Interallelic V(D)J Trans-rearrangement within the
β T Cell Receptor Gene Is Infrequent and Occurs
Preferentially during Attempted Dβ to Jβ Joining

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Summary

Previous work has demonstrated that intergenic V(D)J rearrangement, a process referred to as trans-rearrangement, occurs at an unexpectedly high frequency. These rearrangements generate novel V(D)J combinations which could conceivably have some role in the normal immune system, and since they probably arise through chromosomal rearrangements akin to those associated with lymphoid neoplasia, they may also serve as a model for investigating recombinatorial events which underlie oncogenesis. In view of the existence of a mechanism that permits relatively frequent intergenic trans-rearrangements, it seems reasonable that interallelic trans-rearrangements involving segments belonging to each of the two alleles of a single antigen receptor gene might also occur. To determine the frequency of such rearrangements, we examined thymocytes of F1 progeny of a cross between SWR mice, which have a deletion spanning 10 of the known Vβ segments, and NZW mice, which have a deletion involving all Jβ2 segments. Rearranged TCR-β genes containing Vβ segments from the NZW chromosome and Jβ segments from the SWR chromosome were amplified from the DNA of F1 thymocytes with the polymerase chain reaction. Using this approach, we found that such rearrangements are relatively uncommon, being present in about 1 in 10,000 thymocytes, a frequency lower than that of Vγ/Jβ intergenic trans-rearrangements. The ratio of conventional cis-rearrangement to interallelic trans-rearrangement for any particular Vβ segment appears to be about 104:1. The structure of the junctions in all trans-rearrangements analyzed closely resembles conventional cis-rearrangements, indicating involvement of V(D)J recombinase in the ultimate joining event. However, in contrast to cis-rearrangements, a strong bias for inclusion of Dβ1 segments over Dβ2 segments was noted, suggesting that interallelic trans-rearrangement may occur preferentially during attempted D-J joining. Jβ2 segment usage in trans-rearrangements also appeared to differ from that expected from previously studied cis-rearrangements. The results have implications with respect to the events and timing of conventional cis-rearrangement during thymocyte differentiation, and the prevalence of various types of trans-rearrangements.

Somatic rearrangement of DNA constitutes a fundamental event leading to the production of structurally diverse antigen receptor genes (ARGs). This process, which is believed to be mediated by a lymphoid-specific recombinase, results in the assembly of variable (V), joining (J), and, in some loci, diversity (D) gene segments, into potentially functional Ig and TCR genes. Conserved heptamer and nonamer sequences separated by 11–12 or 22–23 bp flank each type of rearranging segment, and function as critical signal sequences for recombination, which typically occurs within a few base pairs to one side of the heptamer sequence (1, 2).

Cis-scanning of ARG DNA by recombinase during intragenic rearrangement is the simplest model for V(D)J joining. However, certain observations indicate that V(D)J joining occurs, at least some of the time, by a mechanism other than cis-scanning. For example, many lymphoid neoplasms contain chromosomal translocations in which the breakpoint in one of the two participating chromosomes maps cytogenetically to the site of an ARG (3). Sequence analysis of the breakpoints in these translocations has shown that the sites of recombination within ARGs usually lie adjacent to heptamer-nonamer sequences, precisely where normal V(D)J joining occurs during intragenic rearrangement. In some cases, the breakpoint in the second chromosome also lies near sequences with homology to heptamer/nonamer signals (4, 5).
consistent with the action of recombinase on DNA in both chromosomes participating in the translocation.

There is also evidence that intergenic trans-rearrangement occurs routinely in normal lymphoid tissue. Cytogenetic analyses indicate that about 1 in 1,000 spreads of metaphase chromosomes prepared from normal human peripheral lymphocytes show translocations in which both breakpoints map to the site of ARGs (6–8). Using the polymerase chain reaction PCR, several groups have recently detected the presence of chimeric ARG rearrangements within normal thymocytes and peripheral lymphocytes having V and J segments contributed by different ARGs (9, 10). Of note, the frequency at which chimeric sequences are detected, one or more cells in 10^6 total cells, approximates the frequency of translocations that map cytogenetically to these loci, suggesting that chromosomal translocation is the mechanism through which chimeric rearrangements arise.

In view of the relatively high incidence of intergenic trans-rearrangement between ARGs, another type of trans-rearrangement seems possible. These rearrangements would result from recombination between gene segments of allelic ARGs located on each of two chromosome homologues. Such recombination could be relatively frequent yet be overlooked, because rearrangements produced in this fashion would not generally be cytogenetically detectable and would likely contain V(D)J coding junctions that are structurally similar or identical to those produced by conventional cis-rearrangement. Indeed, it may be that intergenic trans-rearrangements merely reflect errors occurring during attempted interallelic trans-rearrangement, since both processes involve recombination between chromosomes. The possibility of interallelic recombination during V(D)J joining is also suggested by the existence of interallelic recombination during isotype switching within IgH genes (11), although the enzyme systems catalyzing these events are presumably different.

