HLA class II and T-cell receptor β chain polymorphisms in Belgian patients with rheumatoid arthritis: no evidence for disease association with the TCRBC2, TCRBV8 and TCRBV11 polymorphisms

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Abstract

***Objectives*—To investigate whether T-cell receptor (TCR) β chain germline alleles, either alone or in combination with a particular HLA-genotype, are associated with rheumatoid arthritis (RA).**

***Methods*—Three restriction fragment length polymorphisms (RFLPs), detected with TCR constant (TCRBC2) and variable (TCRBV8, TCRBV11) gene segments were analysed in a representative group of Belgian, HLA class II-typed patients with RA, and in a group of Belgian control subjects.**

***Results*—The study confirmed the known association of RA with the HLA-DRB1*0401/0404 genotype (RR = 2·14, 95% CI = 1·16–4·00) in the Belgian RA population. This association was even more pronounced in the patients with more severe RA (RR = 3·26, 95% CI = 1·55–6·89). These data suggest that the HLA-DRB1*04 genotype can be used as a marker for disease severity. Similar frequencies in patients and controls were observed for all TCRB RFLPs studied, and this was in spite of subgrouping the RA population according to criteria for disease stratification.**

***Conclusion*—While a clear association with HLA DRB1*0401/0404 is observed, no interactive effects were seen with RA, DR4, TCRBC2 and TCRBV alleles, implying that the combined presence of these polymorphic markers does not cause an increased susceptibility to RA, and does not predispose for more aggressive RA, nor for familial aggregation of the disease. These results argue against the hypothesis that TCRB polymorphisms play a crucial role in the susceptibility for RA.**

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Rheumatoid arthritis (RA) is a common, chronic inflammatory disease affecting the synovial membranes of articulating joints. RA develops in genetic susceptible hosts, and is responsible for severe disability and increased mortality.[1] The aetiology of the disease is still unknown, but there is some evidence that a cell-mediated process involving T cells, antigen-presenting cells, macrophages, synoviocytes, and cytokines, play an important role in the immunopathogenesis.[2]

Familial cases of RA do occur,[3 4] and this, along with an increased rate of concordance in monozygotic, compared with dizygotic, twins,[5 6] suggests that the susceptibility to the disease is inherited, at least in part. It is probable that the susceptibility for RA is determined by a series of genes. The specific loci, however, still needs to be identified.

Part of the genetic susceptibility to RA is accounted for by alleles within the major histocompatibility complex (MHC). This was originally shown by Stastny[7] as a well established strong association between the antigen HLA-DR4, its subtype Dw4, and RA. Disease associations with HLA-DR1,[8 9] specific DR-DQ haplotypes,[10 11] and HLA-DR6,[12 13] have also been reported. However, the extent of the genetic risk to RA accounted for by these HLA region alleles remains uncertain. This leads to the assumption that the true susceptibility genes for RA still have to be identified. It is most likely that other loci, in addition to environmental influences, are also involved. In this respect, RA is similar to most of the HLA class II-associated autoimmune diseases. Recent studies indicate that the presence of DR4 is associated with the risk for more severe disease.[14 15]

Since the antigen-specific T-cell receptor (TCR) plays a central role in immune recognition, variations in the genes encoding for this receptor, could potentially contribute to the development and inheritance of RA. Several reports have described associations of some autoimmune diseases with restriction fragment length polymorphisms (RFLPs) within the TCRA- and TCRB-chain genes. It has been reported that RA may be associated with genetic polymorphisms of the TCRB locus. Gao *et al* found an association between a particular allelic combination of TCRBV8[2] and DR4-positive RA patients.[16 17] These results have been confirmed by the group of Funkhouser *et al.*[18] Other investigators, however, have been unable to confirm this observation.[19 20]

The aim of the present study was to investigate whether the associations with TCRB chain germline alleles previously reported in RA, could be confirmed in a HLA class II-typed, well defined group of Belgian patients with definite RA. We have studied the germline repertoire of the TCRB chain genes in 88 patients with clinically defined RA and we compared this repertoire to that of 67 unselected, unrelated healthy Belgians.

