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 recombinational 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 F_1 progeny of a cross between SWR mice, which have a deletion spanning 10 of the known V_β segments, and NZW mice, which have a deletion involving all $J_\beta 2$ segments. Rearranged TCR-β genes containing Vβ segments from the NZW chromosome and J_β segments from the SWR chromosome were amplified from the DNA of F_1 thymocytes with the polymerase chain reaction. Using this approach, we found that such rearrangements are relatively uncommon, being present in about 1 in 10^5 thymocytes, a frequency lower than that of V_γ/J_β intergenic *trans*-rearrangements. The ratio of conventional *cis*-rearrangement to interallelic *trans*-rearrangement for any particular V_β segment appears to be about 10^4:1. 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 $D_\beta 1$ segments over $D_\beta 2$ segments was noted, suggesting that interallelic *trans*-rearrangement may occur preferentially during attempted D–J joining. $J_\beta 2$ 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).[1] 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),

[1] *Abbreviation used in this paper:* ARG, antigen receptor gene.

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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 10^4 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 F_1 mice resulting from crosses between homozygous NZW and SWR 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 $C_\beta 1$, $D_\beta 2$, and $J_\beta 2.1$ through 2.6 coding segments (13). F_1 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).

Experimental Animals. NZW, SWR, NZW × SWR, and 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 pestil 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 $MgCl_2$, 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 3 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 treated with UV light (254 nm) for 10 min in a UV box (Fotodyne, New Berlin, WI) before addition of Taq polymerase and DNA template (15), and pipette tips 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 6× SSC, 5× Denhardt's solution, 2% formamide, 0.2% sodium pyrophosphate, and 0.5 mg/ml sonicated salmon sperm DNA, then hybridized in 6× SSC, 5× 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-[^{32}P]ATP (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 6× 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 1 *A*), 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 dideoxy 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 V_β coding segments (12). Likewise, NZW mice have also suffered a deletion in this gene which has removed $C_\beta 1$, $D_\beta 2$, and $J_\beta 2.1$–$J_\beta 2.6$ coding segments (13); $J_\beta 2.7$, a pseudogene segment, is also deleted. Since F_1 NZW × SWR mice carry these two deletions in a *trans* configuration, rearranged ARGs composed of V_β segments absent from the SWR chromosome, and $J_\beta 2$ 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 V_β and $J_\beta 2$ segments in PCRs containing F_1 thymic DNA. To increase the likelihood of detecting such products, initial efforts focused on the $V_\beta 8$ subfamily. This subfamily has three highly homologous members, $V_\beta 8.1$, 8.2, and 8.3 (18), which make up more than 10% of all known murine V_β segments. $V_\beta 8$ 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 $V_\beta 8/J_\beta 2$ rearrangements and other rearrangements (discussed later) are listed in Tables 1 and 2. Nested $V_\beta 8$ primers were chosen which are complementary to sequences lying at the 5′ end of the $V_\beta 8$ coding region and which are completely homologous to all three $V_\beta 8$ segments. These primers were paired with nested $J_\beta 2.5$ external and internal primers lying just 3′ to and within the $J_\beta 2.5$ segment. PCR products were then analyzed by Southern blotting, using an internal $V_\beta 8$-specific oligonucleotide probe ($V_\beta 8$ihp).

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 V_β and J_β 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 2×10^5 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 $V_\beta 8/J_\beta 2.5$ rearrangement (~240 bp) predominated in the presence of high concentrations of template, bands approximating the expected position of $V_\beta 8/J_\beta 2.1$ and $V_\beta 8/J_\beta 2.2$ 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 $V_\beta 8/J_\beta 2$ rearrangements of about 1 per 10 cells. Using published data that 25% of BALB/c thymocytes have at least one V(D)J rearrangement involving $V_\beta 8$ segments (19), and assuming that roughly 60% of these rearrangements involve $J_\beta 2.1-2.5$ (20), one would predict that up to 15% of BALB/c thymocytes contain detectable $V_\beta 8/J_\beta 2$ 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 10^5.

