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CONCISE REPORT

Analyis of a T-cell receptor Vβ segment implicated in susceptibility to rheumatoid arthritis: Vβ2 germline polymorphism does not encode susceptibility

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Abstract

***Objectives*—The assessment of allelic polymorphism of the T cell receptor gene segment, TCRBV2S1, in rheumatoid arthritis.**

***Methods*—A total of 136 patients with rheumatoid arthritis (RA) (ACR criteria) and 150 controls were TCRBV2S1 genotyped using a nested PCR amplification strategy followed by single-strand conformation polymorphism (SSCP) analysis.**

***Results*—The SSCP typing method detected two previously unknown alleles of the TCRBV2S1 gene segment. The TCRBV2S1 allele, genotype and inferred phenotype frequencies were similar in the RA patients and controls. No differences were apparent after the RA patients had been partitioned according to their HLA-DR genotypes.**

***Conclusions*—SSCP analysis is a rapid and efficient method of typing T cell receptor germline polymorphisms. Allelic polymorphism of the T cell receptor variable segment, TCRBV2S1, does not influence susceptibility to RA.**

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Rheumatoid arthritis (RA) is strongly associated with a limited range of HLA-DR molecules[1] that form one component of the trimolecular complex (T cell receptor/peptide/HLA) which is integral to specific immune responses.[2] Several lines of investigation also suggest that CD4-positive T cells are important in the pathogenesis of RA.[3] Furthermore, an increasing body of evidence, both from animal models of autoimmunity,[4 5] and human disease,[6] suggests that a restricted repertoire of T cell receptors (TCR) and TCR variable gene segments may be involved in this group of disorders. Recent advances in our understanding of the mechanisms by which the mature TCR repertoire is generated in humans allow insights into how potentially pathogenic T cells may develop in RA.

The involvement of a particular range of TCR in the pathogenesis of RA requires some knowledge of how the mature T cell repertoire is generated. First, enormous diversity is generated from different combinatorial associations of V (variable), D (diversity), J (junctional) and C (constant) segments, particularly since random addition of nucleotides also occurs at the splice sites.[2] Second, there is considerable germline polymorphism (as yet incompletely characterised) at the TCR loci. Although allelic polymorphism was originally described in only four Vβ segments, Vβ1, Vβ6·7, Vβ2 and Vβ6·1, it is now recognised that such variation occurs in the majority of V segments.[7–9] Two of these germline polymorphisms have been implicated in susceptibility to juvenile rheumatoid arthritis[10] and primary Sjögren's syndrome.[11] In both cases, the association was found in only a subset of patients defined either by clinical criteria or HLA typing.

Investigation of TCR usage by activated lymphocytes infiltrating the synovium in RA has yielded conflicting results, some of which may be attributable to the variety of techniques that have been employed.[12] However, one recent study demonstrated increased usage of Vβ2·1 and Vβ3·1 segments by synovial T cells in RA using the inverse polymerase chain reaction (PCR), a technique which overcomes some of the technical difficulties inherent in studying the TCR repertoire.[13] The recent description of a triallelic polymorphism for the Vβ 2·1 segment,[7] together with the suggestion of involvement of this segment in RA[13] prompted us to investigate germline variation of this TCR segment. The single-strand conformation polymorphism (SSCP) method was used for genotyping because of its ability to identify new alleles[7 14] which may be crucial to disease association studies where 'novel' alleles that are rare in the normal population may be enhanced in a disease group.

Patients and methods

PATIENTS

We studied 136 white patients with erosive seropositive RA satisfying the 1987 ACR criteria, and 150 ethnically similar healthy individuals.

