A T cell receptor β chain variable region polymorphism associated with radiographic progression in rheumatoid arthritis

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Abstract

***Objective*—In rheumatoid arthritis (RA) genetic factors influence susceptibility to disease and progression. Identifying these genetic factors may give more insight into the aetiology and pathogenesis of this disease. Furthermore, if these genetic markers can predict progression in an early stage of disease, timely institution of more aggressive treatment in patients with a bad prognosis may help to prevent joint damage. Several studies have shown that HLA-DRB1 alleles are associated with RA, whereas others have indicated that genes not linked to the HLA complex are also involved. Candidates for such genes are the T cell receptor (TCR) α/β genes.**

***Methods*—The association of a polymorphism in a TCR β chain variable region gene (TCR-Vβ8) with both risk for RA and radiographic progression of joint disease was analysed after a three year follow up. A cohort of 118 white patients with a duration of disease shorter than one year at entry, and 110 white controls were typed for this (BamHI) TCR-Vβ8 polymorphism.**

***Results*—The distribution of the two alleles, 2·0 and 23·0 kb, was identical in patients and controls. Radiographic progression (modified Sharp method) after a three year follow up, studied in 111 patients, was significantly less in the group possessing the 2·0 kb allele (p=0·03).**

***Conclusion*—This does not confirm the reported association of the (BamHI) TCR-Vβ8 2·0 kb allele with RA. By contrast with previous findings in smaller studies, in the present study this 2·0 kb allele was protective against radiographic progression. Because well known prognostic variables in RA were corrected for, the findings indicate that the TCR-Vβ8 polymorphism studied is a new prognostic marker for this disease.**

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Rheumatoid arthritis (RA) is a chronic inflammatory disease, preferentially localised in the synovial joints. Although little is known about its aetiology and pathogenesis, several lines of evidence show that genetic factors play an important part in RA. For instance studies on twins show increased concordance in monozygotic *v* dizygotic twins[1–3] and several studies show familial aggregation of RA.[1 4] Although part of this genetic background is explained by the association of HLA-DRB1 alleles with risk of disease in case-control studies,[5] Deighton *et al*[6] calculated that HLA encoded only 37% of the genetic predisposition to RA. Involvement of genes not linked to the HLA complex is also suggested by other studies.[7]

The association of HLA-DRB1 alleles with RA might be related to the function of these molecules, being either the result of the selectivity of DRB1 alleles for certain types of antigen fragments when presenting these to T cells, or the result of the DRB1 allele specific skewing of the T cell repertoire,[8] or both. In either situation, modifications in the T cell repertoire are expected to influence the risk of RA. Such modifications can arise by deletion or mutation in the T cell receptor germline genes. Polymorphisms in the T cell receptor germline genes are therefore good candidates for the non-HLA linked genes involved in RA.

The functional significance of T cell receptor germline deletions has been shown in studies in SWR and AU/ssJ mice.[9 10] In these studies susceptibility to collagen induced arthritis, normally present in mice carrying the H-2^q haplotype, was abrogated by a large deletion of the T cell receptor β variable region gene (TCR-Vβ8). Likewise, the development of lupus nephritis in crosses of NZB × SWR mice seems to be influenced by the inheritance of the SWR T cell receptor haplotype.[11]

Associations of genetic polymorphisms in the T cell receptor germline genes with the risk of disease have been reported for several diseases, including insulin dependent diabetes mellitus,[12] multiple sclerosis,[13] Graves' disease and Hashimoto's thyroiditis,[14] and membranous nephropathy.[15] In multiple sclerosis, the association with T cell receptor β chain polymorphisms has been confirmed by sibpair analysis.[16] Recent reports also showed an association of a TCR β variable region restriction fragment length polymorphism with disease risk in RA.[17 18] Because several studies indicate that genetic factors not only influence risk of disease but also progression of disease, we analysed the association of this TCR-Vβ8 polymorphism in a group of Dutch patients, both with risk of RA, and with radiographic progression. We studied patients in the first

years after the onset of RA because joint damage is especially progressive in this phase of the disease.