To investigate the possible occurrence of trans-rearrangement between alleles of ARGs, we have studied rearrangements within the TCR-β gene of F1 mice resulting from crosses between homozygous NZW and SWK parents. These mice, like several inbred mouse strains, have been shown to have deletions involving various portions of the TCR-β gene. Specifically, SWR mice have deleted almost half of the normal complement of V_β segments (12), while NZW mice have a deletion that spans C_β1, D_β2, and J_β2.1 through 2.6 coding segments (13). F1 mice produced by crossing NZW and SWR strains are thus doubly heterozygous for deletions involving V and J coding segments, with the two deletions being carried in a trans configuration. Using oligonucleotide primers specific for the deleted segments, we have performed PCR analysis to detect and quantify interallelic trans-rearrangements within the TCR-β locus.

Materials and Methods

Materials. Enzymes and phage vector DNAs were obtained from BRL Gibco (Gaithersburg, MD). BALB/c male mice were obtained at 6 wk of age (The Jackson Laboratory, Bar Harbor, ME). Animals were killed within 1 d of receipt, and thymuses were immediately removed and stored at −70°C.

DNA Preparation. Frozen tissue was ground to a powder in a disposable pestle and subjected to proteinase K digestion, phenol/chloroform extraction, and digestion with RNase using a standard method (14). DNA was stored in 10 mM Tris, 1 mM EDTA, pH 8.0, at 4°C.

PCR Conditions. Oligonucleotide primers were synthesized on a DNA synthesizer (model 381A; Applied Biosystems, Foster City, CA). The sequences of individual primers and the combinations of primer pairs used to amplify particular kinds of rearranged ARGs are described in Table 1. All reactions were carried out in 50 μl of 10 mM Tris, pH 8.3, in the presence of 50 mM KCl, 1.5 mM MgCl₂, 0.001% gelatin, 10% dimethyl sulfoxide, 1.25 U of thermostable DNA polymerase (AmpliTaq; Perkin-Elmer Cetus Corp., Emeryville, CA), and 100 ng of each oligonucleotide primer. Generally, two rounds of 30 amplification cycles were performed in an automated thermal cycler (Perkin-Elmer Cetus Corp.). Regardless of the primer pair, during the first round of amplification template DNA was denatured at 94°C for 5 min in the first cycle, and for 1 min in subsequent cycles, and extension was carried out at 72°C for 2 min during the first 29 cycles, and for 8 min during the final cycle. A second round of amplification with one or two internal primers, using 2 μl of initial reaction mixture as template, was then performed in an identical fashion. Annealing temperatures varied depending on the primer pair used, and are given in Table 2. To avoid contamination with previously amplified products, PCRs were prepared in a dedicated laminar flow hood, reaction mixtures were pipetted with aerosol filters (Vanguard, Inc., Neptune, NJ) were employed. All reactions were performed in parallel with appropriate negative controls described in Results.

Analysis of PCR Products. PCR products were electrophoresed in 1.8% agarose gels, stained with ethidium bromide, and transferred to nylon membranes (Plasco, Inc., Woburn, MA) by Southern blotting. Membranes were prehybridized for 1 h in a solution containing 6x SSC, 5x Denhardt’s solution, 2% formamide, 0.2% sodium pyrophosphate, and 0.5 mg/ml sonicated salmon sperm DNA, then hybridized in 6x SSC, 5x Denhardt’s solution, 2% formamide, 0.2% sodium pyrophosphate, 0.5 mg/ml sonicated salmon sperm DNA, 2.5% dextran sulfate at 42°C for 5 h with 100 ng of an internal oligonucleotide probe which had been end-labeled by γ-32P[γATP (New England Nuclear, Boston, MA) using T4 kinase to a specific activity of ~125 μCi/μg. Membranes were then washed twice for 15 min in 6x SSC-0.1% SDS at 54°C, and autoradiograms were prepared at room temperature using exposure times of 15 min to 4 h.

