HLA-D region genes and rheumatoid arthritis (RA): importance of DR and DQ genes in conferring susceptibility to RA

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Abstract

The distribution of HLA-D region antigens was studied in three groups (I, IIa, and IIb) of patients with rheumatoid arthritis (RA): group I comprised 43 patients with mild, non-progressive RA, controlled by non-steroidal anti-inflammatory drugs without progression or erosions; group II comprised 94 patients with severe disease, who had earlier been treated with non-steroidal anti-inflammatory drugs and all had incomplete response requiring treatment with gold (sodium aurothiomalate). Of these, 46 patients (group IIa) responded to gold and the disease was well controlled, and the remaining 48 patients (group IIb) did not respond to gold and developed gold induced toxic reactions, including thrombocytopenia or proteinuria, or both. HLA-D region antigens were defined by serological and molecular (Southern blot analysis and oligonucleotide typing) techniques. The results show that DR4 was significantly increased in all three groups of patients. The prevalence of DR1, of DR1 in DR4 negative patients, and DR3 and DR4 associated DQw7 specificities, however, showed differences in these three groups of patients. The prevalence of DR1 and of DR1 in DR4 negative patients was increased only in patients with mild (group I) RA, but not in patients with severe (groups IIa and IIb) disease. On the other hand, the prevalence of DR4 associated DQw7 was significantly increased in patients with severe disease, but not in patients with mild RA. In addition, DR3 was significantly increased only in patients with severe disease who developed gold induced toxic reactions (group IIb). These data suggest that the HLA-D region genes which cause susceptibility to mild RA may be different from those causing susceptibility to severe RA. The results suggest that both DR and DQ (A, B) genes may be important in conferring susceptibility to RA: DR in mild disease and DQ in severe RA.

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Accepted for publication 19 December 1990

A number of studies have investigated the genetic basis for rheumatoid arthritis (RA). One major focus of these studies has been the attempt to identify genes within the class II region of the major histocompatibility complex—that is, HLA, which confer susceptibility for the disease. A significant association between HLA-DR4 and RA has been noted in most groups studied, though several exceptions have now been reported.[1] In addition, DR1 was significantly increased in patients with RA of Asian, Mexican, Greek, and Israeli origin.[2–5] The prevalence of DR1 in the whole patient population as well as in DR4 negative patients was also significantly increased in white patients with RA.[6 7] A new antigen, MCI, found on most DR4 positive cells and in a subset of subjects positive for DR1, was found to be strongly associated with RA.[8] A number of investigators, however, did not find an increased prevalence of DR1 in patients with RA.[9–12] In fact, inconsistent results for the prevalence of DR1 in patients with RA have been noted in Israeli Jews by two investigators[5 12] and in white patients on two different occasions by at least one laboratory.[6] It is likely that the differences in the prevalence of DR1 in different groups of patients with RA is related to the severity of the disease.

Investigations of the subtypes of DR4 demonstrated a significant increase of Dw4 and Dw14 subtypes in white patients and of Dw15 in Japanese patients with RA.[13 14] On the other hand, Dw10 (the most frequent subtype of DR4 in the Israeli population) and Dw13 were not associated with RA.[5]

A number of investigators have recently examined the prevalence of extended haplotypes bearing DR4 in patients with RA. DR4 is found in linkage disequilibrium with DQw7 or DQw8. These two haplotypes, DR4.DQw7 and DR4.DQw8, have been identified by serology, Southern blot analysis, oligonucleotide typing, and by sequencing of DQB1 genes. Earlier, we reported a significantly higher prevalence of DQw7 in DR4 positive patients with moderate to severe RA.[15] These patients had previously been treated with non-steroidal anti-inflammatory drugs and all had incomplete response requiring further treatment with the disease remitting drug sodium aurothiomalate.[16] These observations have been confirmed by other investigators in patients with severe RA[17 18] and in Felty's syndrome.[19] On the other hand, some studies did not find an increase in the prevalence of DQw7 in DR4 positive patients with RA.[20–24] In these studies the clinical status of the patients was, however, not defined. It is therefore evident that more knowledge is required to understand the complex patterns of associations between HLA-D region genes and RA.