Thymic DNA from F_1 NZW × SWR mice was then

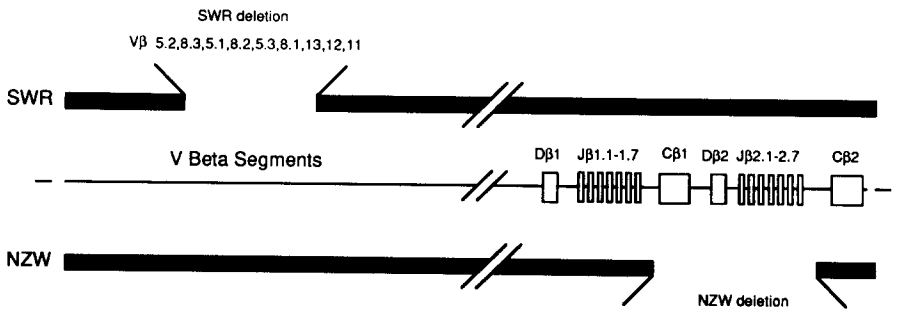

Figure 1. Structure of TCR-β alleles in NZW × SWR mice.

Table 1. *PCR Oligonucleotides*

Oligonucleotide	Sequence (5′–3′)	Restriction site	Reference
Vβ8ext	ATGGAGCTGCAGTCACCCA		38
Vβ8int	ATGAATTCATGTACTGGTATGGGCAGGA	EcoR1	38
Vβ8ihp	GGGCTGAGGCTGATCCATTA		38
Vβ5ext	AAGGATCCAGCAGATTCTCAGTCCAA	BamH1	38
Vβ5ihp	AGTTTGATGACTATCACTCT		38
Jβ2.5ext	ACTGCAGCCCAATCCCGCTGAGAA		39
Jβ2.5int	AAGTCGACGGCCCAAAGTACTGGGTGTC	Sal1	39
Jβ1.5ext	ACACTGCAGGTCCAAAGGACAATGGT	Pst1	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).

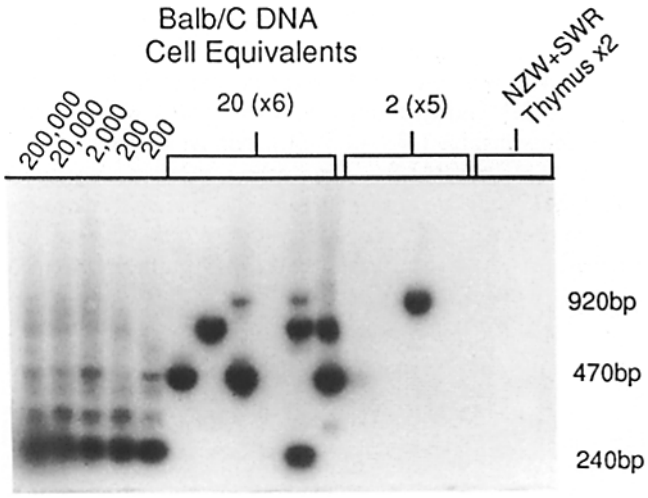

Figure 2. Amplification and detection of $V_\beta8/J_\beta2$ rearrangements in BALB/c thymocytes. Indicated cell equivalents of BALB/c thymic DNA were used as template in PCRs with $V_\beta8$ and $J_\beta2$ specific primers. The total amount of DNA was held constant at 2 μg by adding equimolar amounts 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 $V_\beta8$-specific probe end-labeled with Phosphorous-32. The resultant autoradiogram is shown.