Patients and methods

PATIENT POPULATION

DNA samples from a group of 88 patients, from different parts of Belgium with clinically definite RA[21] were studied (table 1, total group). Patients selected for this study fulfilled the diagnostic criteria of the American Rheumatism Association (ARA).[21] Radiographs of the hands and feet were taken each year as were radiographs of other joints if clinically indicated. They were divided into three groups based on the following criteria: all patients were asked about the presence of deforming RA in close family members (siblings, parents, grandparents, aunts and uncles), and relatives were also examined according to the ARA criteria. Group 1 comprised 28 patients with familial RA. Group 2 comprised 48 patients in whom rheumatoid factor was positive. Rheumatoid factor was measured by RA-latex test and Waaler-Rose test, and considered positive at a titre of > 1/16 and/or > 1/32 respectively. Group 3 comprised 33 patients who had definite erosions in the small joints of the hand and/or forefoot. Sixty seven randomly selected healthy individuals, of European White descent, were used as normal controls. All samples were analysed for the TCRB genes, and for the HLA class II-genotypes.

DNA PROBES AND POLYMORPHISM

The following full-length cDNA probes were used: a *Bgl* II-*Bgl* II fragment (400 bp) of the Jurkat TCRB gene, clone JVR-B_2,[22] kindly provided by Dr T Mak, Ontario Cancer Institute, Canada. DNA probes corresponding to the human TCRBV8 (*Hind III-Eco RI*, 324 bp)[23] and TCRBV11 (*Hind* III-*Eco* RI, 425 bp)[23] were provided by Dr P Charmley, Division of Biology, California Institute of Technology, Pasadena, USA.

Table 1 Clinical characteristics of the Belgian RA population studied

	Patients with rheumatoid arthritis			
	Total group	Group 1 *Familial RA*	Group 2 *RF positive*	Group 3 *Erosive* (duration > 8 yr)
Number of patients	88	28	48	33
Age (year)				
Median	57	55	55	58
Range	22–81	22–72	29–81	35–79
Sex (m/f)	30/58	9/19	20/28	15/18
Disease duration (year)				
Median	7	8	9	14
Range	0–37	1–21	1–37	8–37
Age at disease onset (year)				
Median	47	47	46	44
Range	21–78	21–63	21–78	21–60

Plasmid inserts were prepared by restriction digest, electrophoresis, and isolation of the probe by extracting the appropriate DNA band from the gel with sterile glass wool.

Bgl II blots were used to identify the TCRBC2 gene polymorphisms, with bands present at 9 kb and 10 kb. TCRBV polymorphisms were identified with *Bam* HI blots. The TCRBV8/*Bam* HI polymorphism was identified by bands at 23 kb and 2 kb, while the TCRBV11/*Bam* HI RFLP showed bands at 25 kb and 20 kb.

DNA PREPARATION AND HYBRIDISATION

Genomic DNA was prepared from whole blood and subjected to restriction digests with *Bam* HI or *Bgl* II, using conditions as specified by the manufactures.[24]

Fifteen μg of the restricted DNA was size-separated on 0·8% agarose gels by electrophoresis for 24–28 hours at 22–24 V, and transferred by vacuum blotting to Zeta-Probe GT (Bio-Rad) nylon membranes, according to the manual of the manufacturers. Pre hybridisation was performed for minimal 15 minutes in 0·25 M sodium phosphate pH 7·2, 7% SDS, in the presence of 100 μg/ml denatured herring sperm DNA, at 65°C. Hybridisations were carried out for 15–24 hours at 65°C in a pre hybridisation mixture supplemented with 1–2 ng/ml denatured probe.