Rheumatoid arthritis is a heterogeneous disease with variable severity and may represent a broad spectrum of aetiologically distinct diseases with common clinical manifestations. The objectives of this study were, therefore, to investigate the distribution of HLA–D region antigens in relatively homogeneous groups of patients, based on severity of the disease and on

the patient's response to drug treatment—either non-steroidal anti-inflammatory drugs or a disease remitting drug—for example, sodium aurothiomalate. In this study the HLA-D region antigens were defined by serological and molecular (Southern blot analysis and oligonucleotide typing) techniques.

Materials and methods

One hundred and thirty seven white patients with RA who attended the rheumatology clinics at the McMaster University Medical Centre and the St Joseph's Hospital in Hamilton, and the Wellesley Hospital in Toronto, Ontario were studied. All patients had definite or classical seropositive RA. The patients were split into three groups based on the severity of the disease and on the patient's response to disease remitting drugs. Group I comprised 43 patients with mild, non-progressive RA, controlled by non-steroidal anti-inflammatory drugs without progression or erosions; group II comprised 94 patients with severe disease, who had earlier been treated with non-steroidal anti-inflammatory drugs and all had incomplete response requiring treatment with gold (sodium aurothiomalate). All these patients had a persistent or increasing joint pain and tenderness, and had developed new articular erosions. In addition, several patients had subcutaneous nodules. Of the 94 patients in group II, 46 patients (group IIa) responded to gold and the disease was well controlled. The remaining 48 patients (group IIb) did not respond to gold and developed gold induced toxic reactions, including proteinuria or thrombocytopenia, or both, and many of these complications occurred before completion of the course of 1000 mg of sodium aurothiomalate. Thus it was not possible to determine whether they might have responded to chrysotherapy.

Seventy unrelated white normal healthy subjects served as controls. In addition, 14 Epstein-Barr virus transformed B lymphoblastoid cell lines (homozygous typing cell lines) obtained from the Tenth International Histocompatibility Workshop were used in these studies. These homozygous typing cell lines were as follows: SA=DR1(Dw1).DQw5; KAS116=DR1(Dw1).DQw5; WT100BIS=DR1(Dw1).DQw5; COX=DR3(DRw17).DQw2; VAVY=DR3(DRw17).DQw2; PF97387=DR4(Dw4).DQw7; BM14=DR4(Dw4).DQw8; SAVC=DR4(Dw4).DQw8; JHAF=DR4(Dw13).DQw7; YAR=DR4(Dw10).DQw8; MT14B=DR4(Dw14).DQw8; SWEIG 007=DRw11(Dw5).DQw7; BM16=DRw12(DB6).DQw7; BER=DR7(Dw7).DQw2.

HLA-DR,DQ typing was performed on B cell enriched lymphocyte populations separated from the peripheral blood lymphocytes by the nylon wool method.[25] HLA specificities were defined on the basis of reactivity with antisera in the Canadian Red Cross tissue typing trays.

Genomic DNA was prepared from peripheral blood lymphocytes and from homozygous typing cell lines.[26] Samples of genomic DNA (7 μg) were separately digested with different restriction endonucleases—for example, *Bam*HI, *Eco*RV, *Hin*dIII, *Sst*I, *Taq*I, and electrophoresed on agarose gels.[27] The gels were then transferred (alkaline) to nylon membrane (Biotrace, RP, Gelman) by Southern's method and hybridised with a ^{32}P labelled DRB (p11–β–3), DQα (pDCα107), and DQβ (pII–β–I) cDNA probe. After hybridisation the blots were washed under stringent conditions and autoradiographed with intensifying screens on Kodak XAR film at −70°C for 3–10 days.[15]

DNA was amplified by a polymerase chain reaction as described earlier using primers specific for the second exon of the DRB1 and DQB1 genes.[28] Samples were denatured and slotted onto a Gene Screen Plus nylon membrane and hybridized with oligonucleotide probes.[28] These oligonucleotide probes were derived from published sequences of the subtypes of DR4 (Dw4, Dw10, Dw13, Dw14, Dw15), DQw2, DQw7, and DQw8.[29–31] Table 1 shows the sequences of the primers and oligonucleotide probes. After hybridisation the blots were washed under stringent conditions and autoradiographed for at least 45 minutes using Quanta Blue (Kodak) intensifying screens.