Patients and methods

PATIENTS AND CONTROLS

Included in this study were 122 consecutive adult white patients with RA who visited the department of rheumatology at the University Hospital, Nijmegen, The Netherlands in the period January 1985 to November 1988. Patients were included if they had definite or classical RA according to the American Rheumatism Association criteria, and a duration of disease shorter than one year at entry. Only five patients had had treatment with slow acting antirheumatic drugs before entry into the study; 92% of the patients were treated with these drugs during follow up. As controls 110 healthy white blood donors were analysed.

At entry the median age of the patients with RA was 55 years, 62% were women, 80% were positive for IgM rheumatoid factor (RF, ELISA, <5 IU/ml negative), and 52% carried HLA-DR4. Median erythrocyte sedimentation rate (ESR, Westergren) was 40·5 mm/h, median number of painful and swollen joints 15 (maximum 53) and 16 (maximum 48) respectively, median Ritchie score 11·5 (maximum 79). Of the initial 122 patients, one died, one emigrated, and four were lost to follow up; RF status at entry was not available for one patient and HLA- or TCR typing was not available for four other patients. At their first visit the remaining 111 patients did not differ from the 11 who dropped out with regard to sex, age, RF positivity, *x* ray film score, or disease activity score (a sensitive composite index for disease activity[19] calculated from the Ritchie articular index), erythrocyte sedimentation rate (ESR), number of swollen joints, and general health (visual analogue scale of 10 cm, 0=best possible; 10=worst possible).

RADIOGRAPHIC SCORING

Erosions and joint space narrowing on *x* ray films of hands and feet at entry and after three years of follow up were read blind by one of us (NdV) in a modified way according to Sharp.[20] Erosions were scored in the metacarpophalangeal and proximal interphalangeal joints, the interphalangeal joints of the thumbs, the first metacarpal, radius, ulnar, multangular (trapezium and trapezoid as one unit), navicular and lunate bones, the metatarsophalangeal joints, and the interphalangeal joints of the big toes. Joint space narrowing was assessed in the metacarpophalangeal and proximal interphalangeal joints, the third, fourth, and fifth carpometacarpal joints, the multangular-navicular, capitate-navicular-lunate joints and radiocarpal joints, the metatarsophalangeal joints, and the interphalangeal joints of the big toes. At each location erosions were graded 0–5 in the hands and 0–10 in the feet, according to the fraction of the joint surface involved. An erosion score of 1 represented a discrete interruption of the cortical surface. The maximum erosion score was 80 for each hand and 60 for each foot. Joint space narrowing was graded 0–4 at each location (1=focal, 2=less than 50%, 3=more than 50% or subluxation, 4=ankylosis), resulting in a maximum score of 60 for each hand and 24 for each foot.

Erosion and narrowing progression scores were calculated by subtracting the initial score from the score after a three year follow up. Narrowing and erosion progression scores were summed to give the total radiographic progression score. For analysis of variance these progression scores were transformed to approximate a normal distribution by taking the square root (total radiographic progression after transformation: n=118, kurtosis=0·26, skewness=0·49). The *x* ray films for 39 patients were independently scored by a second observer. Interobserver Pearson's correlation for the radiographic progression scores, taking their square roots, was 0·89 for both erosion and joint space narrowing scores, and 0·94 for total radiographic progression score.

TYPING OF HLA-DRB1 AND TCR-Vβ8

For HLA-DRB1 typing the DRB1 exon 2 was amplified from genomic DNA in a genetic PCR reaction with the sense primer 5'-CCGGTCGACTGTCCCCCCAGCACG-TTTC-3' (with SalI site) and the antisense primer 5'-GAATTCTCGCCGCTGCACT-GTGAAGC-3' (with EcoRI site).[21] The sequence specific oligonucleotide (SSO) probes,[21 22] described in table 1, were end labelled with T4 kinase, hybridised to the dotblotted PCR product, and detected by autoradiography.