The filters were washed (1–2 times) in 20 mM sodium phosphate, pH 7·2 and 5% SDS at 65°C for 30 minutes and 1–2 times in 20 mM sodium phosphate, pH 7·2 and 1% SDS at 65°C for 30 minutes. The blots were autoradiographed using a hyperfilm MP (Amersham, UK), in cassettes with intensifying screens, for 24–72 h.

HAPLOTYPE ASSIGNMENT

The haplotypes for the normal subjects and RA patients were determined for all individuals heterozygous at not more than one locus. Without segregation information the haplotype phase cannot be deducted for persons who were double heterozygous for one locus.

PCR AMPLIFICATION AND SSO-HLA TYPING

All patients and controls were typed for the DRB1, DRB3, DRB4, DRB5 alleles as described previously.[25] Briefly, the four DRB genes were coamplified by the PCR and subsequently hybridised by one single hybridisation assay to DRB sequence specific oligonucleotides (SSO) using a rapid and non-isotopic reverse dot blot. The originally described DRB hybridisation protocols were adapted to yield a reverse hybridisation assay on membrane-based strips with SSOs immobilised in a linewise fashion and the initially described chemiluminescence detection protocol was adapted to yield a colorimetric visualisation of positive hybridisation, using the chromogen 5-Bromo-4-chloro-3-indocylphosphate/nitroblue tetrazolium chloride

(NBT/BCIP) substrates (Innogenetics NV, Gent).

The assay described here could not distinguish the DRB1*1501/1502, DRB1*1601/1602 and DRB1*0701/0702 alleles. Those alleles were respectively grouped as DRB1*1500, DRB1*1600, and DRB1*0700.

For DRB genotyping, some allelic combinations couldn't be deducted because of overlapping sequences. Thus instead of the original coamplification, a group specific PCR was developed for DRB1 (all DRB1 alleles except DR2, DR7 and DR9 groups) and also for DR4 and DR2. The DR4 amplification is described by Buysse *et al.*[25] In the DRB1 locus specific PCR, the previously described DRB1 primer CRX37 (5'-GAATTCCCGCGCCGCGCT-3')[26] was used in combination with the conserved DRB-1 primer (5'-GATCCTTCGTGTCCCCACAGCACG-3').[25] For DR2 group-specific amplification the DR2-DRB1 primer (5'-TTCCTGTGGCAGCCTAAGAGG-3') of the 11th International Histocompatibility Workshop was combined with the conserved DRB-2 primer (5'-GCCGCTGCACTGTGAAGCTCTC-3'). The PCR conditions for the DRB1 locus as well as for the DR2 specific amplification were identical to those of the coamplification.[25]

STATISTICAL ANALYSIS

Allelic, genotypic and haplotypic distributions in the RA patients and control group were compared by using the Chi square and Fisher's exact test of independence.[27] Data were considered significant at $p < 0.05$. Bonferoni correction for multiple testing was done.[28] Additionally, the alleles were examined for a second RA association by the 'Relative Predispositional Effects' (RPE) method.[29] In this method, allele frequencies are examined sequentially and according to strength; thus the problem that a strong association with one allele can create misleading deviations in the frequencies of other alleles is alleviated. Relative Risk (RR, Odds ratio) was calculated using the formula (number of patients with the specific allele/number of patients without this allele)/(number of controls with the specific allele/number of controls without this allele); 95% Confidence limits of RR are given (95% CIs).

Allele frequencies were defined as the number of times an allele (band) appeared and was counted once in a heterozygote and twice in a homozygote person. Genotype frequencies were defined as the number of times a particular combination of alleles (bands) appeared in either a homozygotic pattern or a heterozygotic pattern.