DNA Sequencing. Bands containing PCR products of interest were excised from agarose gels, and DNA was isolated on silica beads (GeneClean II; La Jolla, CA). The purified DNA and M13mp18 or mp19 RF DNA was cut with the appropriate pair of restriction enzymes (Table 1A), mixed, and incubated with T4 ligase for 4–8 h at 16°C. Transformation of competent Escherichia coli strain JM109 with ligation mixtures using a heat shock method, identification of lac’ recombinant phage, and purification of single-stranded template DNA were performed using standard procedures (16). Inserts were sequenced with a kit (U.S. Biochemical, Cleveland, OH) employing the dyeoxy method (17) according to the manufacturer’s instructions.
Results

The basis for the method used to detect interallelic rearrangements within the TCR-β locus is presented schematically in Fig. 1. The TCR-β locus of SWR mice contains a deletion spanning ten of the known murine Vβ coding segments (12). Likewise, NZW mice have also suffered a deletion in this gene which has removed Cβ1, Dβ2, and Jβ2.1-Jβ2.6 coding segments (13); Jβ2.7, a pseudogene segment, is also deleted. Since F1 NZW × SWR mice carry these two deletions in a trans configuration, rearranged ARGs composed of Vβ segments absent from the SWR chromosome, and Jβ2 segments absent from the NZW chromosome can only arise from a recombination event occurring between the NZW and SWR alleles.

We attempted to detect interallelic trans-rearrangement products using crossed pairs of oligonucleotide primers specific for deleted Vβ and Jβ2 segments in PCRs containing F1 thymic DNA. To increase the likelihood of detecting such products, initial efforts focused on the Vβ8 subfamily. This subfamily has three highly homologous members, Vβ8.1, 8.2, and 8.3 (18), which make up more than 10% of all known murine Vβ segments. Vβ8 segments frequently participate in V(D)J recombination, being expressed by about 25% of TCR-positive thymocytes and peripheral T cells (19), and thus might also be expected to be frequently involved in interallelic trans-rearrangements. Primer sequences, combinations of primer pairs, and annealing temperatures used to amplify Vβ8/Jβ2 rearrangements and other rearrangements (discussed later) are listed in Tables 1 and 2. Nested Vβ8 primers were chosen which are complementary to sequences lying at the 5′ end of the Vβ8 coding region and which are completely homologous to all three Vβ8 segments. These primers were paired with nested Jβ2.5 external and internal primers lying just 3′ to and within the Jβ2.5 segment. PCR products were then analyzed by Southern blotting, using an internal Vβ8-specific oligonucleotide probe (Vβ8ishp).

To assess the sensitivity and specificity of our assay, control reactions were carried out with thymic DNA from BALB/c mice that possess the full complement of Vβ and Jβ coding segments, and with NZW and SWR thymic DNA from parental mice. Reaction conditions were chosen that permitted amplification of products from BALB/c DNA, while failing to give positive signals with NZW, SWR, or NZW DNA mixed with SWR DNA. The control reaction containing mixed parental thymic DNAs is particularly important, since it rules out false positives generated by partial extension of primers into regions of homology. Such partial products could conceivably anneal to allelic sequences and be further extended in subsequent rounds of amplification, thereby giving rise to composite sequences that could be mistaken for trans-rearrangements. The absence of such products in the parental mixing control indicates that the PCR method used specifically amplifies only preexistent rearrangements that must have occurred in vivo.

Dilution experiments were then performed with BALB/c thymic DNA mixed with sufficient NZW and SWR parental thymic DNA to hold the total amount of DNA constant at 2 μg, representing about 2 × 10⁶ cell equivalents (Fig. 2). When 200 or more cell equivalents of BALB/c thymic DNA were added to PCRs, a variety of differently sized products were obtained. In contrast, 0 to 3 distinct hybridizing bands ranging from ~240–920 bp were seen in most reactions containing 20 cell equivalents of BALB/c DNA, indicating that this is close to the limiting dilution. It is notable that although bands corresponding to the position of Vβ8/Jβ2.5 rearrangement (~240 bp) predominated in the presence of high concentrations of template, bands approximating the expected position of Vβ8/Jβ2.1 and Vβ8/Jβ2.2 rearrangements (~920 and 720 bp, respectively) were readily detected in some reactions performed with low concentrations of BALB/c DNA. Thus, while smaller PCR products are preferentially amplified, the method is capable of detecting larger products as well. Further dilution revealed a positive signal in 3 of 16 reactions containing two cell equivalents of BALB/c thymic DNA (Fig. 2, and results not shown). Applying this data to the Poisson equation leads to a calculated frequency for Vβ8/Jβ2 rearrangements of ~2 per 10 cells. Using published data that 25% of BALB/c thymocytes have at least one V(D)J rearrangement involving Vβ8 DNA (Fig. 2, and results not shown), one would predict that up to 15% of BALB/c thymocytes contain detectable Vβ8/Jβ2 rearrangements. Therefore, the obtained results are close to the predicted results and indicate that the assay has a sensitivity close to the theoretical maximum of 1 cell in 10⁵.

