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A joining-diversity-joining complex generated by inversion mechanism and a variable-diversity complex in the β-chain gene of the human T-cell receptor

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ABSTRACT

We have analysed an inactive allele of the β-chain gene of the T-cell receptor in a human T-cell line HPB-ALL. Comparison with germline sequences showed that HPB-ALL has a joining (J)-diversity (D)-J complex recombined in head-to-head configuration and a variable (V)-D complex in tail-to-tail configuration. These results demonstrate that the inversion mechanism functions in the β-chain gene of the T-cell receptor. The presence of the V-D complex suggests that V-D recombination could occur prior to D-J recombination although there is no definite proof that the V-D complex is an intermediate to form the V-D-J complex.

INTRODUCTION

The T-cell receptor gene has striking similarities with the immunoglobulin gene not only in its structure but also in the mode of rearrangement (1-13). The T-cell receptor is composed of two polypeptide chains, α and β (14-16), both of which consist of a variable (V) and a constant (C) region of a similar size. The V region of the α and β chains, together with the γ chain with unknown function (9), are encoded by multiple germline gene segments, i.e. V, diversity (D), and joining (J), which are brought together to complete a V-region exon in T lymphocytes.

The conserved heptamer and nonamer sequences flanking the V, D, and J segments of the T cell receptor genes resemble those of the immunoglobulin genes. Recombinations of the V, D, and J segments occur only between the two gene segments, one flanked by the heptamer and nonamer sequences with a 12-bp spacer and the other by the same sequences with a 23-bp spacer (12/23 spacer rule). Since there are multiple members for each segment, random association of those three segments contributes to amplification of V-region diversity of the T-cell receptor as shown in the immunoglobulin. Three mechanisms, deletion, sister chromatid exchange, and inversion have been postulated to operate for V-D-J recombination in both the immunoglobulin and

T-cell receptor genes (17-24).

During course of the study on the structure of the β-chain gene in a human T-cell line HPB-ALL, we found a $J_{\beta 1.5}$-$D_{\beta 1.1}$-$J_{\beta 2.1}$ complex recombined in head-to-head configuration and a pseudo $V_{\beta\cdot HPB}$-$D_{\beta 2.1}$ complex in tail-to-tail configuration. These results demonstrate that the inversion mechanism can occur not only in the immunoglobulin gene but also in the β-chain gene of the human T-cell receptor. Molecular mechanism to create these complexes might include a novel mechanism of recombination such as the violation of the 12/23 spacer rule or an intermediate D-J complex flanked by conserved signal sequences with 12-bp and 23-bp double spacers. In addition, the presence of the V-D complex reserves the possibility that it could be an intermediate to the V-D-J complex. During preparation of this manuscript the inversion mechanism was also demonstrated to operate in a functional V-D-J recombination in a murine T-cell line (24).

MATERIALS AND METHODS

Materials

HPB-ALL was kindly provided by S. Morikawa of Shimane Medical College (25). The karyotype of HPB-ALL was shown to be near-tetraploid with four normal chromosome 7's by conventional banding method (Ohno, H., personal communication). High molecular weight DNA was prepared as described elsewhere (26). Murine β-chain cDNA clone 86T5 (2) was a generous gift of M. M. Davis of Stanford Unviersity. Restriction enzymes, T4 DNA ligase, and M13 sequence kit were purchased from Takara Shuzo (Kyoto) and Toyobo (Osaka), and used according to suppliers' directions.

Methods

Southern blot transfer and hybridization were done as described previously (27, 28). EcoRI or BglII fragments containing germline and rearranged alleles of β-chain gene locus of the T-cell receptor were isolated by agarose gel electrophoresis and ligated with λgtwes·λB or Charon 28 vectors using T4 DNA ligase. The recombinant DNA was packaged *in vitro*, and the phages were screened using murine and human β gene fragments as probes by the method of Benton and Davis (29). DNA was sequenced by the dideoxy method using M13 phage (30) or pUC plasmid (31) as the vector. A part of DNA sequence of $\psi V_{\beta\cdot HPB}$ segment was determined using series of deletion mutants of pUC plasmid (32).