*Table 2a Frequency of HLA-DRB1*04 alleles in the study population*

	Controls	*RA patients Total group*	*Familial*	*RF positive*	*Erosive*
N chromosomes	134	172	56	94	66
n DR4 alleles					
04*	20	47	17	31	24
*04**					
χ^2		*6·07*	*5·05*	*9·36*	*10·63*
p		*0·014*	*0·024*	*0·002*	*0·001*
RR		*2·14*	*2·48*	*2·80*	*3·26*
CIs		*1·16–4·00*	*1·11–5·56*	*1·41–5·60*	*1·55–6·89*
0401	13	35	13	22	17
0402	2	0	0	0	0
0403	2	0	0	0	0
0404	2	8	3	6	6
0405	1	0	0	0	0
0408	0	3	1	2	0
0410	0	1	0	1	1
0401					
χ^2		*5·68*	*5·02*	*6·46*	*7·73*
p		*0·017*	*0·025*	*0·008*	*0·005*
RR		*2·38*	*2·81*	*2·84*	*3·23*
CIs		*1·15–4·98*	*1·12–7·09*	*1·28–6·40*	*1·36–7·70*

RR: relative risk; CIs. 95% Confidence intervals.

Results

HLA CLASS II POLYMORPHISMS

The frequencies of the DRB1*04 polmorphisms are shown in table 2a. It can be seen that the previously observed[30] disease association with HLA-DR4 in White patients with RA is also present in our patient population (RR = 2·14, 95% CI = 1·16–4·00). Within the DR4 positive individuals, the distribution of DRB1*04 alleles did not show any statistically significant difference between RA and controls. In the DR4 positive RA population, about 74% is associated with the allele HLA DRB1*0401 (Dw4), 17% with *0404 (Dw14) and 6% with *0408 (Dw14). No association was found with the other DRB1*04 alleles studied. Only five patients (6%) were homozygous for the HLA-DR4 haplotype.

This disease association with HLA-DRB1*04 was also observed in the patients with familial RA (group 1, table 1). Of 28 patients, 16 expressed at least one disease-associated HLA-DRB1*04 allele (RR = 2·81, 95% CI = 1·12–7·09). The DRB1*0401 subtype was the most frequent variant present in this patient population, and one patient was homozygous for this disease-linked DRB1*0401 allele.

When subgrouping the patients according to clinical symptoms related to more severe RA, the HLA-DR4 association was more pronounced: 27 out of 48 rheumafactor positive patients (group 2, table 1) (RR = 2·80, 95% CI = 1·41–5·60) and 20 of 33 patients with erosive RA (group 3, table 1) (RR = 3·26, 95% CI = 1·55–6·89) were DR4 positive. Also in these subsets the DRB1*0401 allele was most frequently present.

The presence of a strongly predisposing allele, such as DR4 for RA, can mask the presence of a second disease association. The RPE method[29] was therefore applied, but no new RA association was detected.

The previously reported RA association with DRB1*01 and *06 alleles,[31] could not be detected, in spite of subgrouping our RA population. Furthermore, no significant difference in DRB3-4-5 allele distribution among the RA patients and controls was observed (table 2b).

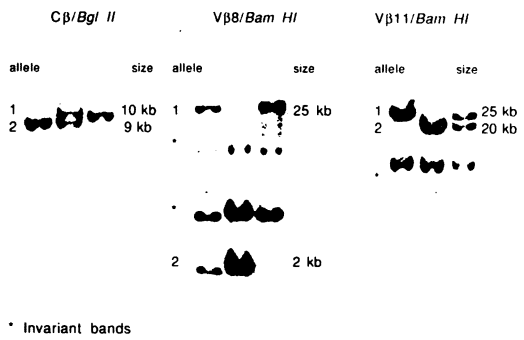
TCRB POLYMORPHISMS

Eighty eight patients with RA (table 3, total group) and 67 healthy controls were investigated for three TCRB RFLPs, TCRBC2/*Bgl* II, TCRBV8/*Bam* HI, and TCRBV11/*Bam* HI

Table 2b Frequencies of HLA class II DRB alleles in Belgian patients with RA and controls

