Brief Definitive Report

Level of Human TCRBV3S1 (Vβ3) Expression Correlates with Allelic Polymorphism in the Spacer Region of the Recombination Signal Sequence

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Summary

One of the causes of variations in the expressed human T cell receptor (TCR) BV (Vβ) repertoire is genetic variation in the germline DNA. Herein evidence is provided that allelic polymorphism may affect recombination frequency for a specific V gene. Two alleles of the TCR BV3 differ only at a single nucleotide position (C/T) within the 23-bp spacer region of the recombination signal sequence. These alleles are associated with variable percentages of BV3 cells in the peripheral blood, as shown in families and in unrelated normal donors. Individuals homozygous for allele 2 have a mean of 8.1% BV3 cells, heterozygous individuals have a mean of 4.7% BV3 cells, and homozygotes for allele 1 have a mean of 1.2% BV3 cells in $CD3^+$ $CD4^+$ peripheral blood T cells. Since the correlation is tight in unrelated individuals and other genetic differences were not found in the vicinity of BV3, we suggest that the spacer region sequence itself modifies recombination efficiency. This allelic system provides an example of a novel mechanism by which *cis*-acting genetic elements may affect recombination in a natural in vivo system.

The vast diversity of TCR and immunoglobulins is created primarily by V(D)J recombination in maturing lymphocytes. As a result of this theoretically random process, a repertoire of highly diverse TCR is generated (1), but the observed repertoire in mature lymphocytes is skewed because of both genetic and environmental factors. Genetic control of the TCR repertoire is apparent because of highly similar profiles of TCR Vα and Vβ usage among monozygotic twins as opposed to unrelated individuals (2–6). Genes that influence the TCR repertoire include the MHC (2) and the TCR genes themselves. MHC genes mold the TCR repertoire because of recognition of peptide Ag in the context of MHC molecules. Thus, certain T cell subsets may be positively or negatively selected in the context of one MHC allele but not another.

TCR V gene segments are deleted in certain mouse haplotypes (7). Genomic deletions in the TCRBV locus also exist in humans (8) and directly affect the number of genomic V gene segments (9). In addition, a number of allelic polymorphisms of TCR V gene exons have been described. Some of these result in null alleles (10, 11) and others result in different amino acid sequences at putative Ag/MHC or SAG-reactive sites (12–15). It is also possible that TCR gene elements fail to rearrange efficiently because of structural constraints on some aspect of the recombinase machinery.

This report describes a new type of polymorphism that can shape the TCR repertoire. Allelic variation in the TCRBV3 recombination signal sequence (RSS) is associated with the level of BV3 gene segment utilization in the peripheral blood (formerly Vβ3, the new WHO-IUSIS recommended nomenclature is used throughout this manuscript. *Bull. WHO* 71:113–115, 1993). Variable expression of BV3 was first mapped to the TCRB locus in family studies and then shown to correlate with allelic variants of the BV3 RSS in unrelated individuals, which suggests a direct role for the RSS in determining the frequency with which the BV3 gene segment undergoes V-DJ recombination. It is surprising that the single point mutation is located in the nonconserved spacer region of the RSS. This allelic system demonstrates a novel mechanism whereby genetic factors affecting recombination may strongly skew expression of certain gene segments.

Materials and Methods

Donors. The unrelated donors are normal adults of various ethnic backgrounds. The families include the D family (Irish), 98, 110, and 23 families, all of northern European ancestry.

Immunofluorescence. Indirect two- and single-color immunofluorescence was performed as described elsewhere (16) using the anti-TCR mAb C1 (anti-BV17), 8F10 (anti-BV3), and others (16).