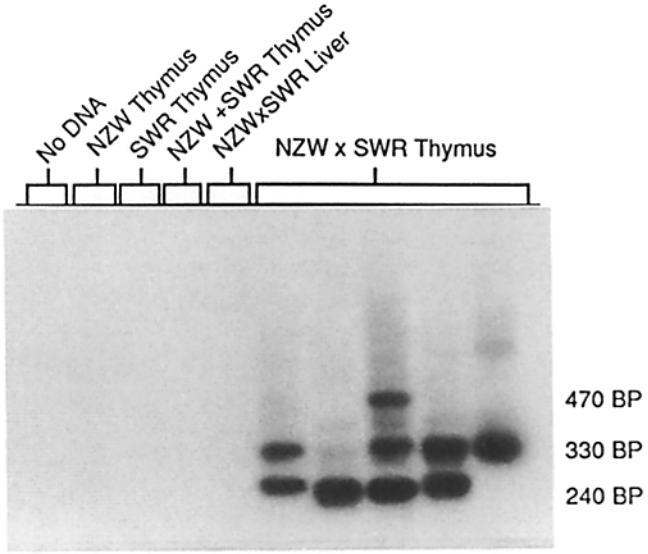

Figure 3. Detection of $V_\beta8/J_\beta2$ *trans*-rearrangements in thymocytes of SWR × NZW mice. PCRs were performed with $V_\beta8$ and $J_\beta2$ primers and the following additions: water only; NZW thymic DNA (2 μg); SWR thymic DNA (2 μg); NZW thymic DNA (1 μg) mixed with SWR thymic DNA (1 μg); NZW × SWR hepatic DNA (2 μg); or NZW × SWR thymic DNA. (2 μg). Amplified *trans*-rearrangements were detected on Southern blots by hybridization with an internal $V_\beta8$-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_1 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 Vβ8ihp probe on Southern blots (Fig. 3). The position of hybridizing bands most commonly corresponded to the expected size of Vβ8/Jβ2.5, Vβ8/Jβ2.4, and Vβ8/Jβ2.3 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 Vβ8/Jβ2 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 ($\sim 4 \times 10^4$ cell equivalents) pooled from three F_1 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 Jβ2.5, Jβ2.4, and Jβ2.3 rearrangements. In contrast, hybridizing bands corresponding in size to rearrangements involving Jβ2.2 and Jβ2.1 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 Vβ8/Jβ2 *trans*-rearrangements in F_1 thymus is 1.4 per 10^5 cells. This is somewhat lower than the true frequency, since Vβ8/Jβ2.6 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 Vβ5 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 Vβ8 primers to ascertain reaction conditions that permit specific and sensitive amplification of Vβ5/Jβ2 rearrangements (not shown). F_1 thymic DNA was then amplified with Vβ5/Jβ2 primer pairs (Fig. 4). 9 of 10 PCRs performed with 2 μg of F_1 thymic DNA contained at least one to as many as three amplification products that hybridized to an internal Vβ5-specific probe (Vβ5ihp). Most of these products approximated the expected size of Vβ5/Jβ2.5 or Vβ5/Jβ2.4 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 Vβ5/Jβ2 rearrangements to be about 1 per 10^5 cells (not shown), close to that observed for Vβ8/Jβ2 rearrangements. Again, rearrangements involving Jβ2.1 and Jβ2.2 were not seen at limiting dilution.

The identity of the interallelic *trans*-rearrangements was

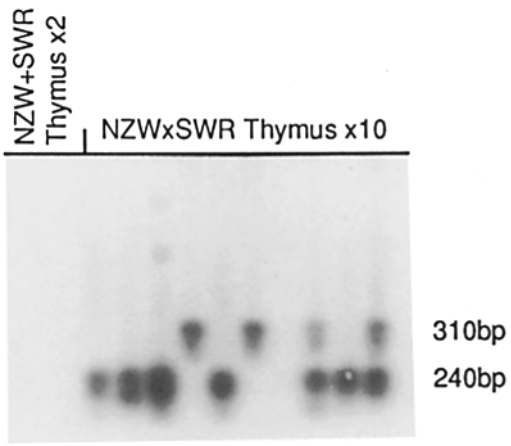

Figure 4. Detection of Vβ5/Jβ2 *trans*-rearrangements in NZW × SWR thymocytes. NZW thymic DNA (1 μg) mixed with SWR thymic DNA (1 μg), or NZW × SWR thymic DNA (2 μg) was used as template in PCRs containing Vβ5- and Jβ2-specific primers. Amplified *trans*-rearrangements were detected on Southern blots by hybridization with an internal Vβ5-specific probe end-labeled with Phosphorous-32. The resultant autoradiogram is shown.