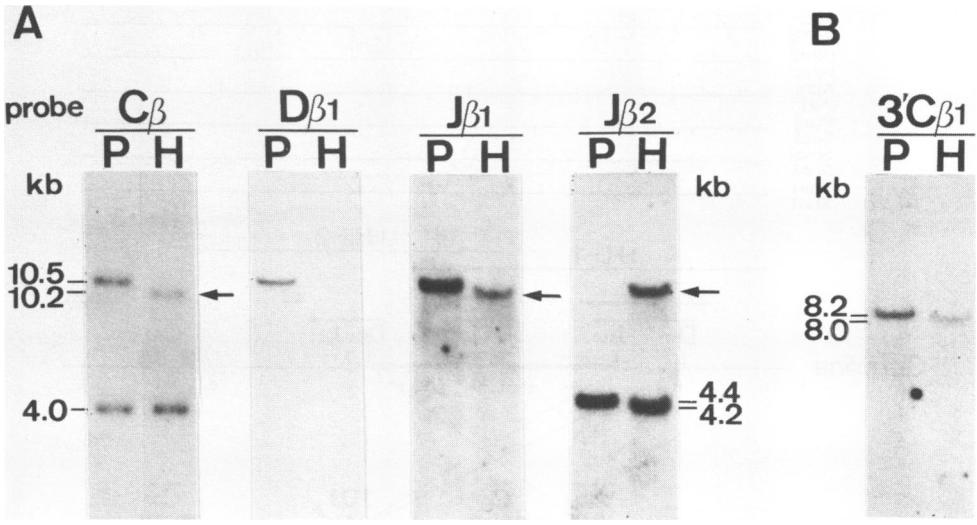

Fig. 1. Rearrangement of the β-chain gene of the T-cell receptor in HPB-ALL DNA. High molecular weight DNA (2 μg each) of human placenta and HPB-ALL were digested with EcoRI (A) and BglII (B). Southern blots of the digests were hybridized with probes as shown in Fig. 2 except that the C_β probe was obtained from the murine β-chain cDNA clone 86T5. Arrows indicate the 10.2-kb EcoRI fragment. Filters were washed at 65 C in 15 mM NaCl/1.5 mM sodium citrate/0.1% Na dodecylSO_4. Origins of DNA in each lane are abbreviated as follows: P, human placenta; H, HPB-ALL.

RESULTS AND DISCUSSION

Rearrangement of the β-chain genes of the T-cell receptor in HPB-ALL

To know the organization of the human β-chain genes of the T-cell receptor in HPB-ALL, Southern blot filters of HPB-ALL DNA digested with EcoRI were hybridized with C_β, $D_{\beta1}$, $J_{\beta1}$, and $J_{\beta2}$ probes (Fig. 1A). Since EcoRI digestion yielded two rearranged fragments (10.2 and 3.8 kb) hybridizing with the $J_{\beta2}$ probe, the $J_{\beta2}$ segments had rearranged on both chromosomes of HPB-ALL. Although deletion model predicts removal of the $J_{\beta1}$ segment upon formation of a V-D-$J_{\beta2}$ complex, the 10.2-kb EcoRI fragment hybridized also with the $J_{\beta1}$ and C_β probes, but not with the $D_{\beta1}$ probe, indicating that the 10.2-kb EcoRI fragment contains both the $J_{\beta1}$ and $J_{\beta2}$ segments which are separated by 7.5-kb DNA and located on two separate EcoRI fragments in the germline chromosome (Fig. 2). The 3' $C_{\beta1}$ probe detected a rearranged 8.0-kb BglII fragment (Fig. 1B), which should be also removed according to the

