *Escherichia coli* MC1061 cells and screening of the subgenomic libraries were as described (15). A genomic CEM library was constructed from *Sau*3A partially digested CEM DNA ligated into the λ bacteriophage vector EMBL 3 (16) according to the methods of Maniatis *et al.* (17). Hybridization, washing, and autoradiography were as described for Southern blots.

DNA Sequence Analysis. DNA was sequenced by the dideoxy method using M13 phage as described in the Amersham M13 sequencing kit.

RESULTS

The Human T-Cell Tumor Line CEM Contains the Products of Unusual β-Chain Gene Segment Rearrangements. We have identified an unusual human thymus β-chain cDNA that probably resulted from a sister chromatid exchange with unequal crossing over between the $J_{\beta 1}$ and $J_{\beta 2}$ gene segment clusters (3). To study this mechanism of β-chain rearrangement, we searched for a T-cell line that contained the products of such a rearrangement. Eight T-cell tumor line DNA samples and nine T-cell leukemic DNA samples were screened by Southern blot analysis for the presence of unusually rearranged β-chain genes. The $C_{\beta 1}$ gene segment and the $J_{\beta 2}$ gene segment cluster are located approximately 4 kilobases (kb) apart in germ-line DNA (Fig. 1*A*). These gene segments are found on two different restriction fragments after *Eco*RI digestion of germ-line or of normally rearranged T-cell DNA. Only the DNA derived from one T-cell tumor line, CEM, yielded a restriction fragment (10.5 kb) that appeared to have hybridized to the $J_{\beta 1}$, $J_{\beta 2}$, and C_β probes (data not shown). To exclude the possibility that two different DNA fragments were comigrating in the agarose gel, the Southern blot analyses were repeated with CEM DNA digested with the restriction enzymes *Hin*dIII, *Bgl* II, and *Xba* I, which detect most rearrangements in both J_β segment clusters when the $J_{\beta 1}$ and $J_{\beta 2}$ probes are used (Fig. 1*A* and refs. 2–6). In these analyses, the $J_{\beta 1}$ probe hybridized to a single fragment, which was the same size as one of the two fragments that hybridized to the $J_{\beta 2}$ probe. An example of these analyses is shown in Fig. 2. Here, the $J_{\beta 1}$ probe hybridized to a 8.3-kb *Hin*dIII fragment while the $J_{\beta 2}$ probe hybridized to the 8.3-kb fragment and a 6.0-kb fragment. The simplest hypothesis to explain this observation was that these gene segments were arranged in the following order: $C_{\beta 1}$-$J_{\beta 1}$-$J_{\beta 2}$-$C_{\beta 2}$. Contributing to this hypothesis were the results of a Southern blot of *Hin*dIII-digested DNA from CEM hybridized to the $D_{\beta 2}$ probe. $D_{\beta 2}$ and $J_{\beta 2}$ gene segments usually reside on the same germ-line or rearranged *Hin*dIII fragment (Fig. 1*A*). In fact, a 5.6-kb *Hin*dIII fragment appeared (data