further confirmed by the sequencing of PCR products cloned into M13. A total of twenty distinct Vβ8 and two Vβ5 clones were analyzed (Table 3). All consist of Vβ8 or Vβ5 coding segments joined to Jβ2 coding segments in a manner resembling standard recombinase-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 Dβ1 segments, no rearrangements bearing the Dβ2-specific sequence GACTG are seen, indicating that participation of Dβ1 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 Vβ8/Jβ1 *cis*-rearrangements was determined by limiting dilution of F_1 thymic DNA in PCRs containing Vβ8 and Jβ1.5 primers (Fig. 5). In these experiments, SWR thymic DNA that lacks Vβ8 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_β	(P_V)-N-(P_D)	$D_\beta 1$	(P_D)-N-(P_J)	J_β
			GGGACAGGGGGC		
V8.1	AGCAGTGATG				
V8.1.a	AGCAGTGATG				GTCAAAACA 2.4
V8.1.b	AGCAGTGAT		GAC		CAAAACA 2.4
V8.1.c	AGCAGTGAT		GGGG		ACA 2.5
V8.1.d	AGCAGTGATG	C	GGGG		AGACA 2.5
V8.1.e	AGCAGT	CAAAGCC	GGACA		AAACACCGG 2.2
V8.1.f	AGCAGT	T	GGGACAGGGG	AGA	TGCAGAAA 2.3
V8.2	AGCGGTGATG				
V8.2.a	AGCGGTG	CTCCC	GGA		GTGCAGAAA 2.3
V8.2.b	AGCGGT	AAG	GGGAC	C	GACA 2.5
V8.2.c	AGCGGTG	TGGC	GGG	T	AGTCAAAACA 2.4
V8.2.d	AGCGGTG	TTC	GGGACAG		CCAAGACA 2.5
V8.2.e	AGCGGTGAT		GC	C	AGTCAAAACA 2.4
V8.2.f	AGCGGTG	TAC	GACAG	A	AAAACA 2.4
V8.2.g	AGCG		GGGACAGGGG	AGGAG	GCTG 2.1
V8.2.h	AGCGGTGATG	CAC	GGACA	A	TGCAGAAA 2.3
V8.2.i	AGCGGTG	C	GACA		ACCAAGACA 2.5
V8.2.j	AGCGG				CAGAAA 2.3
V8.3	AGCAGTGATG				
V8.3.a	AGCAGT		GGGACAGGG	ATACGT	ACCGG 2.2
V8.3.b	AGCAGTGAT		GGGACAGG	AAG	GACA 2.5
V8.3.c	AGCAGTGATG		GGGGG	GAT	AAGACA 2.5
V8.3.d	AGCAGTGAT		GAC	C	AAACA 2.4
V5.1	CAGCTCTCTC				
V5.1.a	CAGCTCTCTC	G	GGGAC		AACCAAGACA 2.5
V5.1.b	CAGCTC	GT	ACAGGGGGC	TG	CCAAGACA 2.5

Germline V_β sequences are indicated with bold labels. Underlined nucleotides represent possible P additions. Ambiguous nucleotides have been arbitrarily assigned to D_β segments and are indicated in italics. For comparison, the germline sequence of $D_\beta 2$ is GGGACTGGGGGGGC.

tions containing F_1 DNA. Under the conditions employed, inclusion of 200 pg (20 cell equivalents) of F_1 thymic DNA resulted in amplification of one to several bands which hybridized to the V_β8ihp probe on Southern blots. Reactions performed with SWR DNA alone were negative. The pattern of bands seen with 20 cell equivalents of F_1 DNA indicates that this is close to the limiting dilution, which is confirmed by further dilution to two cell equivalents. Thus, the frequency of $V_\beta 8/J_\beta 1$ *cis*-rearrangement in F_1 mice is close to 1 in 10 cells. Again, this is somewhat lower than the true frequency since $V_\beta 8/J_\beta 1.6$ rearrangements will be missed. The data indicate therefore that the ratio of intragenic *cis*-rearrangement to interallelic *trans*-rearrangement of $V_\beta 8$ segments is roughly 10^4:1.

To further compare the fine structure of $V_\beta 8$ *cis*- and *trans*-rearrangements, 22 *cis*-rearrangements were amplified from F_1 thymic DNA with $V_\beta 8$ and $J_\beta 1.5$ specific primers, cloned, and sequenced (not shown). All clones consisted of $V_\beta 8$ coding segments joined to $J_\beta 1$ coding segments. Like the $V_\beta 8/J_\beta 2$ *trans*-rearrangements, many coding junctions contained sequences homologous to $D_\beta 1$ 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 $V_\beta 8/J_\beta 2$ *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.