HLA TYPING

HLA-DR genotypes were determined by PCR

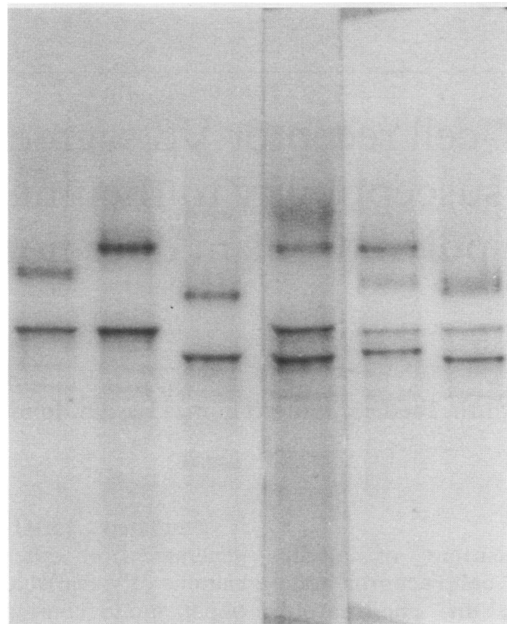

*Figure 1 Genotyping of TCRBV2S1 allelic polymorphism by single-strand conformation polymorphism (SSCP). Lanes 1-3 show the SSCP pattern from individuals homozygous for alleles TCRBV2S1*1, *2, or *3 respectively. Lanes 4 and 5 show patterns suggesting new alleles. Lane 4 has the pattern of allele *2 and the lower band as per lane 3, without the upper band and was sequenced as TCRBV2S1*2/4. Lane 5 has the pattern of allele *2 and two other bands, the lowest migrating higher than the expected lower band as seen in lane 6, and was sequenced as TCRBV2S1*2/5. Lane 6 is TCRBV2S1* 1/3.*

amplification of DRB1 alleles from genomic DNA and serial hybridisation to radiolabelled oligonucleotides as described previously.[15]

TCRBV2S1 (Vβ2) TYPING

Genotypes of the TCR β chain variable region segment family 2 member 1 (TCRBV2S1) were defined in patients and controls by PCR amplification followed by SSCP, as previously described.[7] Briefly, a fragment of the TCRBV2S1 segment was amplified from 200 ng of genomic DNA using the primers V intron (5'-CAAGTGGTGGCCAGCAGGG-3') and V3' (5'-CACTGCAGATGTAGAAGCTGC-3'). In the second round, 1 µl of 1:100 dilution of the initial PCR product was subjected to a further 15 cycles of nested family specific TCRBV2 amplification using the same V3' amplimer as above in combination with the primer V5' (5'-CTCCGGGCTTGGTGC-TGTC-3') to amplify a 296 base pair fragment of the V segment containing coding sequence only. The nomenclature is that of the WHO-IUIS subcommittee on TCR designation. Amplification products were heat-denatured in formamide and loaded on a non-denaturing polyacrylamide gel (10% glycerol, 10% 19:1 polyacrylamide:bisacrylamide) and separated at 10W/gel for 20 hours at 4°C in a Protean II*xi* electrophoresis gel apparatus (Biorad, Richmond, CA 94804). Gels were silver-stained according to the manufacturers instructions (Silver Stain, Biorad, Richmond, CA 94804) and dried under vacuum. Patterns of bands were compared to known alleles.[7] Novel alleles were suggested when bands did not conform to established patterns.

	Nucleotide position 28 Amino acid position 10		Nucleotide position 124 Amino acid position 41
TCRBV2S1*1	T W		C Q
TCRBV2S1*2	A R		C Q
TCRBV2S1*3	A R		A K
TCRBV2S1*4	T W	Position 103 Amino acid 34	A K
TCRBV2S1*5	T W	T to C W to R	C Q

*Figure 2 Allelic variants of TCRBV2S1 showing nonconservative basepair dimorphism at two positions. TCRBV2S1*5 replaces a highly conserved trytophan at position 34 and is probably nonfunctional.*
G = guanidine, A = adenine, T = thymidine, C = cytosine.
W = tryptophan, Q = glutamine, R = arginine, K = lysine.

DNA SEQUENCING OF NOVEL TCRBV2S1 ALLELES

To characterise the sequence of novel alleles a biotinylated 3' primer was used in the second round amplification above. Single-strand DNA was prepared using steptavidin coated magnetic beads (M280, Dynal AS, Oslo, Norway) and used as the sequencing template. Chain termination dideoxy sequencing was performed using Sequenase reagents (US Biochemical, Ohio 44122).

STATISTICAL ANALYSIS

TCRBV2S1 frequencies were compared between patients and controls using chi squared and Fisher's exact test. Comparisons were also made after partitioning the subjects with RA on the basis of HLA-DR genotypes known to be strongly associated with the disease, for example, DRB1*0401/*0404. The subject numbers were calculated in the study design to give 90% power to detect a threefold relative risk significant at $p = 0{\cdot}05$.

Results

ALLELIC VARIATION OF THE TCRBV2S1 GENE SEGMENT

In our RA and control populations we found the SSCP pattern for the three published alleles of TCRBV2S1.[7] In addition we found two new SSCP patterns (fig 1). The results of DNA sequencing of these two new variants, TCRBV2S1*4 and *5, and their relationship to the three previously published alleles is shown in fig 2. The allele TCRBV2S1*4 was found in 3 patients with RA, and in none of the controls ($p = 0{\cdot}11$, NS). The allele TCRBV2S1*5 was found in one control.