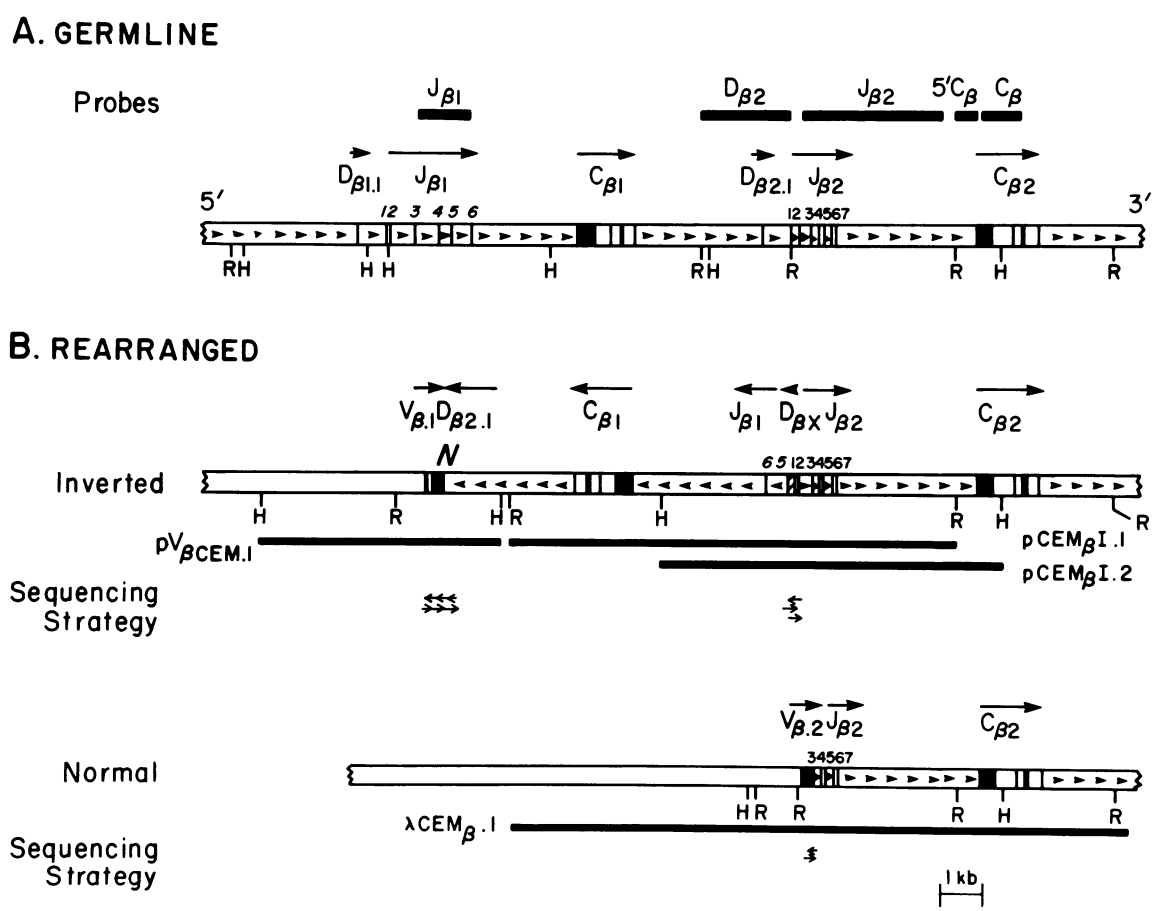

FIG. 1. Organization of the human germ-line β-chain gene locus (*A*) and the rearranged β-chain genes of CEM (*B*). The germ-line organization has been reported (2–6). Exons of the various DNA segments are shown as vertical lines or filled-in boxes. The $J_{\beta 1}$-$D_{\beta X}$-$J_{\beta 2}$ joint of the inverted rearranged β-chain gene is shown by diagonal lines. The 5′ to 3′ orientation of the germ-line DNA segments is indicated both by arrows over the labeled segments and arrowheads between segments. The orientation of the rearranged segments relative to their germ-line orientation is similarly shown by arrows and arrowheads. The six functional $J_{\beta 1}$ gene segments are labeled in italic numbers. The location of *Eco*RI (R) and *Hin*dIII (H) restriction enzyme sites are indicated. The cloned parts of the rearranged β-chain genes of CEM are indicated as bars. The direction and extent of sequencing are indicated by individual arrows. The letter N indicates the 19 nucleotides of unknown origin at the 3′ end of $V_{\beta.1}$. Probes are restriction enzyme fragments; $J_{\beta 1}$ is a 1.3-kb *Pvu* II–*Pvu* II fragment containing $J_{\beta 1.4}$ and $J_{\beta 1.5}$; $D_{\beta 2}$ is a 2.2-kb *Eco*RI–*Eco*RI fragment containing $D_{\beta 2.1}$; $J_{\beta 2}$ are contiguous 1.9-kb *Pvu* II–*Pvu* II and 1.5-kb *Pvu* II–*Eco*RV fragments containing the gene segments $J_{\beta 1.3}$ through $J_{\beta 1.7}$; $5'C_\beta$ is a 0.6-kb *Eco*RI–*Bgl* II fragment containing 0.55-kb of 5′-flanking sequence and 50 bp of coding sequence of the $C_{\beta 2}$ gene segment; and C_β is a 1.0-kb *Bgl* II–*Bgl* II fragment from $C_{\beta 2}$. Both the $5'C_\beta$ and the C_β probes hybridize to both $C_{\beta 1}$ and $C_{\beta 2}$ gene segments.