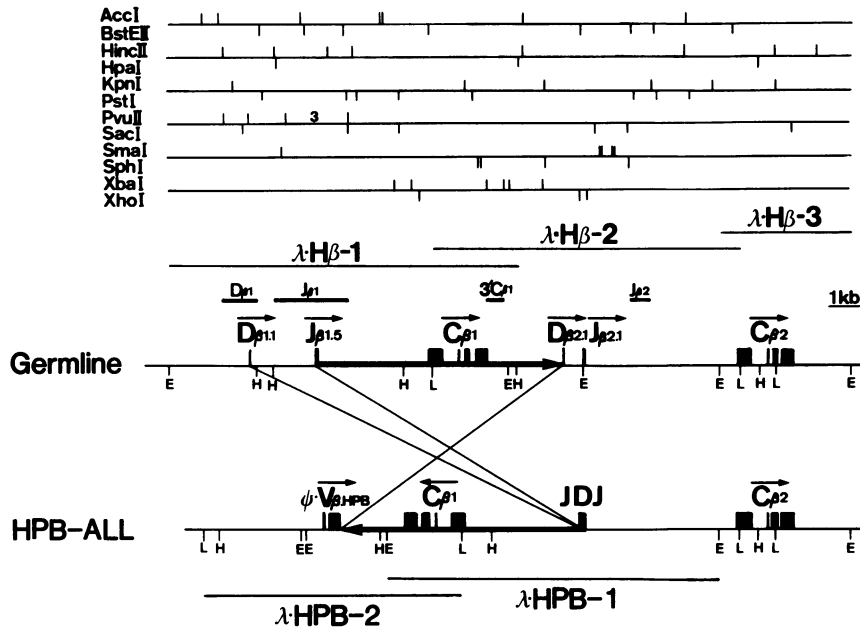

Fig. 2. Comparison of restriction maps of the β-chain genes of the T-cell receptor in the germline and the aberrantly rearranged chromosomes in HPB-ALL. Restriction maps of twelve enzymes of the germline chromosome are also shown above the map. Isolation of human germline Cβ1 fragment (10.5-kb EcoRI) and $C_{\beta 2}$ fragment (4-kb EcoRI) were described elsewhere (Ikuta et al., 1985) and designated as λ·Hβ-1 and λ·Hβ-3, respectively. To complete the organization of human T-cell receptor β-chain genes, we isolated human Jβ2 segments. BglII digestion of human placenta DNA produced a 9.5-kb fragment hybridizing to human Jβ2 probe isolated from H·Vβ·MT1-1 (Ikuta et al., 1985). The BglII fragment was partially purified by agarose gel electrophoresis and cloned with Charon 28 phage (λ·Hβ-2). Restriction site maps of three overlapping clones revealed the organization of human Jβ and Cβ genes. Linkage of Cβ2 gene with the other members in HPB-ALL was deduced from Southern blot hybridization analysis. Filled rectangles indicate exons. Only the Jβ1.5 and Jβ2.1 segments are shown among the Jβ segments. Horizontal arrows show the direction of transcription of each gene segment. Horizontal lines above and beneath the maps indicate the range of phage clones. The broad arrows on the maps indicate the inverted DNA. Fragments used as probes are shown by horizontal bars. The Dβ1, Jβ1, 3'Cβ1, and Jβ2 probes are in 1.1-kb AccI/HindIII, 2.3-kb HindIII/PstI, 0.6-kb XbaI, and 1.0-kb BstEII fragments, respectively. Oblique lines linking two maps indicate corresponding recombination points between germline and HPB-ALL sequences. The numeral in the map of PvuII is the number of unassigned sites in this region for the same enzyme. Abbreviated restriction sites are as follows: E, EcoRI; H, HindIII; L, BglII.

deletion model. The results suggest that the 10.2-kb EcoRI fragment containing the $J_{\beta 1}$ sequence was created by a mechanism other than the deletion mechanism. On the other hand, the other rearranged $J_{\beta 2}$ fragment (3.8 kb)