Thymic DNA from F1 NZW × SWR mice was then

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Figure 1. Structure of TCR-β alleles in NZW × SWR mice.
Table 1. PCR Oligonucleotides

| Oligonucleotide | Sequence (5'-3') | Restriction site | Reference |
|-----------------|------------------|------------------|-----------|
| Vβ8ext          | ATGGAGCTGCAGTCACCCA |                  | 38        |
| Vβ8int          | ATGAATTCATGTACTGGATATGGGCAGGA | EcoR1 | 38        |
| Vβ8ihp          | GGGCTGAGGCTGATCCATTA |                  | 38        |
| Vβ5ext          | AAGGATCCACGAGATTCTCAGTCCAA | BamH1 | 38        |
| Vβ5ihp          | AGTTTGATGACTATCAGCTCT |                  | 38        |
| Jβ2.5ext        | ACTGCAGCCCAATCCCGCTGAGAA |                  | 39        |
| Jβ2.5int        | AAGTCGACGGCCTAAGTACTGGTTGTC | SalI | 39        |
| Jβ1.5ext        | ACCACTGAGTCCGAGGACAATGGT | PstI | 40        |
| Vγ2ext          | AAGGAATTCATCGAAAGCTTTAGGAG | EcoR1 | 41        |
| Vγ2ihp          | ACCATACACTGGTACCGGCA |                  | 41        |

Underlined nucleotides denote noncomplementary sequences included to create restriction sites.

Table 2. Primer Pairs and Reaction Conditions

| Target rearrangement | Primers (Round 1) | Primers (Round 2) |
|----------------------|-------------------|-------------------|
| Vβ8/Jβ2              | Vβ8ext/Jβ2.5ext (60°C) | Vβ8int/Jβ2.5int (60°C) |
| Vβ5/Jβ2              | Vβ5ext/Jβ2.5ext (55°C) | Vβ5ext/Jβ2.5int (55°C) |
| Vγ2/Jβ2              | Vγ2ext/Jβ2.5ext (60°C) | Vγ2ext/Jβ2.5int (60°C) |
| Vβ8/Jβ1              | Vβ8ext/Jβ1.5ext (60°C) | Vβ8int/Jβ1.5ext (60°C) |

Annealing temperatures are given in parentheses next to each primer pair. Vγ nomenclature is according to Garman et al. (41).

Figure 2. Amplification and detection of Vβ8/Jβ2 rearrangements in BALB/c thymocytes. Indicated cell equivalents of BALB/c thymic DNA were used as template in PCRs with Vβ8 and Jβ2 specific primers. The total amount of DNA was held constant at 2 μg by adding equimolar amounts of NZW and SWR thymic DNA. Control reactions contained 1 μg of NZW thymic DNA mixed with 1 μg of SWR thymic DNA. PCR products were electrophoresed in 1.8% agarose gels, transferred to nylon membranes, and hybridized to an internal Vβ8-specific probe end-labeled with Phosphorous-32. The resultant autoradiogram is shown.

Figure 3. Detection of Vβ8/Jβ2 trans-rearrangements in thymocytes of SWR x NZW mice. PCRs were performed with Vβ8 and Jβ2 primers and the following additions: water only; NZW thymic DNA (2 μg); SWR thymic DNA (2 μg); NZW thymic DNA (1 μg) mixed with SWR thymic DNA (1 μg); NZW x SWR thymic DNA (2 μg); NZW x SWR thymic DNA (2 μg). Amplified trans-rearrangements were detected on Southern blots by hybridization with an internal Vβ8-specific probe end-labeled with Phosphorous-32. The resultant autoradiogram is shown.
amplified in the same fashion. Results obtained with three different animals were similar. When 2 µg of F, thymic DNA was used as template, electrophoresis of PCR products on agarose gels consistently revealed one to several intense ethidium bromide-stained bands that were always found to hybridize to the Va5/ja2p probe on Southern blots (Fig. 3). The position of hybridizing bands most commonly corresponded to the expected size of Va5/ja2.5, Va5/ja2.4, and Va5/ja2.3 rearrangements. Control reactions run concomitantly containing no added template, NZW thymic DNA, SWR thymic DNA, mixed NZW and SWR thymic DNA, or NZW × SWR liver DNA uniformly failed to produce hybridizing bands.

Detection of only a few of the five possible Va5/ja2 products in most amplifications suggested that the frequency of such rearrangements is relatively low. To better quantify this type of rearrangement, multiple PCRs were performed using 0.4 µg of thymic DNA (~4 x 10^6 cell equivalents) pooled from three F1 animals as template. With this amount of DNA, hybridizing bands were observed in 10 of 24 reactions (not shown), most often in the positions expected for ja2.5, ja2.4, and ja2.3 rearrangements. In contrast, hybridizing bands corresponding in size to rearrangements involving ja2.2 and ja2.1 were not observed. Since such bands were readily detected when BALB/c thymic DNA was used as template (Fig. 2), particularly near limiting dilution, this is unlikely to be an artifact stemming from preferential amplification of smaller products. Using the Poisson equation, the calculated frequency of Va5/ja2 trans-rearrangements in F1 thymus is 1.4 per 10^5 cells. This is somewhat lower than the true frequency, since Va5/ja2 rearrangements are not detected by the method.