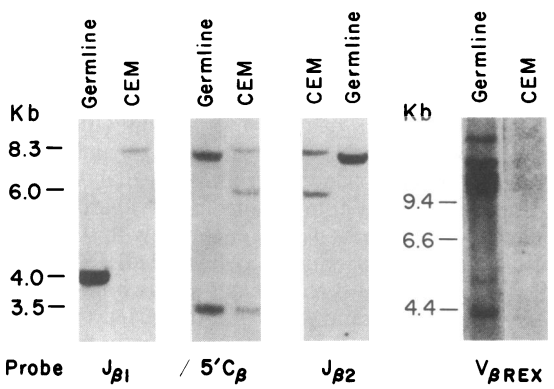

FIG. 2. Demonstration by Southern blot analysis of a rearranged DNA fragment of CEM hybridizing to the $J_{\beta 1}$, $J_{\beta 2}$, and C_β probes. Ten micrograms of DNA from B cells (Germline) and CEM were digested with *Hin*dIII, fractionated on a 0.6% (first six lanes) or 0.8% (last two lanes) agarose gel, transferred to nitrocellulose, hybridized with the probes indicated below the lanes, and analyzed by autoradiography. $V_{\beta REX}$ is a 1.1-kb *Eco*RI–*Sma* I fragment, obtained from a cloned rearranged β-chain gene, and contains one of the members of the *REX* V_β gene segment family (7) and $J_{\beta 1.2}$ (unpublished data). The sizes, in kb, of the hybridizing fragments are indicated to the left of the lanes.

not shown), suggesting physical dissociation of the $D_{\beta 2}$ and $J_{\beta 2}$ gene segments and that the gene segments were ordered $D_{\beta 2}$-$C_{\beta 1}$-$J_{\beta 1}$-$J_{\beta 2}$-$C_{\beta 2}$ at one of the two CEM rearranged β-chain loci.

Characterization of the Rearranged β-Chain Genes of CEM. To better understand the recombination mechanism that generated the rearranged β-chain genes in CEM, we have isolated bacteriophage and plasmid clones containing these genes. Unfortunately only clones (e.g., λCEM$_\beta$.1, Fig. 1*B*) containing the 6.0-kb *Hin*dIII fragment that hybridized to the $J_{\beta 2}$ probe were isolated from a CEM genomic library (unpublished results). The 10.5-kb *Eco*RI fragments and 8.3-kb *Hin*dIII fragments that hybridize to the C_β, $J_{\beta 1}$, and $J_{\beta 2}$ probes were partially purified by agarose gel electrophoresis and ligated to pBR322. The appropriate clones obtained from subgenomic libraries were labeled pCEM$_\beta$I.1 and pCEM$_\beta$I.2 (Fig. 1*B*). A plasmid containing the 5.6-kb *Hin*dIII fragment that hybridized to the $D_{\beta 2}$ probe was isolated from a similarly constructed subgenomic library and named pV$_{\beta CEM.1}$. All the cloned fragments were subjected to restriction enzyme mapping and Southern blot analysis (data not shown). One of the CEM rearranged β-chain genes (labeled "inverted" in Fig. 1*B*) contains $J_{\beta 1.5}$, $J_{\beta 1.6}$, and all seven functional and one pseudo $J_{\beta 2}$ segments. Apparently a 6-kb fragment of DNA containing $J_{\beta 1.5}$, $J_{\beta 1.6}$, $C_{\beta 1}$, and $D_{\beta 2.1}$ had inverted, placing the $J_{\beta 1}$ and $J_{\beta 2}$ gene segments in a head-to-head configuration, i.e., $D_{\beta 2.1}$-$C_{\beta 1}$-$J_{\beta 1}$-$J_{\beta 2}$-$C_{\beta 2}$. The "normally" rearranged β-chain gene has deleted the $J_{\beta 1}$ gene cluster, $C_{\beta 1}$, $J_{\beta 2.1}$, and $J_{\beta 2.2}$, retaining the other $J_{\beta 2}$ gene segments.