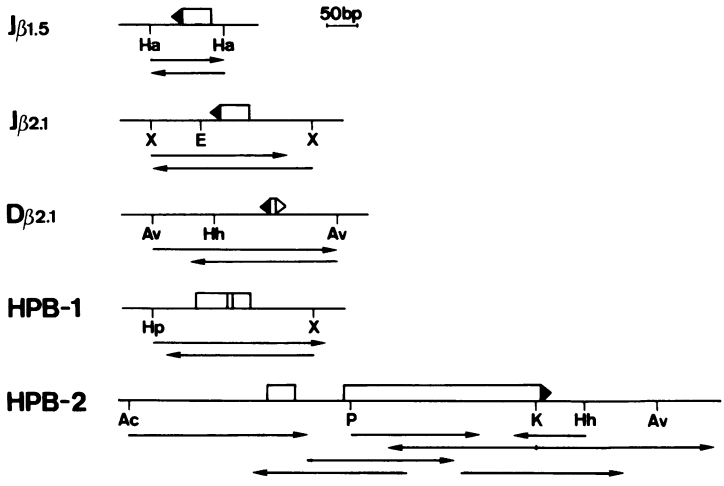

Fig. 3. Sequence strategy for the germline and rearranged Jβ and Dβ segments. Open rectangles indicate the coding region of Jβ, Dβ, and ψVβ segments. Open and filled triangles indicate the recombination signals with 23-bp and 12-bp spacer, respectively. The direction of transcription is from left to right. The direction and ranges of nucleotide sequences determined are shown by horizontal arrows. Restriction sites in addition to those shown in Fig. 2 are abbreviated as follows: Ac, AccI; Av, AvaII; Ha, HaeIII; Hh, HhaI; Hp, HpaII; K, KpnI; P, PstI; X, XhoI.

did not contain either $J_{\beta 1}$ or C_{β} sequences as expected by the deletion mechanism.

Isolation of the J-D-J complex of the aberrantly rearranged chromosome

The 10.2-kb EcoRI fragment hybridizing with the C_{β}, $J_{\beta 1}$ and $J_{\beta 2}$ probes and the 8.0-kb BglII fragment hybridizing with the $3'C_{\beta 1}$ probe were partially purified by agarose gel electrophoresis and cloned in λ phage vectors. Clones containing the 10.2-kb EcoRI and 8.0-kb BglII fragments were named as λ·HPB-1 and λ·HPB-2, respectively. Restriction maps and Southern blot hybridization of the two clones showed that they overlapped with each other (Fig. 2). Comparison of the restriction map of the cloned rearranged J_{β} allele with that of the germline clones (13) suggested that the rearranged allele contained the inverted $C_{\beta 1}$ segment.

Nucleotide sequences of the J-D-J complex and recombination points

To make clear the mode of rearrangement, nucleotide sequences of the regions surrounding the two recombination points and their germline counterparts were determined according to strategies shown in Fig. 3. The D_{β}, J_{β}, and V_{β} segments were identified by their homology with sequences de-