HLA alleles	*Patients (N = 172 chromosomes)*		*Controls (N = 134 chromosomes)*		*p value*	*RR (CIs)*
	n	frequencies	n	frequencies		
DRB1						
0101	20	0·116	9	0·067		
0102	1	0·006	4	0·030		
0301	21	0·122	13	0·097		
0401	35	0·203	13	0·097		
0402	0	0	2	0·015		
0403	0	0	2	0·015		
0404	8	0·046	2	0·015		
0405	0	0	1	0·007		
0408	3	0·017	0	0		
0410	1	0·006	0	0		
0700	14	0·081	15	0·111		
0801	2	0·012	3	0·022		
0901	7	0·041	1	0·007		
1001	6	0·035	2	0·015		
1101	16	0·093	16	0·119		
1102	0	0	1	0·007		
1103	7	0·041	3	0·022		
1201	1	0·006	4	0·030		
1202	1	0·006	0	0		
1300	14	0·081	22	0·164		
1401	6	0·035	6	0·045		
1500	8	0·047	11	0·082		
1600	1	0·006	4	0·030		
0400	47	0·273	20	0·149	0·014	2·14 (1·16–4·0)
DRB3						
0101	19	0·110	19	0·142		
0200	34	0·198	37	0·276		
0202	3	0·017	0	0		
0301	9	0·052	9	0·067		
no DRB3	107	0·622	69	0·515		
DRB4						
0101	69	0·401	36	0·269		
no DRB4	103	0·599	98	0·731		
DRB5						
0101	9	0·052	11	0·082		
0200	1	0·006	3	0·030		
no DRB5	162	0·942	119	0·888		

DRB1*0400 group contains all DRB1*04 alleles.
DRB1*1300 group contains DRB1*1301/1302/1303/1305 alleles.
DRB1*1501/1502. DRB1*0701/0702 and DRB1*1601/1602 were grouped at DRB1*1500.
DRB1*0700 and DRB1*1600 respectively.
RR, relative risk; CIs 95% confidence intervals.



T-cell receptor β chain polymorphisms. The hybridisation pattern at each locus is displayed by Southern blots for both homozygotes and heterozygotes.

Table 4 The distribution of the TCRBV8/Bam HI-TCRBV11/Bam HI-TCRBC2/Bgl II haplotypes in RA and control group

TCRB-V8-V11-C2 haplotype	*Observed*		*Frequencies*	
	RA	*Control*	*RA*	*Control*
A: 23/25/10*[a]	2	3	0·02	0·04
B: 2/25/10	27	17	0·25	0·24
C: 23/20/10	19	12	0·18	0·17
D: 2/20/10*	6	4	0·06	0·06
E: 23/25/9*	1	3	0·01	0·04
F: 2/25/9	14	11	0·13	0·16
G: 23/20/9	35	17	0·32	0·24
H: 2/20/9*	4	3	0·04	0·04
Total number of haplotypes	108	70		

$\chi^2 = 4·269$, $p > 0·5$ (7 df).
$\chi^2 = 2·443$, $p > 0·5$ (4 df)[a].
[a] Data for the 4 lowest (*) frequencies were combined before Chi-square analysis.

(figure). These three TCRB RFLPs did not exhibit significant differences in allele or genotype frequencies between RA patients and controls. No extra bands or aberrant restriction fragment sizes were identified among the RA patients, indicating that no major TCRB gene deletions or duplications could be found in these patients.

Although these TCRB RFLPs are associated with only two alleles, their combination results in haplotypes with a limited linkage disequilibrium between the various markers in the TCRBV and TCRBC regions.[32] Analysis of the haplotypes assembled from our data also failed to reveal differences between RA patients (Total group, table 1) and controls (table 4).

We also analysed these TCRB RFLPs in the RA patient subsets (table 1), neither the allele nor the genotype frequencies for the different groups of patients were significantly different from the control data (table 3).