Statistical analysis of the distribution of HLA-DR, DQ antigens in patients with RA and normal controls was performed by the χ^2 test with Yates's correction. Relative risk values were calculated by the method of Woolf.[32]

Results

It was possible to type all subjects (137 patients with RA and 70 normal healthy controls) for DR (DR1-DRw10, DRw15, DRw16), DR supertypic (DRw52, DRw53), and DQ (DQw1-3, 7, 8) specificities by a serological technique. Southern blot analysis of genomic DNA with DRβ, DQα, and DQβ cDNA

Table 1 Sequence specific oligonucleotides used for analysis of Dw subtypes associated with DR4, and of DQw2, DQw7, and DQw8

Oligonucleotide	*Sequence*	*Amino acid position*	*Specificity*
DRB1-616	5′ GGA GCA GAA GCG GGC CGC G 3′	68–74	DR4 (Dw4)
DRB1-522	5′ GCT CGT CTT CCA GGA TGT C 3′	66–71	DR4 (Dw10)
DRB1-524	5′ CCG CGG CCC GCC TCT GCT C 3′	69–75	DR4 (Dw14)
DQB1-516	5′ GCT TCT GCT CAC AAG ACG CA 3′	24–30	DQw2
DQB1-517	5′ GGT ACA CCT CCA CGT CGC TG 3′	41–47	DQw7
DQB1-518	5′ CAG TAC TCG GCG GCA GGC GG 3′	55–61	DQw8
PCR primers			
DRB1	5′ CCG GAT CCT TCG TGT CCC CAC AGC ACG 3′ (+ve)		
	5′ CTC CCC AAC CCC GTA GTT GTG TCT GCA 3′ (−ve)		
DQB1	5′ CTC GGA TCC GCA TGT GCT ACT TCA CCA ACG 3′ (+ve)		
	5′ GAG CTG CAG GTA GTT GTG TCT GCA CAC 3′ (−ve)		

probes confirmed the results obtained with serological typing and extended these for the subspecificities of DR2 (DRw15, DRw16), DR3 (DRw17, DRw18), DRw6 (DRw13, DRw14), DQw1 (DQw5, DQw6), and for DQw4 and DQw9. The molecular sizes for restriction fragment lengths were the same as those defined by us and other investigators, including the joint reports in the Tenth International Histocompatibility Workshop.[15 33–37] In addition, molecular analysis of genomic DNA from DR3 positive homozygous B lymphoblastoid lines and members of 35 DR3 positive families (20 normal, 15 with RA) with DR and DQ (A and B chain) cDNA probes showed that HLA-B, DR, DQ alleles in all examples of three different DR3 haplotypes (B8,DR3; B18,DR3; non-B8; non-B18,DR3) were fixed.[34 38] We found complete concordance between DR (A1, B1, B3), DQ (A1, A2, B1, B2) alleles and serologically defined extended haplotypes bearing DR3. It was unequivocally possible to identify these extended haplotypes bearing DR3 by restriction fragment length polymorphism in the remaining DR3 positive subjects.

Oligonucleotide typing of genomic DNA amplified by a polymerase chain reaction confirmed data obtained by serological and Southern blot techniques. In addition, it was possible to define DR4 associated Dw subtypes by this methodology. In all instances concordant typing results were obtained for HLA-D region specificities by these three techniques.

Table 2 shows the distribution of HLA-D region antigens, which showed significant differences in normal healthy controls and the three groups of patients with RA. Figure 1 shows the results from Southern blot and oligonucleotide hybridisation experiments with genomic DNA from four patients with mild RA (group I). The prevalence of DR1 and of DR4 was significantly higher in patients with mild RA than in normal controls (DR1=32·6% *v* 14·3%, relative risk (RR)=2·90, $p<0·04$; DR4=44·2% *v* 22·9%, RR=2·67, $p<0·03$). In addition, the prevalence of DR1 in DR4 negative patients was even higher than that in normal healthy subjects (58·3% *v* 16·7%, RR=7·0, $p<0·0006$). The prevalence of DR4 associated Dw subtypes (Dw4, Dw14) was also significantly higher in patients with mild RA (Dw4=34·2% *v* 12·9%, $p<0·008$; Dw14=14·6% *v* 4·3%, $p<0·034$). On the other hand, the prevalence of DR3 and of DR4 associated DQw7 in patients with mild RA was not different from that in normal controls (DR3=13·9% *v* 20·0%, $\chi^2=0·32$, NS; DR4 associated DQw7=39·1% *v* 24·4%, $\chi^2=2·68$, NS).