For TCR typing BamHI digested genomic DNA was subjected to electrophoresis on 0·8% agarose gels and transferred to nylon membrane by the Southern blotting technique. The filters were hybridised to a ^{32}P-labelled human T cell receptor Vβ8 probe VβYT35 (provided by Dr L Hood, California Institute of Technology, Pasadena, USA).[23] After washing with 40 mM PO_4/1% SDS at 65°C for 45 minutes, the 2·0 *v* 23·0 kb polymorphism was visualised by autoradiography.

STATISTICAL METHODS

A review of publications showed that female sex and a positive RF state are variables that

Table 1 DRB1 SSO probes used in this study

*Probe**	*DRB1 specificity†*	*Sequence*	*AA‡*
88–148	0100	5'-TGTGGCAGCTTAAGTTTGAA-3'	8–14
1002	1500 1600	5'-CAGCCTAAGAGGGAGTGT-3'	11–16
89–60	0300 1100 1200 1300 1400 0800	5'-GTTTCTTGGAGTACTCTACG-3'	6–12
88–150	0400	5'-TGGAGCAGGTTAAACATGAG-3'	8–14
88–151	0700	5'-TGTGGCAGGGTAAGTATAAG-3'	8–14
88–153	0900	5'-TGAAGCAGGATAAGTTTGAG-3'	8–14
89–55	1001	5'-GAAAGACGCGTCCATAACCA-3'	28–34

*Probe 1002 has been described in the 11th international histocompatibility workshop; probe 88–153[22] and the other probes[21] have also been described.
†00, recognised sequence is shared by all subtypes of the broad specificity defined by the first two figures.
‡Amino acid position of recognised sequence.

indicate a poor prognosis.[24] In a recent article on prognostic factors radiographic progression after two years of follow up correlated with ESR, C reactive protein (CRP, mg/l), the number of swollen joints, and *x* ray film score at entry, age, and the presence of IgM RF, HLA-DR4, and HLA-DR2.[25] As in our material CRP and ESR at entry showed a strong correlation, CRP was left out of the analysis. Likewise, of the two HLA-DR alleles, only DR4 was included. Thus in the analysis of covariance of the association of this T cell polymorphism with radiographic progression after three years, we included the prognostic variables: age group (55 and younger *v* 56 and older), sex, HLA-DR4, and RF positivity, number of swollen joints, ESR, and *x* ray film score at entry. The ESR and *x* ray film scores were transformed to approximate a normal distribution by taking the square root. Relative risk (RR) was calculated as odds ratio (OR) in 2 × 2 tables. Higher dimensional tables were analysed with log linear models.

Results

ASSOCIATION WITH RISK OF DISEASE

To study the association of the BamHI generated 2·0 kb restriction fragment of the TCR-Vβ8 gene with risk for RA, 110 controls and 118 patients were typed for this polymorphism. Table 2 gives the frequencies of the individual genotypes and alleles. These were virtually identical between patients and controls.

HLA-DR4, present in 61 of the 118 (52%) patients with RA typed for HLA-DR, and in 30 of the 88 (34%) controls, was significantly associated with RA (RR=2·07; p<0·02). In a multidimensional table analysis, we did not find any association between the 2·0 kb fragment and risk for RA, even when considering the influence of HLA-DR4. In DR4 positive patients the distribution of the T cell receptor polymorphism under study did not differ from that in DR4 negative patients or controls (table 2).

Table 2 Distribution of BamHI TCR-Vβ8 RFLP in patients with RA and controls

	*Genotypes**			*Alleles**	
	23·0/23·0 kb	*23·0/2·0 kb*	*2·0/2·0 kb*	*23·0 kb*	*2·0 kb*
Controls (n=110)	29 (32)	50 (55)	21 (23)	54	46
Patients (n=118)	26 (31)	48 (57)	25 (30)	50	50
DR4+ patients (n=61)	25 (15)	56 (34)	20 (12)	52	48
DR4– patients (n=57)	28 (16)	40 (23)	32 (18)	48	52

*Percentages (absolute numbers in parentheses). No significant differences were found.