GENOTYPING OF TCRBV2S1 IN RA PATIENTS AND CONTROLS

The results of TCRBV2S1 typing are shown in the table. TCRBV2S1*1 indicating the TCR β chain variable segment family 2, member 1,

Table Distribution of TCRBV2S1 alleles in patients with rheumatoid arthritis and controls

	Rheumatoid arthritis (n = 136)	*Controls (n = 150)*
Allele		
TCRBV2S1*1	136(50%)	145(48%)
TCRBV2S1*2	104(38%)	108(36%)
TCRBV2S1*3	29(11%)	46(15%)
TCRBV2S1*4	3(2%)	0
TCRBV2S1*5	0	1(0·7%)
Genotype		
TCRBV2S1*1/1	35(26%)	39(26%)
TCRBV2S1*1/2	52(38%)	50(33%)
TCRBV2S1*1/3	14(10%)	17(11%)
TCRBV2S1*2/2	19(14%)	20(13%)
TCRBV2S1*2/3	11(8%)	17(11%)
TCRBV2S1*2/4	3(2%)	0
TCRBV2S1*2/5	0	1(0·6%)
TCRBV2S1*3/3	2(1%)	6(4%)
Inferred phenotype		
TCRBV2S1*1	101(74%)	106(71%)
TCRBV2S1*2	85(63%)	88(59%)
TCRBV2S1*3	27(20%)	40(27%)
TCRBV2S1*4	3(2%)	0
TCRBV2S1*5	0	1

Values are absolute counts followed by percentages for alleles, genotypes and inferred phenotypes.
There was no statistically significant difference between RA patients and controls.

allele 1. Eighty one per cent (110/136) of the patients with RA possessed one or more of the RA susceptibility alleles, HLA-DRB1*0401, DRB1*0404 or DRB1*0101. Comparison of TCRBV2S1 allele, genotype and inferred phenotype frequencies showed no significant differences between the patients with RA and controls (table). TCRBV2S1 frequencies were also very similar between the groups after partitioning for HLA alleles and genotypes strongly associated with RA (for example, HLA-DRB1*0401, *0404 and *0401/*0404).

Discussion

The SSCP technique is advantageous in population studies because it is relatively rapid and does not require the use of radiolabelled probes. It also allowed us to identify 2 novel alleles, in addition to assigning known genotypes at the TCRBV2S1 segment. These studies have hinted at the extent of polymorphism that probably exists for TCR V segments, with five variants already identified for TCRBV2S1. The TCRBV2S1*4 allele represents the fourth possible combination between the 2 variable positions of the TCRBV2S1 segment suggesting that polymorphism at these positions is likely to have been generated by gene conversion events. Consequently the coding polymorphism does not retain a tight linkage disequilibrium with genetic markers located on either side of the functional sequence. We would therefore infer that restriction fragment length polymorphism (RFLP) investigation of TCR genes is not necessarily appropriate for disease association studies, and that candidate V segments should be assessed with their coding sequences and not with a nearby RFLP. A somewhat analogous situation has been described in RA where RFLP can define the HLA-DR4 haplotype associated with the disease but not the individual subtypes of DR4 which have been generated by limited gene conversion events.[1]

The TCRBV2S1*5 allele differs from TCRBV2S1*1 by one nucleotide change (T_{103} to C) resulting in an amino acid substitution at position 34. Tryptophan at position 34 is a highly conserved residue in Vβ and Vα sequences and its replacement by arginine probably results in a non-functional segment. This might lead to reduced use of the TCRBV2S1 segment in the TCR repertoire of individuals with this allele.

The results clearly showed no difference between the RA patients and controls for the allele, genotype and inferred phenotype frequencies at the TCRBV2S1 segment, even when the patients were partitioned according to HLA genotypes known to be strongly associated with RA.[1] In particular, in view of the previous report of a DR4 positive patient with an increased usage of Vβ2·1 in synovial lymphocytes,[13] there was no suggestion of an increase in the frequency of the TCRBV2S1*1 allele, the equivalent of the Vβ2·1 sequence, in any of our DR4 positive groups. The possibility that rearranged TCR with particular VDJ sequences, and including the TCRBV2S1 segment, might be involved in RA cannot be answered by the methods that we have used in this study but only by analysing the rearranged TCR genes.[6 12 13] Therefore this negative result does not exclude a pathogenic role for T cells bearing Vβ2 but any possible effect is not attributable to a disease related allelic polymorphism of the germline DNA.

In conclusion, the use of SSCP methods has allowed rigorous investigation of a TCR allelic polymorphism in RA. Although, there was preliminary evidence incriminating T cells bearing particular TCR V segments in RA,[13] the analysis of TCRBV2S1 did not reveal a disease association. We were also able to define two new variants which were rapidly characterised. The extent of such allelic variation (five alleles currently identified in TCRBV2S1) suggests that this source of germline polymorphism is more extensive than previously recognised, and further studies will be needed to determine their role in disease susceptibility.

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