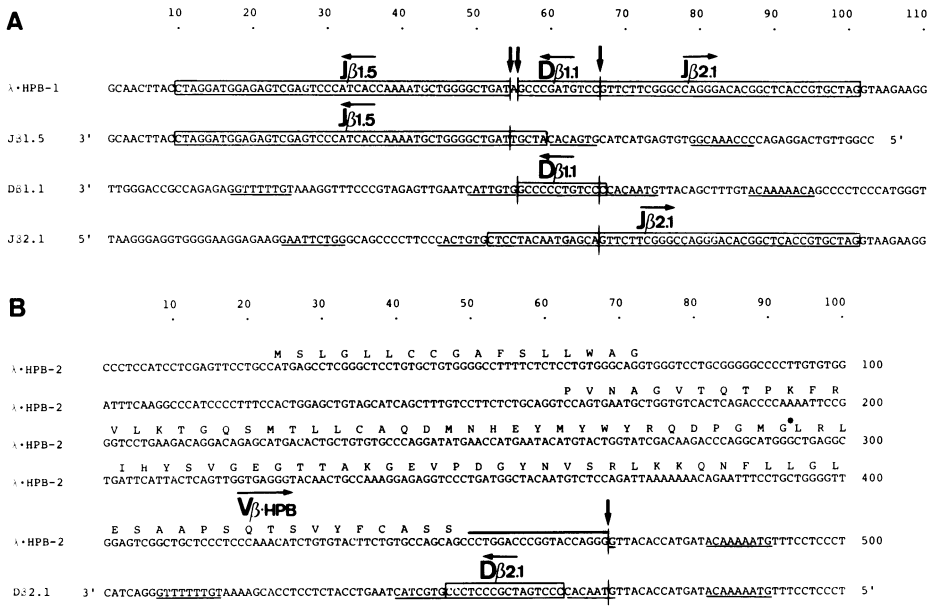

Fig. 4. Nucleotide sequences of germline and rearranged Jβ and Dβ segments. (A) λ·HPB-1, germline Jβ1.5, Dβ1.1, and Jβ2.1; (B) λ·HPB-2 and germline Dβ2.1. Sequences of germline Jβ1.5, Dβ1.1 (Clark et al., 1984), and Dβ2.1 are aligned with 3' to 5' direction from left to right. The direction of transcription is also shown by arrows. Conserved nonamer and heptamer sequences are underlined. Coding regions of the Dβ and Jβ sequences are boxed. An asterix above the nucleotide sequence of λ·HPB-2 shows the one nucleotide deletion in the ψVβ·HPB segment. Amino acid sequence (one letter code) shown above the nucleotide sequence was deduced from the nucleotide sequence of λ·HPB-2 ignoring the frame shift mutation. Vertical arrows and lines indicate the recombination points. Overlined region of λ·HPB-2 has no known origin.

scribed previously (7, 13). The germline $J_{\beta 1.5}$ segment was assigned as such by the homology with the murine $J_{\beta 1.5}$ segment.

The nucleotide sequence surrounding the recombination site in λ·HPB-1 revealed that the 10.2-kb EcoRI fragment contained the J-D-J complex derived from the germline $J_{\beta 1.5}$, $D_{\beta 1.1}$ (6), and $J_{\beta 2.1}$ (33) fragments as shown in Fig. 4A. Although the sequences of germline $J_{\beta 1.5}$ and $J_{\beta 2.1}$ segments were identical to those in the J-D-J complex, the sequence of germline $D_{\beta 1.1}$ agreed with that in the J-D-J complex only at 9 out of 11 bp. The difference could be due to polymorphism. Since no other D_{β} segments except for the $D_{\beta 1.1}$ and $D_{\beta 2.1}$ sequences were identified in the 5' flanking regions of the murine $J_{\beta 1}$ and $J_{\beta 2}$ segments, we considered it most likely that the 12-bp

sequence in the J-D-J complex was derived from the $D_{\beta 1.1}$ segment although we were unable to exclude the possibility that it was derived from another unknown D_{β} sequence in the human genome or N sequence (19).

The nucleotide sequence of the right of the recombination point of λ·HPB-2 was identical to the inverted sequence of the 5' flanking region of the germline $D_{\beta 2.1}$ segment up to the first nucleotide of the heptamer of the 12-bp spacer signal (Fig. 4B). The left of the recombination site of λ·HPB-2 contained a pseudoV_{β} segment ($\psi V_{\beta \cdot HPB}$) because it had one nucleotide deletion (position 293 in Fig. 4B) in the coding sequence, which resulted in a frameshift mutation. Though we do not know the sequence of the germline $\psi V_{\beta \cdot HPB}$, this pseudo V_{β} segment seems to be about 20 bp longer than known V_{β} segments. The origin of the 20 nucleotides between the $\psi V_{\beta \cdot HPB}$ and $D_{\beta 2.1}$ sequences is unknown. It is possible that this sequence was derived from a germline $\psi V_{\beta \cdot HPB}$, an unknown D_{β} segment or N sequence.

The results demonstrate that the cloned β-chain gene allele is aberrantly rearranged. The other J_{β} allele (3.8 kb EcoRI fragment) is expected to be an active V-J complex as predicted from analysis of a cDNA clone (8).