Table 3 Median erosion, narrowing, and total radiographic progression scores after three year follow up in different patient groups, showing the effect of the genetic markers TCR-Vβ8 2·0 kb and HLA-DR4

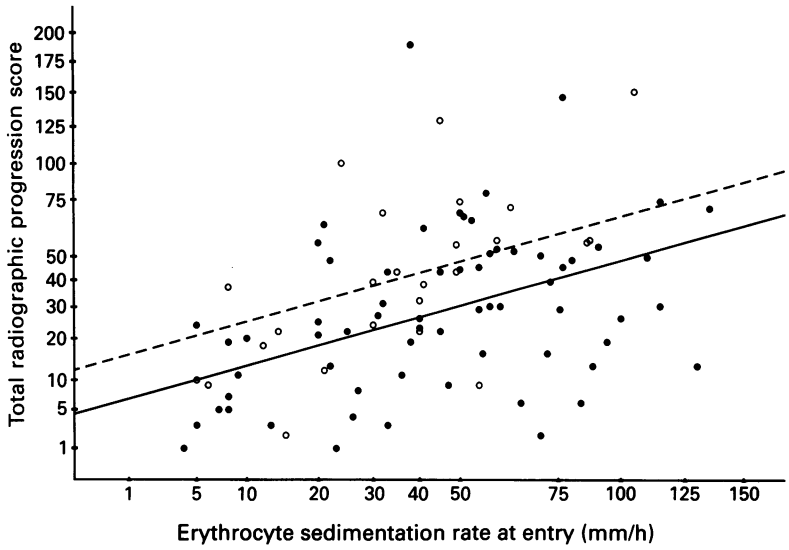
Marker	*Patients*			*Radiographic progression scores*					
	*Subgroup**	*Numbers*		*Erosions*		*Narrowing*		*Total x ray film*	
		M+†	*M–†*	*M+*	*M–*	*M+*	*M–*	*M+*	*M–*
2·0 kb	All patients	82	29	9·5	14·0	9·5	19·0‡	20·5	37·0‡
	ESR≤28	28	11	4·5	6·0	2·5	6·0	6·0	10·0
	ESR>28	54	18	13·0	18·0	16·5	35·0	29·5	49·0
	RF+	65	25	12·0	15·0	13·0	22·0‡	26·0	39·0‡
	ESR≤28	20	8	7·5	8·0	3·5	7·0	12·0	15·0
	ESR>28	45	17	14·0	19·0	19·0	35·0	31·0	55·0
DR4	All patients	56	55	11·5	8·0§	11·5	13·0	22·5	22·0

*ESR=Erythrocyte sedimentation rate at entry (Westergren, mm/h); RF+=rheumatoid factor positivity at entry (IU>4).
†Marker (2·0 kb TCR-Vβ8 allele or HLA-DR4 allele) is present (M+) or absent (M–).
‡Analysis of covariance (See results section) M+ *v* M–: p<0·05.
§One-sided *t* test (See results section): p<0·05.

ASSOCIATION WITH OUTCOME OF DISEASE

At entry, the patient group possessing the 2·0 kb TCR-Vβ8 BamHI fragment did not show a significant difference in sex, age, RF positivity, presence of HLA-DR4, *x* ray film score, or disease activity score (DAS) from the patients homozygous for the 23·0 kb fragment. At entry median scores for erosions, joint space narrowing, and total *x* ray film score were 1, 0, and 3 respectively. After three years of follow up, median increases in erosion, joint space narrowing, and total *x* ray film score were 10, 12, and 22 respectively. Taking into account the prognostic variables mentioned in the patients and methods section, the analysis of covariance showed a significant association between the presence of the 2·0 kb T cell receptor BamHI fragment and lower total radiographic progression score (p=0·03). Explorative analysis showed that the 2·0 kb fragment was significantly associated with a lower joint space narrowing score (p=0·03). The 2·0 kb group also showed less erosions after three years; however, this effect was not significant (p=0·12). The association of HLA-DR4 with total radiographic progression did not reach significance in our study; if no correction for the prognostic variables mentioned earlier was made, the association of HLA-DR4 with a higher increase in the erosion score, reported in earlier studies,[25 29] was confirmed (one sided *t* test: p<0·05).