To show that this frequency for interallelic trans-rearrangement within the TCR-β locus is likely to be representative, a less extensive series of amplifications were also performed with primers complementary to Va5 subfamily sequences. This subfamily, which is also deleted in SWR mice, consists of two homologous V segments and one pseudogene (18) which are used in about 8% of thymocyte β chain transcripts (21). Positive and negative control experiments were performed with BALB/c thymic DNA as described for Va8 primers to ascertain reaction conditions that permit specific and sensitive amplification of Va5/ja2 rearrangements (not shown). F1 thymic DNA was then amplified with Va5/ja2 primer pairs (Fig. 4). 9 of 10 PCRs performed with 2 µg of F1 thymic DNA contained at least one to as many as three amplification products that hybridized to an internal Vα5-specific probe (Va5/ja2p). Most of these products approximated the expected size of Va5/ja2.5 or Va5/ja2.4 rearrangements. Hybridizing bands were absent from control reactions performed in parallel with NZW thymic DNA mixed with SWR thymic DNA. Limiting dilution experiments revealed the frequency of Va5/ja2 rearrangements to be about 1 per 10^6 cells (not shown), close to that observed for Va5/ja2 rearrangements. Again, rearrangements involving ja2.1 and ja2.2 were not seen at limiting dilution.

The identity of the interallelic trans-rearrangements was further confirmed by the sequencing of PCR products cloned into M13. A total of twenty distinct Va5 and two Va5 clones were analyzed (Table 3). All consist of Va5 or Va5 coding segments joined to ja2 coding segments in a manner resembling standard recombine-mediated ARG rearrangement. Specifically, the breakpoints within both segments lie close to their respective heptameric signal sequences, exonucleolytic digestion appears to have occurred at the 3' and 5' ends of the V and J coding segments, respectively, and random addition of N nucleotides is apparent in most rearrangements in the region between V-D and D-J coding junctions. 19 of 22 V(D)J junctions contain at least a 3 bp sequence homologous to a Dβ segment. Unexpectedly, although 10 of the rearrangements contain unambiguous Dα1 segments, no rearrangements bearing the Dβ2-specific sequence GACTG are seen, indicating that participation of Dα1 segments is strongly favored. While the three rearrangements that lack sequences homologous to Dβ segments could represent examples of direct Vβ to Jβ joining, they also can be explained by complete exonucleolytic removal of Dβ segments before ligation. Apparent misincorporation of nucleotides by Taq polymerase was detected in flanking Vβ and Jβ coding segments at a frequency that varied from 1 in 200 to 1 in 1,000 bp, and thus are unlikely to contribute substantially to the observed V(D)J junctional heterogeneity.

The possible contribution of interallelic trans-rearrangement to diversity among rearranged ARGs was assessed by comparing the frequency of cis and trans-rearrangements for given Vβ segments. To do this, the frequency of Va5/ja1 cis-rearrangements was determined by limiting dilution of F1 thymic DNA in PCRs containing Va5 and Ja1.5 primers (Fig. 5). In these experiments, SWR thymic DNA that lacks Va5 segments was used as a negative control and as diluent to hold the amount of total DNA constant at 2 µg in reac-
Table 3. Sequences of TCR-β Trans-Rearrangements

| $V_8$ | (Pv)-N-(Pd) | $D_0$1 | (Pd)-N-(Pj) | $J_8$ |
|-------|-------------|--------|-------------|-------|
| V8.1  | AGCAGTGATG  | GGGACAGGGGC | GTCAAAACA  | 2.4   |
| V8.1.a| AGCAGTGATG  |         |             |       |
| V8.1.b| AGCAGTGATG  | GAC     | CAAAACA     | 2.4   |
| V8.1.c| AGCAGTGATG  | GGGG    | ACA         | 2.5   |
| V8.1.d| AGCAGTGATG  | GGGG    | AGACA       | 2.5   |
| V8.1.e| AGCAGTGATG  | CAAAGCC | GGACA       | AAACACGG | 2.2   |
| V8.1.f| AGCAGT      | T       | GGGACAGGGGC | AGA   | TGCAGAAA | 2.3   |
| V8.2  | AGCGGTGATG  |         |             |       |
| V8.2.a| AGCGGTG     | CTCCC   | GGA         |       |
| V8.2.b| AGCGGTG     | AAG     | C           | GCA   | 2.5   |
| V8.2.c| AGCGGTG     | TGGG    | GG           | T     | AGCACA  | 2.4   |
| V8.2.d| AGCGGTG     | TTC     | GGGACAG     |       |
| V8.2.e| AGCGGTG     | GC      | C           | AGCACA | 2.4   |
| V8.2.f| AGCGGTG     | TAC     | GACAG       | A     | AAAACA | 2.4   |
| V8.2.g| AGCG         | GGGACAGGGG | AGGAG     | GCTG  | 2.1   |
| V8.2.h| AGCGGTG     | CAC     | GGACA       | A     | TGCAGAAA | 2.3   |
| V8.2.i| AGCGGTG     | C       | GACA        |       |
| V8.2.j| AGCGG       |         |             |       |
| V8.3  | AGCAGTGATG  |         |             |       |
| V8.3.a| AGCAGT      | GGGACAGGG | ATACGT     | ACCGG | 2.2   |
| V8.3.b| AGCAGTGATG  | GGGACAGGG | AAG     | GCA   | 2.5   |
| V8.3.c| AGCAGTGATG  | GGGGG    | GAT         | AAGACA| 2.5   |
| V8.3.d| AGCAGTGATG  | GAC      | C           | AAAACA | 2.4   |
| V5.1  | CAGCTCTCTC  |         |             |       |
| V5.1.a| CAGCTCTCTC  | G       | GGGAC       |       |
| V5.1.b| CAGCTCTCTC  | GT      | ACAGGGGGGC  | TG    | CCAAGACA | 2.5   |