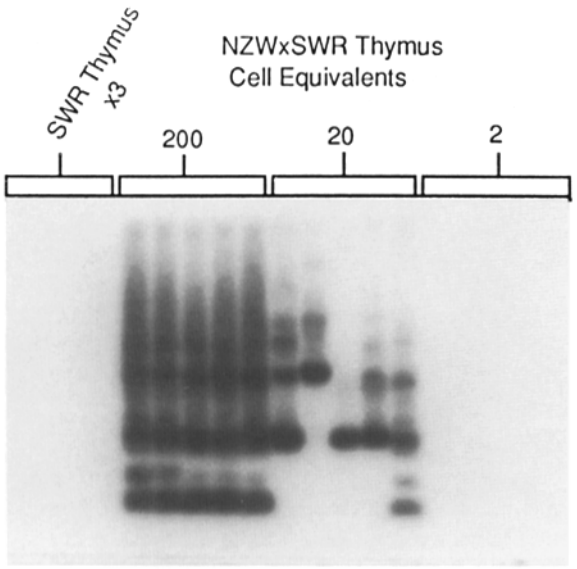

Figure 5. Frequency of $V_\beta 8/J_\beta 1$ *cis*-rearrangement in NZW × SWR thymocytes. SWR thymic DNA (2 μg) or indicated amounts of NZW × SWR thymic DNA mixed with SWR thymic DNA were used as template in PCRs containing $V_\beta 8$- and $J_\beta 1$-specific primers. Amplified *cis*-rearrangements were detected on Southern blots by hybridization with an internal $V_\beta 8$-specific probe end labeled with Phosphorous-32. The resultant autoradiogram is shown.

The calculated frequency of interallelic *trans*-rearrangements in the F_1 mice is about an order of magnitude lower than that previously reported for other types of *trans*-rearrangement which produce chimeric receptors, such as V_γ/J_β and V_γ/J_δ 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 $V_\gamma/J_\beta 2$ *trans*-rearrangements was investigated in F_1 thymuses. In these experiments, NZW thymic DNA that lacks $J_\beta 2$ segments was used as a negative control. Addition of 0.2 μg (2×10^4 cell equivalents) of DNA to the PCR consistently resulted in amplification of several bands which hybridized to an internal $V_\gamma 2$ probe ($V_\gamma 2$ihp) 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 $V_\gamma 2$ coding segments to $J_\beta 2$ coding segments without interposed D_β segments (Table 4). Further dilution revealed positive signals in 11 of 24 reactions containing 0.04 μg (4×10^3 cell equivalents) of DNA (not shown), giving a frequency for $V_\gamma 2/J_\beta 2$ *trans*-rearrangements of ~15 per 10^5 cells. The size of most rearrangements approximated that expected for joining with $J_\beta 2.5$, $J_\beta 2.4$, or $J_\beta 2.3$. Bands corresponding to the size of $V_\gamma 2/J_\beta 2.1$ rearrangements were not seen. Once again, the calculated frequency for $V_\gamma 2/J_\beta 2$ *trans*-rearrangements represents a lower limit, since rearrangements involving V_γ segments other than $V_\gamma 2$ or $J_\beta 2.6$ will not be detected by the primers used. With this caveat, it can be concluded that the frequency of intergenic V_γ/J_β *trans*-rearrangements is about one order of magnitude higher than that of interallelic V_β/J_β *trans*-rearrangements.

Table 4. *Sequences of $V\gamma 2/J\beta 2$ Trans-Rearrangements*

$V_\gamma 2$	P_V-N-P_J	J_β
GTTCCTACGG	G	GACA 2.5
GTTCCTACG		AAACAC 2.4
GTTC		CAAGACA 2.5
GTTCCTACGG	CTGAGG	GTCAAACAC 2.4
GTTCCTACGG	CTAAAGT	AACCAAGACA 2.5
GTTCCTACGG	CTATT	CAAGACA 2.5

Underlined nucleotides represent possible P additions.

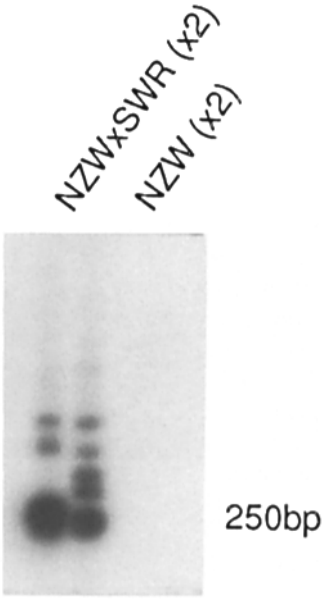

Figure 6. Detection of $V_\gamma 2/J_\beta 2$ *trans*-rearrangements in NZW × SWR thymocytes. NZW or NZW × SWR thymic DNA (2 μg) was used as template in PCRs containing primers specific for $V_\gamma 2$ and $J_\beta 2$. Amplified *trans*-rearrangements were then detected on Southern blots by hybridization with an internal $V_\gamma 2$-specific probe end labeled with Phosphorous-32. The resultant autoradiogram is shown.