HLA-DR4 POSITIVE INDIVIDUALS

Forty two of 87 (48%) of the Belgian patients with RA used in this study were DR4 positive, compared with 18 of 37 (27%) of the control individuals. To determine if HLA contributes to differences in TCRB allele or genotype frequencies, we analysed TCRB RFLPs for both RA patients and controls positive for DR4 (table 5). We observed an increase in frequency of the 23 kb TCRBV8 allele in the RA populations (0·51 vs. 0·32) which, however,

Table 3 Human TCRB RFLP allele and genotype frequencies in Belgian patients with RA and controls

				Rheumatoid arthritis patients							
Probe/enzyme	Allele (kb)	*Controls*		*Total group*		*Familial*		*RF positive*		*Erosive*	
		Obs.	Freq.	Obs.	Freq.	Obs.	Freq.	Obs.	Freq.	Obs.	Freq.
TCRBC2/*Bgl* II	1 (9)	72	0·54	91	0·55	35	0·62	42	0·44	37	0·56
	2 (10)	62	0·46	85	0·48	21	0·38	54	0·56	29	0·44
TCRBV8/*Bam* HI	1 (2)	74	0·55	85	0·48	28	0·50	46	0·48	31	0·47
	2 (23)	60	0·45	91	0·52	28	0·50	50	0·52	35	0·53
TCRBV11/*Bam* HI	1 (20)	71	0·53	98	0·56	32	0·57	54	0·56	40	0·61
	2 (25)	63	0·47	78	0·44	24	0·43	42	0·44	36	0·39
	Genotype										
TCRBC2/*Bgl* II	9,9	21	0·31	28	0·32	11	0·39	10	0·21	10	0·30
	9,10	30	0·45	35	0·42	13	0·46	22	0·46	17	0·52
	10,10	16	0·24	25	0·28	4	0·14	16	0·33	6	0·20
TCRBV8/*Bam* HI	2,2	21	0·31	26	0·30	10	0·36	13	0·28	9	0·27
	2,23	32	0·48	33	0·38	8	0·29	20	0·42	13	0·39
	23,23	14	0·21	29	0·33	10	0·36	15	0·31	11	0·33
TCRBV11/*Bam* HI	20,20	21	0·31	32	0·36	12	0·43	18	0·38	15	0·45
	20,25	29	0·43	34	0·39	8	0·29	18	0·38	10	0·30
	25,25	17	0·25	22	0·25	8	0·29	12	0·25	8	0·24

Table 5 Human TCRB RFLP allele and genotype frequencies in Belgian DR4 positive RA patients and controls

		Rheumatoid arthritis patients									
Probe/enzyme	Allele (kb)	*Controls* Obs.	Freq.	*Total group* Obs.	Freq.	*Familial* Obs.	Freq.	*RF positive* Obs.	Freq.	*Erosive* Obs.	Freq.
TCRBC2/*Bgl* II	1 (9)	18	0·56	44	0·52	21	0·66	24	0·44	23	0·55
	2 (10)	14	0·44	40	0·48	11	0·34	30	0·56	19	0·45
TCRBV8/*Bam* HI	1 (2)	23	0·68	41	0·49	16	0·50	27	0·50	23	0·55
	2 (23)	11	0·32	43	0·51	16	0·50	27	0·50	19	0·45
TCRBV11/*Bam* HI	1 (20)	15	0·47	48	0·57	20	0·63	30	0·56	23	0·55
	2 (25)	17	0·53	36	0·43	12	0·38	24	0·44	19	0·45
	Genotype										
TCRBC2/*Bgl* II	9,9	6	0·38	13	0·31	7	0·44	6	0·22	6	0·29
	9,10	6	0·38	18	0·43	7	0·44	12	0·44	11	0·52
	10,10	4	0·25	11	0·26	2	0·13	9	0·33	4	0·19
TCRBV8/*Bam* HI	2,2	7	0·41	13	0·31	6	0·38	8	0·30	7	0·33
	2,23	9	0·53	15	0·36	4	0·25	11	0·41	9	0·43
	23,23	1	0·06	14	0·33	6	0·38	8	0·30	5	0·16
TCRBV11/*Bam* HI	20,20	4	0·25	17	0·40	8	0·50	11	0·41	8	0·38
	20,25	7	0·44	14	0·33	4	0·25	8	0·30	7	0·33
	25,25	5	0·31	11	0·26	4	0·25	8	0·30	6	0·29