Figure 2 shows the results from Southern blot and oligonucleotide experiments with DNA from four patients with severe RA, in whom the disease was relatively well controlled with gold treatment (group IIa), and table 2 gives the prevalence of some DR,DQ antigens. It is evident that the prevalences of DR4 and of DR4 associated DQw7 were significantly higher than those in normal healthy controls (DR4=63·0% *v* 22·9%, RR=5·76, $p<0·00004$; DR4 associated DQw7=69·0% *v* 24·4%, RR=6·89, $p<0·0006$). In addition, the prevalences of Dw4 (38·1%) and of Dw14 (26·2%) were significantly ($p<0·002$, $<10^{-6}$ respectively) higher than in normal subjects. On the other hand, the prevalence of DR1 (21·7%, $\chi^2=0·62$, NS), DR3 (23·9%, $\chi^2=0·07$, NS), and of DR1 in DR4 negative patients (33·3%, $\chi^2=1·38$, NS) was not different from that in controls.

Figure 3 shows the results from Southern blot and oligonucleotide experiments with DNA from four patients with severe RA who developed gold induced toxic reactions (group IIb), and table 2 gives the prevalence of some DR, DQ antigens. It can be seen that the prevalences of DR3, DR4 and DR4 associated DQw7 were significantly higher in these patients than in controls DR3=58·3% *v* 20·0%, RR=5·60, $p<0·0002$; DR4=43·8% *v* 22·9%, RR=2·63, $p<0·03$; DR4 associated DQw7=78·9% *v* 24·4%, RR=11·63, $p<0·0003$). Subtypes of DR4 could only be determined in 13 (10 Dw4, three Dw14) of 21 DR4 positive patients owing

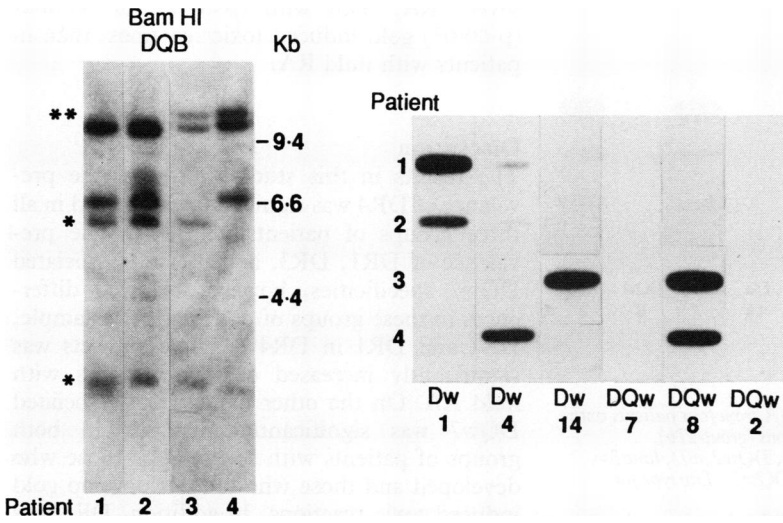

*Figure 1 Southern blot and oligonucleotide typing of genomic DNA from four patients with mild rheumatoid arthritis (group I). Lane 1=No 1 (DR1,w15; DQw5,w6); lane 2=No 2 (DR1,w14; DQw5); lane 3=No 3 (DR4(Dw14), w16; DQw5,w8); lane 4=No 4 (DR4(Dw4), w15); DQw6,w8). *indicates DQw5; ** indicates DQw8.*