Models for the inversion in the aberrantly rearranged chromosome

To explain the formation of the J-D-J complex from the germline $J_{\beta 1.5}$, $D_{\beta 1.1}$, and $J_{\beta 2.1}$ segments, two alternative models were proposed as shown in Fig. 5. The first model assumes that rearrangement of the β-chain gene can violate the 23/12 spacer rule. First, the $D_{\beta 1.1}$-$J_{\beta 1.5}$ complex was formed according to the 23/12 spacer rule. We know that the recombination signals of the $D_{\beta 1.1}$, $D_{\beta 2.1}$, $J_{\beta 1.5}$, and $J_{\beta 2.1}$ segments are not different from the consensus sequence. Then, the $D_{\beta 1.1}$-$J_{\beta 1.5}$ complex was invertedly recombined with the $J_{\beta 2.1}$ segment somehow between the 12/12 spacer pair. The $J_{\beta 1.5}$-$D_{\beta 1.1}$-$J_{\beta 2.1}$ complex formed has deletion of the 5' 15 bp of the $J_{\beta 2.1}$ segment by a possible misalignment at the recombination. A hypothetical reciprocal product is composed of two 12-bp spacer signals and the 5' 15 bp of the $J_{\beta 2.1}$ segment. The $\psi V_{\beta \cdot HPB}$ segment recombined with the inverted $D_{\beta 2.1}$ segment between the two 23-bp spacer signals deleting the hypothetical reciprocal product. Here we assume that the recombination signal of the germline $\psi V_{\beta \cdot HPB}$ segment contains the 23-bp spacer just like many other V_{β} segments. On the $\psi V_{\beta \cdot HPB}$-$D_{\beta 2.1}$ recombination, the 22-bp sequence of the $D_{\beta 2.1}$ segment (16 bp from the coding sequence and 6 bp from the heptamer) was assumed to be deleted. In addition, $\psi V_{\beta \cdot HPB}$-$D_{\beta 2.1}$ complex contains about 20-bp sequence of unknown origin between the V and D segments.

The second model assumes that the 23/12 spacer rule is strictly abided

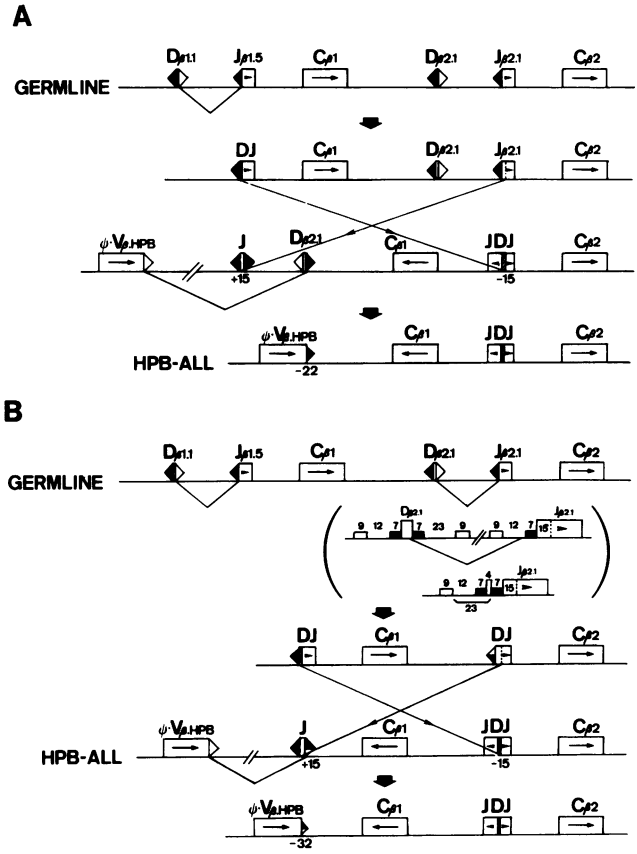

Fig. 5. Two models to explain the aberrantly rearranged chromosome in HPB-ALL. Vertical lines and boxes indicate Dβ, Jβ, and Cβ segments. Horizontal arrows and arrow heads show the direction of transcription. Oblique lines and arrows indicate the recombination by deletion and inversion, respectively. Recombination signals with 12-bp, 23-bp and double spacers are shown by filled, open, and half filled triangles, respectively. Numerals with plus and minus under the recombination product indicate deviation of the recombination point from the expected site. (A) Overrule model. The violation of 12/23 spacer rule is assumed. (B) Double spacer model. This model assumes that the intermediate Dβ2.1-Jβ2.1 complex has a signal sequence with 12-bp and 23-bp spacers (double spacer) composed of the nonamer and heptamer of the Dβ2.1 segment, and the heptamer of the Jβ2.1 segment. Structure of the double spacer is shown in parentheses with nucleotide length by numbers.