The fine structure of the recombination joints was determined by their nucleotide sequences (Fig. 3). These sequences were compared to their germ-line J_β and D_β counterparts (unpublished results and refs. 3, 5, and 6). The $J_{\beta 1.5}$ and $J_{\beta 2.1}$ gene segments were present in a head-to-head configuration and were identical to their germ-line genes, with 5 and 15 nucleotides deleted from their 5′ ends, respectively (Fig. 3*A*). It has been shown that both *D-J* and *V-D* recombination often occurs with loss of nucleotides from *D* and *J* segments (reviewed in refs. 1, 8, and 9). Twelve base pairs separate $J_{\beta 1.5}$ from $J_{\beta 2.1}$; 9 of these 12 bp are homologous to the germ-line sequence of $D_{\beta 1.1}$. Although we cannot exclude the possibility that this represents either a $D_{\beta 1.1}$ polymorphism or even less likely an *N* gene segment (7), we have chosen to label this sequence $D_{\beta X}$, corresponding to unidentified D_β sequences in the human genome.

A second recombination event in this β-chain locus joined a V_β gene segment (designated $V_{\beta.1}$) to the inverted $D_{\beta 2.1}$ gene segment, deleting all of its coding sequence but retaining most of its 5′-flanking sequences (Fig. 3*B*). This V_β segment is homologous to other V_β segments but appears to be a pseudogene because it has a nucleotide deletion, resulting in a frameshift mutation (designated X in Fig. 3*B*). Between $V_{\beta.1}$ and the start of germ-line $D_{\beta 2.1}$ sequence are 19 nucleotides of unknown origin; they could be part of the germ-line $V_{\beta.1}$ gene segment, a D_β segment, an *N* gene segment, or a combination of the aforementioned.

The other rearranged β-chain gene of CEM, the product of a recombination between a V_β gene segment (designated $V_{\beta.2}$), a D_β gene segment, and the $J_{\beta 2.3}$ gene segment, contains no unusual features (Fig. 3*C*). Although we have not sequenced the complete rearranged $V_{\beta.2}$ gene segment, its identity as a V_β segment is inferred from its homology to the 3′ end of other V_β segments. If the remainder of the $V_{\beta.2}$ sequence is in frame, then this rearranged β-chain gene codes for a functional β-chain polypeptide. There is no reason presently to suspect that the recombination events in the two β-chain genes of CEM did not occur independently of each other.

DISCUSSION

We have characterized the rearranged β-chain genes of the human T-cell tumor line CEM. One of its β-chain genes has undergone an inversion of a 6-kb DNA fragment from the $J_{\beta 1.5}$ segment to the $D_{\beta 2.1}$ segment. This inversion has led to the formation of two recombination products, $J_{\beta 1.5}$-$D_{\beta X}$-$J_{\beta 2.1}$, with the J_β gene segments in a head-to-head configuration and $V_{\beta.1}$-$D_{\beta 2.1}$ in a tail-to-tail configuration. The other β-chain gene has undergone what appears to be a normal recombination, generating the product, $V_{\beta.2}$-D_β-$J_{\beta 2.3}$. The frequency of this particular aberrant rearrangement in both fetal and adult thymic T cells and peripheral blood T cells must be low as no unique rearranged bands were detected in Southern blot analysis of these samples (A.D.D., William M. Strauss, J.G.S., unpublished results). Furthermore, although we have demonstrated the existence of an aberrant V_β-D_β product, it remains to be seen whether this represents a legitimate intermediate in a productive rearrangement of the β-chain gene or a common aberrant rearrangement of V_β and D_β gene segments. We note that *V-D* rearrangement without *D-J* rearrangement would not be detected in Southern blot analysis using standard C or J probes.