Germline $V_8$ sequences are indicated with bold labels. Underlined nucleotides represent possible $P$ additions. Ambiguous nucleotides have been arbitrarily assigned to $D_0$ segments and are indicated in italics. For comparison, the germline sequence of $D_02$ is GGGACTGGGGGGGC.

To further compare the fine structure of $V_8$ cis- and trans-rearrangements, 22 cis-rearrangements were amplified from F1 thymic DNA with $V_8$ and $J_8$1.5 specific primers, cloned, and sequenced (not shown). All clones consisted of $V_8$ coding segments joined to $J_8$1 coding segments. Like the $V_8$/$J_8$2 trans-rearrangements, many coding junctions contained sequences homologous to $D_01$ diversity segments, consistent with derivation from the NZW chromosome. The extent of exonucleolytic digestion and size and content of $N$ insertions did not differ significantly from that observed in the $V_8$/$J_8$2 trans-rearrangements. Thus, interallelic trans-rearrangements do not appear to possess any distinct structural features that would allow them to be readily distinguished from cis-rearrangements.
Table 4. Sequences of Vγ2/Jβ2 Trans-Rearrangements

| Vγ2 | Pγ-N-Pj | Jβ |
|-----|---------|----|
| GTTCCTACGG | G      | GACA 2.5 |
| GTTCCTACGG | AAACAC 2.4 |
| GTTC | CAAGACA 2.5 |
| GTTCCTACGG | GTGAGG | GTCAACACAC 2.4 |
| GTTCCTACGG | AACCAAGACA 2.5 |
| GTTCCTACGG | CAAGACA 2.5 |

Underlined nucleotides represent possible P additions.

The calculated frequency of interallelic trans-rearrangements in the F1 mice is about an order of magnitude lower than that previously reported for other types of trans-rearrangement which produce chimeric receptors, such as Vγ/Jδ and Vγ/Jβ rearrangements (9, 10). This difference could be due to strain variation or could represent a real difference in the frequency of these types of rearrangements. To differentiate between these possibilities, the prevalence of Vγ/Jβ trans-rearrangements was investigated in F1 thymuses. In these experiments, NZW thymic DNA that lacks Jβ2 segments was used as a negative control. Addition of 0.2 μg (2 × 10⁴ cell equivalents) of DNA to the PCR consistently resulted in amplification of several bands which hybridized to an internal Vγ2 probe (Vγ2ihp) on Southern blots (Fig. 6), while NZW DNA never produced any positive signals. Identity of the hybridizing bands was confirmed by DNA sequencing, which demonstrated direct joining of Vγ2 coding segments to Jβ2 coding segments without interposed Dδ segments (Table 4). Further dilution revealed positive signals in 11 of 24 reactions containing 0.04 μg (4 × 10³ cell equivalents) of DNA (not shown), giving a frequency for Vγ2/Jβ2 trans-rearrangements of ~15 per 10⁶ cells. The size of most rearrangements approximated that expected for joining with Jβ2.5, Jβ2.4, or Jβ2.3. Bands corresponding to the size of Vγ2/Jβ2.1 rearrangements were not seen. Once again, the calculated frequency for Vγ2/Jβ2 trans-rearrangements represents a lower limit, since rearrangements involving Vγ segments other than Vγ2 or Jβ2.6 will not be detected by the primers used. With this caveat, it can be concluded that the frequency of intergenic Vγ/Jβ trans-rearrangements is about one order of magnitude higher than that of interallelic Vγ/Jβ trans-rearrangements.