was not statistically significant. When analysing the TCRBV8 genotypes, a trend towards a predominant expression of the homozygous 23 kb genotype was observed in the total RA population (Total group, $p = 0{\cdot}0291$), and in the RA patients with familial RA (group 1, $p = 0{\cdot}0270$).

When the haplotype frequencies, defined the two TCRBV probes and one TCRB probe in the DR4 positive subset of RA patients, were compared with the distribution in the DR4 positive control group, no significant difference was found (data not shown).

Discussion

As RA is assumed to be an immunopathological disease, developing in a genetic susceptible host, the search for genomic regions predisposing to RA has focused on genes coding for components of the immune system.

So far the role of the MHC has been most extensively studied, because of the central position of the MHC gene products in the antigen presentation. In this study, as well as in previous studies of patients of northern European White ancestry with RA,[7-9] a prominent association with the HLA-DR4 haplotype (48% of RA patients *v* 27% of controls) was detected. Furthermore, the Belgian patients with RA appeared to be genetically very homogeneous, 74% of the DR4 positive patients express the allele DRB*0401 (Dw4) and 23% DRB1*0404/*0408 (Dw14). No association was found with the other *04 alleles studied. These results are in good agreement with previous observations.[33]

These disease-associated HLA-DRB1*04 alleles (Dw4, Dw14) share a common sequence (amino acid positions 70–74 of the HVR3), which is not present in the HLA-Dw10 and HLA-Dw13 subtypes.[34] Thus as this sequence QKRAA (Dw4)/QRRAA (Dw14/DR1) is expressed in the HVR3 of the DRβ1 chain in most RA patients, it has been proposed that this sequence motif is responsible for the disease susceptibility. This phenomenon is known as the 'shared epitope hypothesis'.[35] It has been postulated that, as peptides from the same area of MHC class II-molecules are able to modulate the T-cell repertoire by deleting self-reactive T cells, RA may originate from a particular T-cell repertoire, imposed by the sequence QRKAA/QRRAA.[35 36]

We subsequently analysed the allelic combination of HLA-DRB1*0401 and *0404 alleles in individual patients and controls, to address the question whether the second haplotype influences the presentation of the disease. Only five patients (6%) were homozygous for the HLA-DR4 haplotype. This is much lower than observed by Weyand *et al*,[14] 27% of their RA patients were homozygous for HLA-DR4.

To correlate the HLA-DR4 haplotype with the inheritance and clinical manifestation of the disease, we defined three groups of RA patients. Familial RA was highly associated with the HLA-DRB1*04 alleles. 57% of these patients (16 of 28) expressed at least one of the disease-associated alleles. Also in the two patient subsets representing the more severe forms of RA, a clear association with the molecular polymorphisms of the HLA-DRB1*04 gene was observed. The high frequency of the HLA-DR4 genotype in these two disease categories, strongly supports the association of RA with this haplotype. The strong association in these patient subgroups (RR of 2·80 in group 2 and RR of 3·46 in group 3 *v* RR of 2·14 in the total RA patient population), is most likely due to the patient selection, and suggests that RA patients with severe disease show a more pronounced association with the HLA-DRB1*0401 and *404 alleles, as has been suggested by others.[14 15 37] Severe RA therefore seems to be immunogenetically different from the milder forms of the disease.