To explain the formation of $J_{\beta 1.5}$-$D_{\beta X}$-$J_{\beta 2.1}$ and $V_{\beta.1}$-$D_{\beta 2.1}$, we propose the model depicted in Fig. 4. In the first step (Fig. 4), $D_{\beta X}$ recombines with $J_{\beta 1.5}$, correctly using the 3′- and 5′-recombination signals of $D_{\beta X}$ and $J_{\beta 1.5}$, respectively. Next (Fig. 4, stage I), $D_{\beta X}$-$J_{\beta 1.5}$ recombines by inversion with $J_{\beta 2.1}$. Finally (Fig. 4, stage II), $V_{\beta.1}$ recombines with the inverted $D_{\beta 2.1}$, deleting its 3′-recombination signal, the $D_{\beta 2.1}$ exon, and six of the nucleotides of the 5′ heptamer. Although the location of the germ-line $V_{\beta.1}$ segment relative to the J_β and C_β segments is not known, the deletion of all members of the V_β gene segment family hybridizing to a V$_{\beta REX}$ probe (Fig. 2) probably places $V_{\beta.1}$ on the 5′ side of the J_β and C_β gene segments. Recombination of the $V_{\beta.1}$ gene segment with the inverted $D_{\beta 2.1}$ gene segment probably occurs by a looping out/deletion mechanism. We note that the last two recombination steps violate the 12/23 rule. One can speculate that the last two recombination steps would obey the rule if either the 12-bp recognition signal on the 5′ side of the $D_{\beta X}$-$J_{\beta 1.5}$ intermediate recognized a cryptic 23-bp recognition signal on the 5′ side of $J_{\beta 2.1}$ or the recognition signal on the 5′ side of $D_{\beta X}$ was in fact a 23-bp signal. Furthermore, the recognition