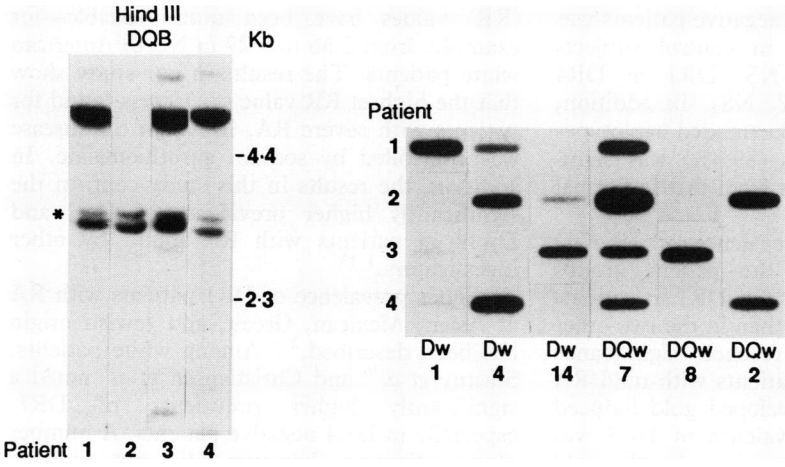

*Figure 2 Southern blot and oligonucleotide typing of genomic DNA from four patients with severe rheumatoid arthritis who responded to gold treatment and in whom the disease was well controlled (group IIa). Lane 1=No 1 (DR1,4(Dw4); DQw5,w7); lane 2=No 2 (DR3,4(Dw4); DQw2,w7); lane 3=No 3 (DR4(Dw14); DQw7,w8); lane 4=No 4 (DR3,4(Dw4); DQw2,w7. *indicates DQw7.*

Table 2 Prevalence (%) of relevant HLA-DR, DQ antigens in normal healthy controls and in patients with rheumatoid arthritis (RA)

Antigen	Normal healthy controls (n=70)	Patients with mild RA	Patients with severe RA, treated with gold	
			No side effects	Thrombocytopenia, proteinuria
		Group I (n=43)	Group IIa (n=46)	Group IIb (n=48)
DR1	14·3	32·6* (2·90)† (p<0·04)	21·7	10·4
DR3	20·0	13·9	23·9	58·3 (5·60) (p<0·0002)
DR4	22·9	44·2 (2·67) (p<0·03)	63·0 (5·76) (p<0·00004)	43·8 (2·63) (p<0·03)
DR1 in DR4 negative	16·7	58·3 (7·00) (p<0·0006)	33·3	12·5
DR4 associated DQw7	24·4	39·1	69·0 (6·89) (p<0·0006)	78·9 (11·63) (p<0·0003)

*Frequency; †relative risk.

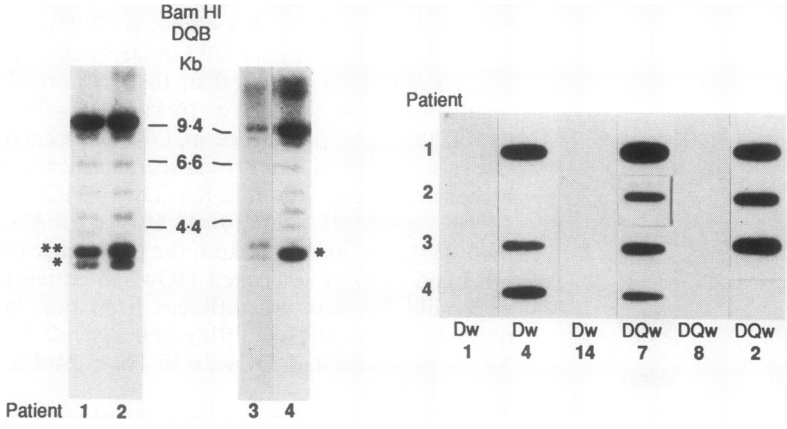

Figure 3 Southern blot and oligonucleotide typing of genomic DNA from four patients with severe rheumatoid arthritis who developed gold induced toxic reactions (group IIb). Lane 1=No 1 (DR3,4(Dw4); DQw2,w7); lane 2=No 2 (DR3,4; DQw2,w7); lane 3= No 3 (DR3,4(Dw4); DQw2,w7); lane 4=No 4 (DR4(Dw4),5; DQw7). Dw type for patient
*No 2 could not be determined owing to the failure to amplify the DRB1 chain by a polymerase chain reaction. *Indicates DQw7; **indicates DQw2.*

to the failure to amplify the DRB1 gene by a polymerase chain reaction in these cases on more than two occasions. The prevalence of DR1 and of DR1 in DR4 negative patients was not different from that in control subjects (DR1=10·4% χ^2=0·11, NS; DR1 in DR4 negative=12·5%, χ^2=0·02, NS). In addition, the prevalence of B8, DR3 extended haplotypes in DR3 positive patients (89·3%) was significantly (p<0·02) different from that in normal controls (65·6%).