We could not confirm the disease association with the DRB1*0101 allele as has been observed earlier.[8 9] There seems to be an increase in the frequency of this allele among our RA patients, but this increase is not statistically significant. This was also the case in the different patient subsets. Furthermore, no significant difference in the HLA-DRB3-4-5 allele distribution among the RA patients and controls was observed, suggesting that the RA-associated HLA-genotype do not extend to

these regions.

Because the inheritance of predisposing HLA-alleles cannot account for all of the genetic susceptibility to RA, and since T-lymphocytes play a central role in immune recognition through the antigen specific TCRA and TCRB chains, a number of investigators have tested the hypothesis that genetic variations in the TCRA and TCRB germline genes may play a role in the susceptibility to autoimmune diseases.[38 39] The identification of a critical TCR in autoimmune diseases could provide a specific site for clinical intervention.[40]

In 1988 Gao *et al*[16 17] reported an association between the DR4 haplotype and the 2 kb TCRBV8 allele from a *Bam* HI digest. These results were confirmed by some investigators,[18] but could not be confirmed by others, in spite of various ways of subgrouping patients according to the HLA-phenotype and clinical symptoms.[19 20 41]

In summary, contradictory data have been reported by different groups, and a clear cut disease association with RFLP-defined alleles of the TCR genes is not evident. Furthermore, until now there has been no evidence that these germline genes actually play a functional role in the pathogenesis of RA or other autoimmune diseases.

A well-characterised RA patient population was studied, using established molecular genetic techniques, to evaluate TCRB chain RFLPs. We could not identify any significant difference in allele or genotype frequencies between RA patients and controls for the three TCR-RFLPs studied, and this in spite of subgrouping our RA population according to clinical symptoms. Despite the fact that only two alleles are associated with these TCR-RFLPs, their combination results in haplotypes in a limited linkage disequilibrium with the various markers in the TCRBV and TCRBC regions.[42] However, the distribution of the haplotypes defined by TCRBV8, TCRBV11 and TCRBC2 RFLP alleles in our group of RA patients was not different from that found in the control group.

In the RA population studied, 42 of 87 (48%) patients were DR4 positive, compared with 18 of 67 (27%) control persons. Analysis of only this DR4 positive subgroup showed an increase in frequency of the 23 kb TCRBV8 allele: 51% compared with 32% in the DR4 positive control population, in whom the 2 kb TCRBV8 allele was more frequently used. This difference, however, was not statistically significant. When analysing the TCRBV8 genotypes, a trend towards an over-representation of the homozygous 23 kb genotype in the total DR4+ RA population ($p = 0{\cdot}0291$), and in the RA patients with familial RA ($p = 0{\cdot}0270$) was observed. These data are at variance with the findings of Funkhouser *et al*,[18] who found a significant difference in the TCRBV8/*Bam* HI allele frequency between this RA population and controls; in their hands the 2 kb fragment occurred with much greater frequency than the 23 kb fragment in the DR4 positive RA patients (n = 26). This over-representation was reflected in a predominance of the homozygous 2 kb genotype. We failed to confirm these findings, in spite of subgrouping our DR4+ RA patients according to clinical symptoms. The conflicting results obtained in our study and those who reported positive associations with TCRB polymorphisms can be explained by, first, disease heterogeneity, leading to contamination in the population study and second, by statistical errors due to insufficient correction for multiple comparisons, and also by genetic differences between the RA populations studied.

In conclusion, we have been able to confirm the known association of RA with the HLA-DRB*04 genotype in the Belgian RA population. This association was also present in familial RA, and was even more pronounced in patients with more severe RA. We could not show any statistical significant association of RA with RFLP-defined allelic patterns of the TCRB chain genes, in spite of various ways of subgrouping patients according to clinical symptoms and HLA-genotype. Furthermore, a combined presence of different polymorphic markers, such as DR4, TCRBC, TCRBV8 and TCRBV11, is not essential for increasing the susceptibility to RA. This study indicates that there is no evidence for an association of clinically definite RA with RFLPs of the TCRB chain in patients of White ancestry.

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