PCR and Sequencing. For typing of alleles of the BV3-RSS, PCR was performed with predetermined optimal amounts of genomic DNA, primers at 200 nM, 1.5 mM $MgCl_2$, dNTPs at 0.5 mM in 0.05 ml of 10 mM Tris (pH 9 at 25°C), 50 mM KCl, 0.01% gelatin, and 0.1% Triton X-100 (36 cycles at 94°C for 1 min, 51°C for 1 min, 72°C for 1.5 min, with a final cycle at 72°C for 10 min). The primers used were based on a sequence of the BV3 gene of the cosmid clone 46.1 (17). They were BV3-intron 5′ CCTTGATGGCCTGTTTTTCAC 3′ (sense) and BV3-3′ of RSS 5′ GTGCCATCGGAGCCAGCAC 3′ (antisense). These primers amplify a 431-bp fragment including the entire second BV3 exon and the RSS. The PCR product was then digested with PvuII to distinguish between the two alleles.

For sequencing of genomic BV3, overlapping PCR fragments were amplified and cloned. The downstream fragment is described above and the upstream fragment (392 bp), including 209 bp of promoter region, the first BV3 exon, and most of the intron was amplified with the following primers: 5′GCTGTGTCAACTCTTTTCTA 3′ (sense) and 5′ TGAGAGACAGAAAGCAAGG 3′ (antisense). 35 clones were sequenced from the various members of the D family, usually with at least three replicates to recognize Taq errors. The Taq error rate was ~1/2,000 bp.

Additional nucleotide sequences extending 5′ and 3′ of the BV3 gene were determined by cycle sequencing on two cosmids, H46.1 and HVB19.1 (17a). To test for polymorphism in these flanking regions, genomic DNA corresponding to 842 bp 5′ of the ATG codon of the BV3 gene and 100 bp 3′ of the RSS heptamer were separately amplified from 14 unrelated individuals. PCR bands were separated on low melting agarose and directly sequenced (18) on both strands.

Haplotyping. Segregation of allelic forms of TCRBV genes was followed in families. The allelic markers carried by each parental haplotype were determined and haplotypes were assigned to family members. In family D, TCRBV alleles, including TCRBV6S1, TCRBV12S2, TCRBV12S3, TCRBV20, and TCRBC3′, were characterized by single stranded conformational polymorphism (SSCP) as described (19). In other families, RFLPs of TCRBV8S2/BamHI, TCRBV7S2/BamHI, TCRBC/BglII, TCRBC/KpnI, and microsatellites within the intron of TCRBV6S7 and TCRBV6S1 (20) were used to determine haplotypes.

HLA Typing. HLA typing was performed according to previously defined methods (21) and by PCR (22). Part of the typing for families 23, 98, and 110 was kindly provided by Dr. R. Spielman (University of Pennsylvania, Philadelphia, PA).

Results and Discussion

While screening normal donors for TCRBV expression with various specific mAb, we observed several donors with low percentages of BV3 T cells per total CD3-positive T cells. The family of one such individual (family D, Fig. 1) was tested for BV3 expression, demonstrating that only the blood of the mother (M) and one sibling (S4) contained about 4% BV3 T cells, whereas that of the father (P) and S1-S3 contained <0.6% BV3 T cells (Fig. 1). By contrast, BV17 (and 11 other Vβ tested) were expressed at similar levels in all family members (Fig. 1). These results are consistent with prior work by Malhotra et al. (6) in which PCR was used to assay BV3 expression in family studies.

Four families were analyzed (Table 1). In each family BV3 expression correlated exactly with TCRB haplotypes. The maternal "c" haplotype in family D (M, S4), and the paternal "a" haplotype in family 98 (98.1 P, 98.3), both segregate with high BV3 expression. By contrast, BV3 expression does not segregate with MHC haplotypes in family D. These data indicate that a gene within the TCRB locus is controlling the frequency of BV3-positive cells.