Discussion

By making use of strain-specific deletions, we have detected and analyzed rearranged murine TCR-β genes which appear to have been created by interallelic V(D)J recombination, a process we refer to as interallelic trans-rearrangement. Our results indicate that this type of rearrangement occurs relatively infrequently, the ratio of cis- to trans-rearrangement being about 10⁴:1. In NZW × SWR mice, the frequency of interallelic trans-rearrangements involving Vγ8 and Vδ5 segments within the TCR-β locus is around 1 per 10⁶ thymocytes. Assuming that the ratio of cis- to trans-rearrangement is also about 10⁴:1 for other Vδ segments, the cumulative frequency of all interallelic trans-rearrangements in TCR-β is probably not greater than 1 per 10⁶ thymocytes in these animals.

Previously, evidence supporting the occurrence of interallelic trans-rearrangement in other ARGs has been obtained.
from serologic studies performed on rabbit Ig. Rabbits preferentially rearrange a single \( V_n \) segment (22), \( V_n \alpha \), which shows strain-specific variation, and also possess variation in \( C_n \) segments. Therefore, it is possible to breed animals that are doubly heterozygous for allotypic \( V_n \) and \( C_n \) sequences. About 1% of Ig molecules from the peripheral blood of such animals appear to contain \( V_n \) and \( C_n \) allotypes encoded by alleles carried in a trans configuration in the germline DNA (23, 24). Additionally immunofluorescent studies have demonstrated colocalization of both allotypes in about 1% of plasma cells (25). Recently, a single rearranged IgH gene from a doubly heterozygous rabbit has been cloned and shown to have a sequence consistent with a trans-rearrangement (26).

These data, though mostly indirect, when considered in the context of the present studies imply that the incidence of interallelic trans-rearrangement might be several orders of magnitude greater within the IgH locus than within the TCR-\( \beta \) locus. However, a number of observations indicate that recombination events other than V(D)J recombinase-mediated trans-rearrangement could partly or wholly explain the observations made in rabbits. Homozygous rabbits presumed to lack certain \( V_n \) allotypes can be induced to express them after immunization with antiallotype antibody (27), and pseudogenes potentially capable of contributing \( V_n \) allotype-specific sequences through gene conversion events have been detected in allotype-negative rabbits (28). Recent data suggest that gene conversion plays an important role in generating sequence diversity in the rabbit IgH gene (22), and therefore may participate in creation of doubly allotypic molecules. Finally, since one site of allotypic variation lies within \( C_n \) segments, the serological data could also be explained by trans-switching (11) or trans-splicing (29), subsequent to recombinase-mediated \( C_n \) cis-rearrangement. Therefore, the high apparent incidence of trans-rearrangement within the rabbit IgH locus may be due to the summation of several kinds of genetic events, some involving recombinase and some not, and as a result, the true incidence of interallelic trans-rearrangement involving the IgH gene is uncertain.

Alternatively, it is possible that deletions within murine TCR-\( \beta \) genes might somehow suppress the participation of remaining gene segments in interallelic trans-rearrangements, thus leading to an unrepresentatively low incidence of such events in the mouse cross used in this study. For example, it could be argued that deletion of \( C_3 \alpha 1 \), \( D_\beta 2 \), and \( J_\beta 2 \) segments from the NZW allele diminishes trans-rearrangement of residual \( D_\beta 1 \) and \( J_\beta 1 \) segments. This seems unlikely for several reasons. Model systems for studying recombinase-mediated recombination have produced little evidence of promotion or suppression of recombination by flanking sequences (30, 31). More directly, it seems likely that any suppressive influence of deletions would extend to cis-rearrangements and intergenic trans-rearrangements. However, cis-rearrangement appears to proceed normally in NZW mice, and intergenic \( V_n/J_\beta 1 \) rearrangements occur at similar frequencies in NZW and wild-type mice (data not shown), indicating that deletions within TCR-\( \beta \) do not inhibit other types of interchromosomal recombination.

Three issues arising from our work concern the role of trans-rearrangements in normal immune function, the mechanism by which they are produced, and factors which tend to promote or suppress their occurrence. With regard to the first issue, the current work does not seem to support a major role for interallelic rearrangement in augmentation of the immune repertoire. The detected trans-rearrangements occur at low frequency and generate coding junctions similar to standard cis-rearrangements. Further, except for unusual situations, such as the double-deletion mice used by us to detect the existence of trans-rearrangements, it seems unlikely that novel V(D)J combinations will be generated by this mechanism. On the other hand, the possibility that chimeric ARGs created by intergenic trans-rearrangements may have novel properties remains open to question.

The close resemblance of the coding junctions of trans-rearrangements, whether interallelic or intergenic, to those seen in conventional recombinase-mediated cis-rearrangements, strongly implicate recombinase in the ultimate V(D)J joining event. The simplest way for this to occur would be for recombinase to directly catalyze chromosomal translocation. Indirect evidence linking intergenic trans-rearrangements to chromosomal inversions and translocations supports this mechanism (9, 10, 32, 33).