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 10^4:1. In NZW × SWR mice, the frequency of interallelic *trans*-rearrangement involving $V_\beta 8$ and $V_\beta 5$ segments within the TCR-β locus is around 1 per 10^5 thymocytes. Assuming that the ratio of *cis*- to *trans*-rearrangement is also about 10^4:1 for other V_β segments, the cumulative frequency of all interallelic *trans*-rearrangements in TCR-β is probably not greater than 1 per 10^4 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_H segment (22), V_H1, which shows strain-specific variation, and also possess variation in C_H segments. Therefore, it is possible to breed animals that are doubly heterozygous for allotypic V_H and C_H sequences. About 1% of Ig molecules from the peripheral blood of such animals appear to contain V_H and C_H 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-β 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_H allotypes can be induced to express them after immunization with antiallotype antibody (27), and pseudogenes potentially capable of contributing V_H 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_H segments, the serological data could also be explained by *trans*-switching (11) or *trans*-splicing (29), subsequent to recombinase-mediated $C_H\mu$ *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-β 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 $C_\beta1$, $D_\beta2$, and $J_\beta2$ segments from the NZW allele diminishes *trans*-rearrangement of residual $D_\beta1$ and $J_\beta1$ 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 $V_\gamma/J_\beta1$ rearrangements occur at similar frequencies in NZW and wild-type mice (data not shown), indicating that deletions within TCR-β 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*-rearrangement 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_β or J_β 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_β 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_λ sequences in chickens (34, 35) and V_H 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_β 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-β would have been expected to result in recombinant V_β 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 $D_\beta2$ segments from interallelic *trans*-rearrangements, since $D_\beta2$ segments participate in about 50% of *cis*-rearrangements containing $J_\beta2$ segments (20, 36). In contrast, a *trans*-joining mechanism mediated by recombinase could produce this result if interallelic *trans*-rearrangement within TCR-β is limited to D_β to J_β joining. *Trans*-rearrangements occurring during attempted $V_{\beta NZW}$ to $D_\beta J_{\beta SWR}$ joining can contain either $D_\beta1$

or $D_\beta 2$ segments, since the SWR allele contains both $D_\beta 1$ and $D_\beta 2$ segments. In contrast, the NZW allele contains only a $D_\beta 1$ segment, so that all *trans*-rearrangements occurring during attempted $D_{\beta NZW}$ to $J_{\beta SWR}$ joining must involve $D_\beta 1$ segments and cannot contain $D_\beta 2$. The lack of $D_\beta 2$ segments in the interallelic *trans*-rearrangement products is therefore consistent with restriction of such rearrangements within TCR-β to D_β to J_β joining, with subsequent V_β to $D_\beta J_\beta$ joining occurring only in *cis*.

Our data also suggest that J_β usage differs when D_β to J_β joining occurs in *trans* rather than *cis*. $J_\beta 2.1$ segments normally participate in about 20–30% of *cis* D_β to $J_\beta 2$ rearrangements (20, 36). In contrast, hybridizing bands of the expected size of a $V_\beta/J_\beta 2.1$ *trans*-rearrangement were quite infrequent, not being seen at all in PCRs performed at limiting dilution. The reason for this difference in J_β usage is unclear, but it may be a general feature of *trans*-rearrangements involving $J_\beta 2$ segments, since V_γ/J_β *trans*-rearrangements also appear to only rarely involve $J_\beta 2.1$. These data further support restriction of interallelic *trans*-rearrangement to D_β to J_β joining, since *trans*-rearrangements formed by joining of V_β segments to $D_\beta J_\beta$ segments previously rearranged in *cis* would be expected to frequently contain $J_\beta 2.1$.

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 V_β to $D_\beta J_\beta$ interallelic *trans*-rearrangement might thus be the result of the temporal separation of V_β to $D_\beta J_\beta$ rearrangement events in the two alleles. The situation is analogous to what has been proposed to occur during V_H to $D_H J_H$ joining in pre-B cell lines (2), during which temporally 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 V_γ/J_β *trans*-rearrangements appear to occur more frequently than interallelic D_β/J_β *trans*-rearrangements. Additional studies assessing the spatial relationship of ARGs in differentiating lymphoid cells may help to resolve this question.

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