Expression of BV3 was never totally absent since brightly stained cells could always be observed even in donors with only 0.3% positive cells. Moreover, similar results were obtained with another BV3-specific mAb, 5E4 (data not shown). Since BV3-expressing cells were apparently present, even in individuals with very low percentages of BV3 cells, it was important to demonstrate the presence of a normal BV3 gene in the various members of family D. The initial approach was to clone and sequence genomic BV3 from each family member. PCR-amplified DNA was subcloned into a TA over-

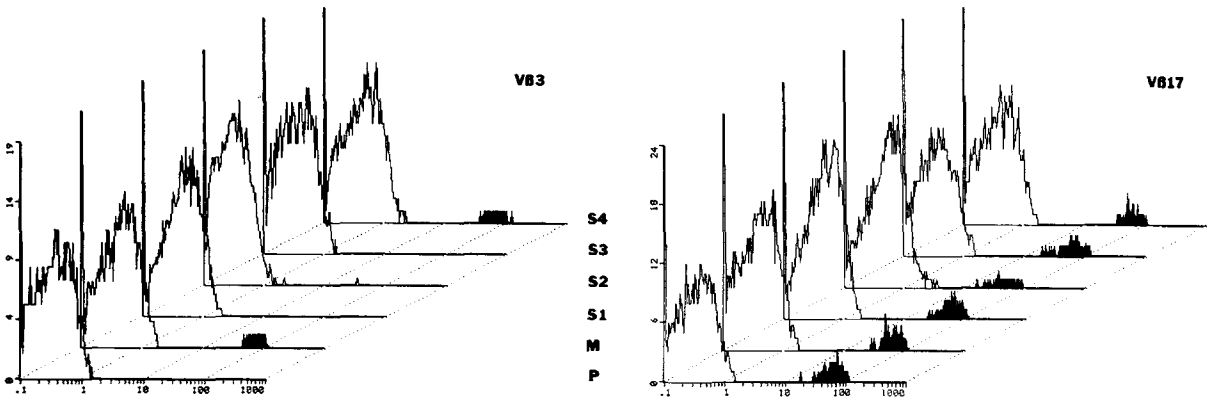

Figure 1. Staining for BV3 and BV17 in family D.

Table 1. *Analysis of BV3-positive T Cells in Four Families and Correlations with TCR Haplotypes*

Family	BV3-RSS PvuII	TCRB haplotype	MHC haplotype	BV3
				%
D-P	1/1	a/b	a/b	0.6
D-M	1/2	c/d	c/d	3.7
D-S1	1/1	a/d	a/d	0.3
D-S2	1/1	a/d	a/c	0.4
D-S3	1/1	a/d	a/c	0.3
D-S4	1/2	a/c	a/c	4.2
98.1 P	1/2	a/b	a/b	3.9
98.2 M	1/1	c/d	c/d	0.7
98.3	1/2	a/c	b/c	5.5
98.4	1/1	b/d	a/c	1.3
98.5	1/1	b/c	b/c	0.5
98.6	1/1	b/c	b/c	0.6
98.7	1/1	b/c	b/d	1.1
110.1 P	2/2	a/b	a/b	7.1
110.2 M	2/2	c/d	c/d	7.4
110.3	2/2	a/d	b/c	8.7
110.5	2/2	b/d	b/d	9.6
110.6	2/2	a/c	b/d	6.5
110.7	2/2	a/d	b/c	7.3
23.1 P	1/1	a/b	a/b	1.0
23.2 M	2/2	c/d	c/d	9.1
23.2	1/2	b/d	b/d	4.7
23.4	1/2	b/d	a/c	4.0
23.5	1/2	b/c	a/c	5.2
23.6	1/2	a/d	b/d	4.5

BV3 percentages are per total CD3+CD4+ cells except for family D where the results are expressed per total CD3+ cells. The HLA haplotypes were:

Family D
(a) A32-B62-Cw3-DRB1*1301
(b) A2-Bx-Cw5-DRB1*1301
(c) A24-B7-Cw7-DRB1*0401
(d) A1-B8-Cw7-DRB1*1302

Family 23
A24-B35-DR1
A24-B39-DR8
A1-B8-DR7
A2-B44-DR4

Family 110
(a) A2-B40-DR4
(b) A29-B12-DR7
(c) A3-B7-DR1
(d) A3-DR6

Family 98
A1-B17-DR7
A2-B27-DR1
Aw23-B12-DR5
A1-B18-DR5

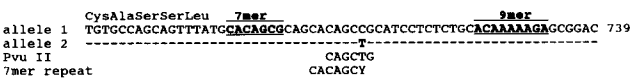

Figure 2. Sequences of the BV3-RSS demonstrating the difference between two alleles. The nucleotide numbering is based on 903 bp of genomic sequence data available from EMBL/GenBank/DDBJ under accession number U08314.