Alternatively, \( V_\beta \) or \( J_\beta \) sequences could be moved from a trans-orientation to a cis-orientation by some other type of recombination event between homologous chromosomes, either before or after conventional intrachromosomal cis-V(D)J-rearrangement. In the case of interallelic trans-rearrangements, reorientation of coding segments by gene conversion, homologous mitotic recombination, reinsertion of sequences excised from one allele during cis-rearrangement, or \( V_\beta \) replacement after cis-rearrangement seem possible. A number of considerations, however, make these possibilities less likely. While gene conversion commonly occurs in trans and is believed to play an important role in diversification of Ig \( V_\lambda \) sequences in chickens (34, 35) and \( V_n \) sequences in rabbits (22), within Ig genes it typically results in transposition of short stretches of DNA sequence ranging from 10 to 120 bp. Since the \( V_\beta \) primers used to amplify V(D)J trans-rearrangements lie 150–200 bp 5' of the recombination signal sequences, similar conversion events occurring in TCR-\( \beta \) would have been expected to result in recombinant \( V_\beta \) sequences, which were not observed. Moreover, gene conversion has not yet been described in the TCR genes.

More importantly, some features of the interallelic trans-rearrangements appear to directly support involvement of V(D)J recombinase. Specifically, models requiring reorientation of segments by a mechanism not involving recombinase do not readily explain the absence of \( D_\beta 2 \) segments from interallelic trans-rearrangements, since \( D_\beta 2 \) segments participate in about 50% of cis-rearrangements containing \( J_\beta 2 \) segments (20, 36). In contrast, a trans-joining mechanism mediated by recombinase could produce this result if interallelic trans-rearrangement within TCR-\( \beta \) is limited to \( D_\beta \) to \( J_\beta \) joining. Trans-rearrangements occurring during attempted \( V_\beta_{NZW} \) to \( D_\beta_{JSW} \) joining can contain either \( D_\beta 1 \)
or Dα2 segments, since the SWR allele contains both Dα1 and Dα2 segments. In contrast, the NZW allele contains only a Dα1 segment, so that all trans-rearrangements occurring during attempted DαNZW to JαSWA joining must involve Dα1 segments and cannot contain Dα2. The lack of Dα2 segments in the interallelic trans-rearrangement products is therefore consistent with restriction of such rearrangements within TCR-β to Dα to Jα joining, with subsequent Vβ to DαJα joining occurring only in cis.

Our data also suggest that Jα usage differs when Dα to Jβ joining occurs in trans rather than cis. Jα2.1 segments normally participate in about 20–30% of cis Dα to Jα2 rearrangements (20, 36). In contrast, hybridizing bands of the expected size of a Vβ/Jα2.1 trans-rearrangement were quite infrequent, not being seen at all in PCRs performed at limiting dilution. The reason for this difference in Jα usage is unclear, but it may be a general feature of trans-rearrangements involving Jα2 segments, since Vα/Jα trans-rearrangements also appear to only rarely involve Jα2.1. These data further support restriction of interallelic trans-rearrangement to Dα to Jα joining, since trans-rearrangements formed by joining of Vβ segments to DαJα segments previously rearranged in cis would be expected to frequently contain Jα2.1.

Given this possible restriction in interallelic trans-rearrangement, one might ask what factors determine the frequency of joining of various ARG segments in trans. It seems reasonable that concomitant accessibility of gene segments to recombinase is necessary, albeit perhaps not sufficient, to promote trans-rearrangement. The low incidence of Vβ to DαJα interallelic trans-rearrangement might thus be the result of the temporal separation of Vβ to DαJα rearrangement events in the two alleles. The situation is analogous to what has been proposed to occur during Vα to DαJα joining in pre-B cell lines (2), during which temporarily staggered rearrangement of alleles is believed to contribute to the process of allelic exclusion. Our data suggest that a similar mechanism may promote allelic exclusion in TCR-β.

Aside from the timing of rearrangement, a separate factor which could effect the frequency of trans-rearrangement is the physical localization of rearranging gene segments in the interphase nucleus, since topological proximity of ARGs would seem to be an absolute requirement for trans-rearrangement to occur. In most mammalian cells, chromosomal homologs are usually spatially separated from one another in interphase (37). If true of thymocytes as well, this could also act to diminish the chance of interallelic trans-rearrangement. Topological constraints could conceivably explain, for example, why Vα/Jβ trans-rearrangements appear to occur more frequently than interallelic Dα/Jα trans-rearrangements. Additional studies assessing the spatial relationship of ARGs in differentiating lymphoid cells may help to resolve this question.

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