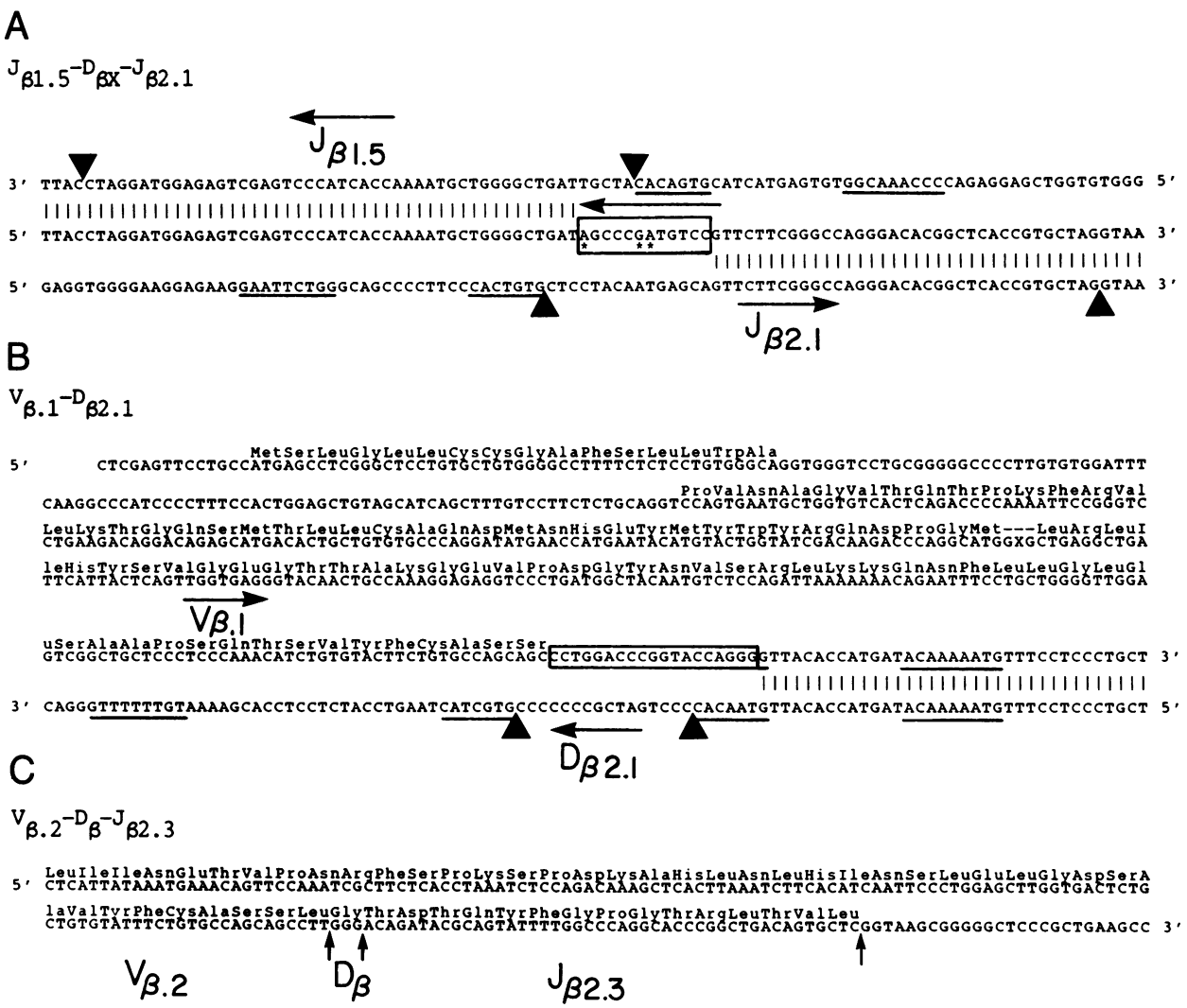

FIG. 3. Nucleotide sequences of the recombination points of the rearranged β-chain genes of CEM. The germ-line $J_{\beta 1.5}$, $J_{\beta 2.1}$, $D_{\beta 1.1}$, and $D_{\beta 2.1}$ sequences have been reported (3–5). Nucleotide sequences of the recombination points are shown in 5′ to 3′ polarity as is that of germ-line $J_{\beta 2.1}$; germ-line $J_{\beta 1.5}$ and $D_{\beta 2.1}$ sequences are displayed in 3′ to 5′ polarity. The boundaries of germ-line gene segment exons are shown by arrowheads. Nonamer and heptamer recognition sequence signals are underlined. Areas of homology between sequences are indicated by vertical lines. (*A*) Nucleotide sequence of $J_{\beta 1.5}$-$D_{\beta X}$-$J_{\beta 2.1}$ (*Middle*) is compared to germ-line $J_{\beta 1.5}$ (*Top*) and $J_{\beta 2.1}$ (*Bottom*). The sequence of $D_{\beta X}$ is boxed; it is homologous to that of $D_{\beta 1.1}$ except for the three starred nucleotides. (*B*) The nucleotide sequence of $V_{\beta.1}$–$D_{\beta 2.1}$ is compared to the germ-line sequence of $D_{\beta 2.1}$. The probable nucleotide deletion is indicated by the letter X. Amino acid sequence of the leader and exon of $V_{\beta.1}$ is shown by the three-letter code above the nucleotide sequence. The 19 nucleotides of unknown origin at the 3′ end of $V_{\beta.1}$ are boxed. (*C*) The boundaries of the probable origins of coding gene segments of $V_{\beta.2}$-$D_{\beta X}$-$J_{\beta 2.3}$ from germ-line D_{β} and $J_{\beta 2.3}$ gene segments is indicated below the nucleotide sequence by vertical arrows. Deduced amino acid sequence is shown above the nucleotide sequence.

signal on the 3′ side of $V_{\beta.1}$ could have been "compatible" with the signal on the 3′ side of $D_{\beta 2.1}$. Knowledge of the recognition signals of $D_{\beta X}$ and $V_{\beta.1}$, especially if they differed from classical ones, would add to our understanding of the sequence requirements for DNA recombination. Although another mechanism not using recombination signals might have produced the unusually rearranged β-chain gene of CEM, this is unlikely as the recombination joints do not appear to be random.