hang vector and several subclones (total 35) were sequenced from each individual of family D. All clones from P, S1, S2, and S3 yielded the same sequence (allele 1), but clones from M and S4 yielded two sequences, allele 1 and allele 2 (Fig. 2). For instance, three clones represented allele 1 and four clones represent allele 2 for DNA derived from M. The two alleles of BV3 differed by a single base pair mutation in the 23-bp spacer region of the RSS downstream of BV3 (Fig. 2). A total of 800 bp of sequence, including 209 bp from the promoter, both BV3 exons, the intron, and 96 bp of 3'UT, were otherwise identical in the various members of family D. Thus, all members of family D possess an apparently normal BV3 gene.

The mutation in the BV3-RSS results in a PvuII restriction enzyme site specific for allele 2, CAGCTG (Fig. 2). This allowed us to determine genotypes at this site and test for correlation with percentages of BV3-expressing T cells in the blood. Fig. 3 shows PvuII digests of PCR-amplified DNA using the primers flanking the BV3 gene with its RSS. PvuII cleaves the 431-bp PCR product from allele 2 into 352- and 79-bp fragments. The presence of allele 2 correlates with expression of larger percentages of BV3 cells in family D (Fig. 3) and in the other families (Table 1). In family 23, for example, the father is homozygous 1/1 (1.3% BV3 cells), the mother is homozygous 2/2 (8.8% BV3 cells), and all siblings are heterozygous and express intermediate percentages of BV3 cells (3.8–4.9%). In family 110, all members are homozygous 2/2 and have similar high percentages of BV3 cells.

These data confirm that a gene within the TCRB locus

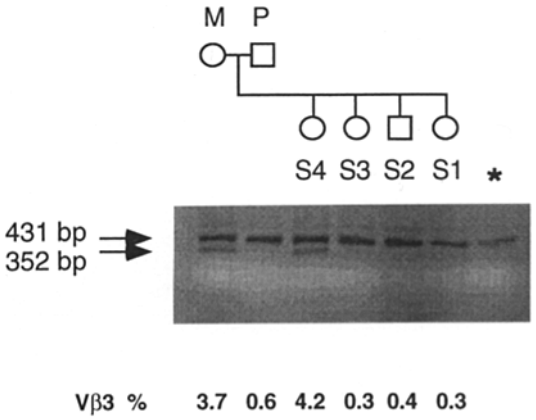

Figure 3. PvuII digest of PCR amplified BV3-RSS from family D showing correlation with percentage of BV3 cells in the blood. The lane marked with (*) is an undigested control. The lesser relative intensity of the lower band is likely due to incomplete PvuII digestion.