by the β-chain gene. As the first step of a second model, two D-J complexes ($D_{\beta 1.1}$-$J_{\beta 1.5}$ and $D_{\beta 2.1}$-$J_{\beta 2.1}$) were formed. We postulate that the $D_{\beta 2.1}$-$J_{\beta 2.1}$ complex was joined slightly off the coding region so as to have a signal which had not only 12-bp but also 23-bp spacer (double spacers).

Then, the $D_{\beta 1.1}$-$J_{\beta 1.5}$ and $D_{\beta 2.1}$-$J_{\beta 2.1}$ complexes recombined invertedly according to the 12/23 spacer rule, giving rise to the $J_{\beta 1.5}$-$D_{\beta 1.1}$-($D_{\beta 2.1}$)-$J_{\beta 2.1}$ complex with deletion of the 5' 15 bp of the $J_{\beta 2.1}$ segment. A hypothetical reciprocal product is composed of the three elements, the 12-bp spacer signal, the double spacer signal, and the 5' 15 bp of the $J_{\beta 2.1}$ segment. Finally, the $\psi V_{\beta \cdot HPB}$ segment recombined with this reciprocal product, deleting the 5' 32-bp sequence of the reciprocal product (15-bp from the coding sequence of the $J_{\beta 2.1}$ segment and 17-bp from the double spacer signal).

According to the first model, the violation of 12/23 spacer rule occurred twice successively. Since the violation of the rule has never been reported, the probability of two sequential violations might be very low. From this point of view, the second model is more favorable. Another advantage of the second model is that the intermediate D-J complex with the double spacer signal explains the formation of both the J-D-J complex and the ψV_{β}-D complex. Whether the double spacer signal could be generated or not might be tested when many other D-J complexes are sequenced.

Recombination to form the J-D-J complex deleted 15 bp from the $J_{\beta 2.1}$ sequence. Recombination of the $\psi V_{\beta \cdot HPB}$ and the $D_{\beta 2.1}$ segments seem to have deleted 22 and 32 bp in the first and second models, respectively. V-D-J and D-J recombinations are often accompanied by partial deletion of the coding sequence of the J and D segments. In average, 4.3 bp (total 90 bp in 21 cases, 10 bp at maximum) were deleted in the immunoglobulin heavy-chain gene (17, 34, 35), and 2.5 bp (total 92 bp in 36 cases, 14 bp at maximum) in the β-chain gene (3, 4, 7, 8, 36-38). Since the reciprocal products do not necessarily contain the deleted coding sequences (21, 23), 32-bp deletion of the $\psi V_{\beta \cdot HPB}$-$D_{\beta 2.1}$ recombination in the second model could be overestimated. The deletions associated with the J-D-J and ψV_{β}-D complex formations in HPB-ALL are thus considered to be slightly larger than reported but not extraordinary.

It is proved that the D-J complex is an intermediate of the V-D-J formation in the heavy-chain gene of the immunoglobulin (39, 40). The $\psi V_{\beta \cdot HPB}$-$D_{\beta 2.1}$ complex suggests the possibility that a V_{β} segment might have recombined directly with a germline D_{β} segment (Fig. 5A). However, there is another possibility that not yet found D_{β} segment(s) had recombined with the inverted $D_{\beta 2.1}$ segment (Fig. 5A) or the J-D complex (Fig. 1B) prior to V-D recombination. This might explain the origin of the 20 nucleotides between

$\psi V_{\beta\cdot HPB}$ and $D_{\beta 2.1}$ sequences. In any case, it remains to be seen whether or not the V-D complex is an intermediate to the V-D-J complex.

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