To show the size of the effect of the presence of this 2·0 kb fragment on radiographic progression and thus its potential clinical relevance, we had to take into account the effect of both ESR at entry and RF positivity, as these were the most influential variables, reducing the residual variance by 23% and 20% respectively. Table 3 presents the median erosion, narrowing, and radiographic progression scores for patient groups with and without the 2·0 kb fragment. The median total radiographic progression score was almost twice as high for patients homozygous for the 23·0 kb fragment compared with patients encoding the 2·0 kb fragment when analysing all patients, and 50% higher when considering the RF positive group only. In the RF positive group the differences were greater for the subgroup with a higher ESR at entry (ESR>28 mm/h), although this effect was not significant. Pooling the patients according to their 2·0 kb marker state generated smaller differences in median erosion progression scores than in the median joint space narrowing scores; however, these differences in median erosion progression scores were still larger than those generated with the HLA-DR4 allele. The figure shows the influence of the 2·0 kb fragment on radiographic progression for the RF positive group as a function of the ESR at entry.



Influence of T cell receptor polymorphism on total radiographic progression score in seropositive patients after a three year follow up. Total radiographic progression score in seropositive patients with (●) and without (○) the 2·0 kb fragment as a function of the erythrocyte sedimentation rate at entry into the study. Regression lines (analysis of covariance) are shown for patients with (straight line) and without (dashed line) the 2·0 kb fragment.

Discussion

Several smaller studies have reported an association of the TCR-Vβ8 BamHI 2·0 kb restriction fragment with risk for RA.[17 18] The results from the present study do not, however, confirm this association (RR=1·15; 95% CI=0·64–2·06), not even after incorporating the effect of HLA-DR4. This lack of association cannot be explained by the fact that we analysed a group of patients with recent onset of disease; if the 2·0 kb allele is only associated with more severe, progressive disease, then in our study we would expect to find it associated with more radiographic progression. By contrast a significant association of this allele with less radiographic progression was found.

Conflicting results have also been reported for associations of TCR germline restriction fragment length polymorphisms with disease risk in multiple sclerosis, insulin dependent diabetes mellitus, and thyroid disease.[26 27] Our study argues against the explanation that clinical differences might be responsible for these conflicting results, at least in RA. To explain the discrepancies Steinman *et al*[26] suggest that the expression of disease susceptibility encoded within the TCR germline genes is critically dependent on the T cell repertoire, which may differ from one population to another owing to differences in genetic background. Another explanation is that the TCR polymorphism studied is not itself involved, but is only a marker for a stronger association with a susceptibility gene nearby. Such linkage disequilibrium has been found for genes within the T cell receptor β chain germline, albeit to a limited extent.[28] In this situation, as this linkage disequilibrium is expected to differ from one population to another,[27] the associations of the disease with the marker polymorphism will be unstable from one investigation to another. Further studies will be needed to elucidate this point.

Several studies indicate that genetic factors are associated with the more severe, progressive or erosive forms of RA. For instance, Lawrence[1] found that familial aggregation is more pronounced if the proband has a more severe, erosive form of disease. In several studies HLA-DR4 is associated with more erosive disease.[25 29] Also, although increased in hospital based series, the frequency of HLA-DR4 is not higher in population based series.[30] If indeed genetic polymorphisms are associated with disease progression,[31] these genetic markers might be used for making a prognosis in the individual patient in an early phase of disease. Timely institution of more aggressive treatment based on these prognostic markers may help to prevent radiographic damage, as many patients develop radiographic damage early in the course of the disease[32] and the highest rate in radiographic progression takes place in the first years,[32] whereas in later years relatively few unaffected joints become radiographically damaged.[33]

This study shows that patients not carrying the 2·0 kb fragment, and thus homozygous for the 23·0 kb fragment, have a significantly higher radiographic progression score after a three year follow up. The effect is independent of well known prognostic factors like RF positivity and ESR at entry. If confirmed, this polymorphism might therefore be used as a new prognostic marker, additive to the existing markers, to predict progression of disease in individual patients. This might be the more important as we recently showed that effective antirheumatic treatment in early RA could slow down radiographic progression,[20] an effect that was still present at a three year follow up.[34]

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