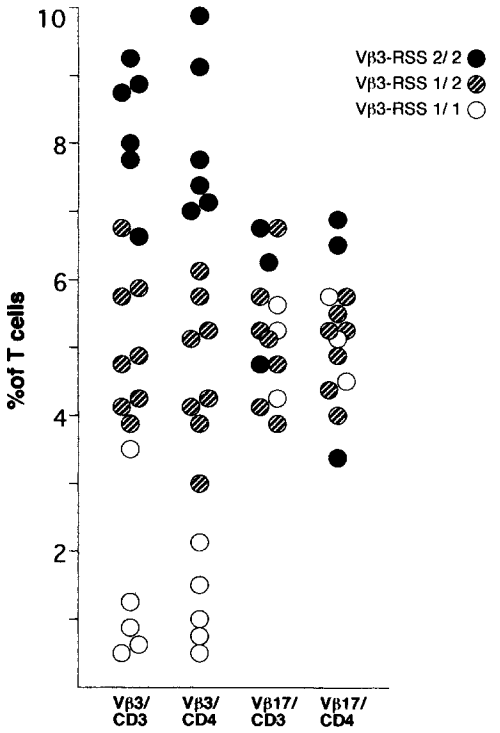

Figure 4. Correlation between percentage of BV3-positive T cells and BV3-RSS genotype in unrelated individuals. Both BV3- and BV17-positive cells were counted as a percentage of either all $CD3^+$ cells or of $CD3^+CD4^+$ cells. The means of BV3 percentages (per total CD3 or per total $CD3^+CD4^+$) in the three genotype groups were significantly different from one another ($p < 0.0001$) by one-way analysis.

is responsible for the variable frequency of BV3-expressing T cells, but do not distinguish between several possibilities: (*a*) a mutation in the promoter of the BV3 gene might result in deficient BV3-DJ recombination, since recombination is dependent on transcriptional activity in the recombining locus (23); (*b*) a large insertion/deletion polymorphism near the BV3 gene or a structural defect elsewhere in the TCRB locus may affect BV3-DJ recombination by an unknown mechanism; and (*c*) decreased recombination frequency of allele 1 may be due to the point mutation in the spacer region of the BV3-RSS.

To rule out large insertion/deletion polymorphisms, we screened genomic DNA from the parents of family D by hybridization using two cosmid clones as probes (17). The cosmid clones both contain the BV3 gene segment and span in excess of 50 kb of genomic DNA including sequences both up- and downstream of BV3. On Southern blots using five different restriction enzymes, both probes failed to uncover any large insertion/deletion variants (data not shown). Moreover, sequencing 842 bp 5′ of the ATG codon and 100 bp 3′ of the heptamer in 14 unrelated individuals, including the parents of family D, revealed no further polymorphism suggesting that the promoter region of BV3 was not polymorphic.

Unrelated individuals have a large number of different TCRB haplotypes (24, 25), even if they share allelic markers at a single site such as in the BV3-RSS. Thus, it was reasoned that if the BV3-RSS mutation was the direct cause for decreased BV3 usage among T cells, the correlation between the PvuII-defined alleles and percentages of BV3 cells should also hold true in unrelated donors. Fig. 4 shows a striking correlation between inheritance of TCR BV3 alleles and the percentage of BV3 cells. Generally, a genotype of 1/1 is associated with low percentages of BV3 cells (mean 1.2% of $CD3^+CD4^+$ cells), a genotype of 2/2 is associated with high percentages of BV3 cells (mean 8.1%), and a heterozygous genotype of 1/2 is associated with intermediate percentages of BV3 cells (mean 4.7%). Thus, the cumulative data suggest that the single mutation in the BV3-RSS spacer affects V-DJ recombination for the BV3 gene segment.

The RSS mediating V-J recombination in the mouse κ and λ loci are directly responsible for dramatic differences in recombination frequency observed in plasmid recombination substrates (26). Previous data had suggested that a fixed length of 23 or 12 bp was required for the RSS spacer region, but that the nonconserved spacer sequence itself was of no import. In contrast, mutations in the conserved heptamer and nonamer reduce recombination frequency in artificial plasmid recombination substrates transfected into pre-B cells (27). However, recombination of genomic DNA in a physiologic in vivo setting cannot be assessed by these methods.

It may be relevant that genomic DNA is often methylated at CpG islands and that methylation of plasmid recombination substrates has been shown to inhibit recombination (28). Close scrutiny of the BV3-RSS spacer of allele 1 reveals the sequence GCCGC, which may be methylated in vivo, whereas allele 2 contains the sequence GCTGC at this site. Also of interest is the simple repeat of the heptamer observed in the BV3-RSS, as well as in many other RSS (29). Thus the sequence CACAGCGnnnCACAGCT (allele 2) may allow functional binding of a required component of recombinase, whereas the sequence of allele 1 CACAGCGnnnCACAGCC does not. A future goal will be to determine the exact mechanism by which the C/T substitution in the BV3 RSS affects recombination using plasmid recombination substrates.

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