This unusual rearrangement of a β-chain gene bears a striking resemblance to an aberrant rearrangement in an immunoglobulin H-chain gene described by Alt and Baltimore (10). These authors isolated a J_{H1}-D_{H1}-J_{H3} recombination product with the J_{H1}-D_{H1} joined head-to-head with the J_{H3} gene segment, and a 12- and 23-bp recognition signals joined in a tail-to-tail configuration, on the 5′ side of the former product. The authors speculate that these two products formed as a result of inversion of the DNA segment containing J_{H1}. Because of a different pattern of recombination signals present in the flanking regions of J_H and D_H segments compared to J_{β} and D_{β} segments, the 12/23 rule was not violated. Both the orientation and the structure of these H-chain gene products are analogous to those of the β-chain rearrangement described here.

We have shown that β-chain genes, like immunoglobulin genes, can undergo unusual rearrangements. The physiological significance of these rearrangements remains to be determined. Several groups have suggested that the β-chain locus may be involved in chromosomal translocations in T cells of normal individuals as well as those from individuals with the autosomal recessive disease, ataxia telangiectasia (18–20). These chromosomal translocations may be due to errors in the rearrangement process of β-chain genes similar to those described here. The normal rearrangement process has an inherent flexibility that is thought to play an important role in generating diversity of rearranged β-chain genes. The advantages of this flexibility appear to outweigh the disadvantages of nonproductively and defectively rearranged β-chain genes and chromosomal translocations that do occur. Further study of the mechanism by which β-chain genes

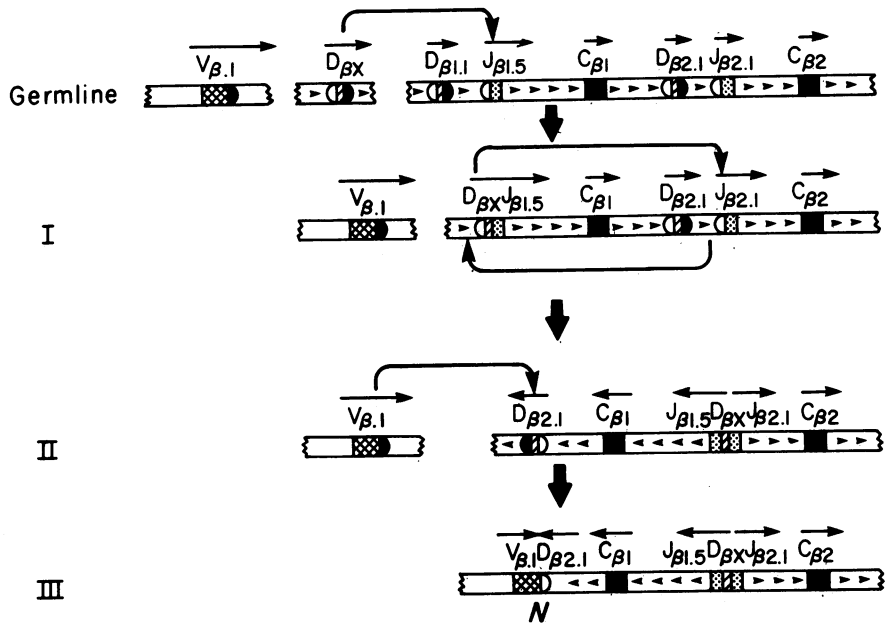

FIG. 4. A model involving inversion to explain the "inverted" β-chain gene of CEM. The organization of the germ-line gene segments, intermediate (I, II), and final (III) products of the model are depicted. Gene segments for V_β, D_β, J_β, and C_β are shown by cross-hatched, hatched, dotted, and solid rectangles, respectively. The 5'- and 3'-recombination signals are shown as open and solid half-circles, respectively. The 5' to 3' orientation of the gene segments is indicated by arrows above and arrowheads between gene segments. Curved arrows depict DNA recombination between the gene segments at either end of the arrows. The unknown distance between $V_{\beta.1}$ or $D_{\beta X}$ and $D_{\beta 1.1}$ is indicated by a break in the bar. The 19 nucleotides of unknown origin at the 3' end of $V_{\beta.1}$ are indicated by the letter *N*. The model is not drawn to scale.

rearrange will explain both the apparent specificity and flexibility of this process.

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