Comparison of the prevalence of HLA-D region antigens among the patient groups showed that the prevalence of DR1 in patients with mild RA was higher than in the two other groups, but the data reached significance (p<0·02) only between patients with mild RA and the patients who developed gold induced toxic reactions. The prevalence of DR3 was significantly higher in patients with gold induced toxic reactions than in patients with mild RA (p<0·00004) and patients in whom the disease was well controlled by gold (p<0·002). Finally, the prevalence of DR4 associated DQw7 was significantly higher in patients with severe RA, both with (p<0·03) or without (p<0·03) gold induced toxic reactions, than in patients with mild RA.

Discussion

The results in this study show that the prevalence of DR4 was significantly increased in all three groups of patients with RA. The prevalence of DR1, DR3, and of DR4 associated DQw7 specificities, however, showed differences in these groups of patients. For example, DR1 and DR1 in DR4 negative subjects was significantly increased only in patients with mild RA. On the other hand, DR4 associated DQw7 was significantly increased in both groups of patients with severe RA—those who developed and those who did not develop gold induced toxic reactions. In addition, DR3 was significantly increased only in patients who developed gold induced toxic reactions.

A number of studies have noted a significant association between DR4 and RA.[1] Although this association has been found by several investigators in white patients, the relative risk (RR) values have been quite variable—for example, from 2·66 to 9·29 in North American white patients. The results in our study show that the highest RR value (5·76) was found for patients with severe RA, in whom the disease was controlled by sodium aurothiomalate. In addition, the results in this study confirm the significantly higher prevalence of Dw4 and Dw14 in patients with RA found by other investigators.[1 13]

Higher prevalence of DR1 patients with RA of Asian, Mexican, Greek, and Jewish origin has been described.[2–5] Among white patients, Stastny *et al* [6] and Christiansen *et al*[7] noted a significantly higher prevalence of DR1, especially in DR4 negative patients. A number of investigators, however, did not find an increased prevalence of DR1 in patients with RA.[9–12] In fact, inconsistent results for the prevalence of DR1 in patients with RA has been noted in Jewish patients,[5 12] and in white

patients on two separate occasions by a single laboratory.[6 10] The results in our study show that DR1 was significantly increased only in patients with mild RA, and no such increase was seen in patients with severe disease. In fact, the prevalence of DR1 in patients with RA with gold induced toxic effects was decreased, but the results did not reach significance. In addition, the prevalence of DR1 in DR4 negative subjects was increased only in patients with mild disease. Thus it is likely that the differences in the prevalence of DR1 in different groups of patients with RA may be related to the severity of the disease.

The prevalence of DR3 was significantly higher in these rheumatoid patients who developed gold induced toxic reactions than in normal healthy controls. The results in this study are in agreement with earlier reports which showed that patients with RA carrying DR3 are at a higher risk of developing gold induced adverse reactions.[9 38–46] Two reports, however, did not find an association between DR3 and gold induced toxicity.[47 48] These differences may be due to patient selection (racial factors, seropositive, or seronegative) and to the gold preparation used. In addition, the results in the present investigation confirm our earlier report in that the B8, DR3 haplotype was present in a significantly higher proportion in DR3 positive patients with gold induced toxic reactions than in normal controls or in DR3 positive patients without these side effects.[38]

Recently, a number of studies have examined the prevalence of extended haplotypes bearing DR4 in patients with RA. DR4 is found in linkage disequilibrium with either DQw7 or DQw8. These DR4 associated DQw7 and DQw8 specificities have the same DQA1 chain, but differ in the DQB1 chain. The DQw7 specificity is also associated with DR5. The DR4 associated DQw7 and the DR5 associated DQw7 specificities carry the same DQB1 chain, but differ in the DQA1 chain. Thus DR4 associated DQw7 carries a unique DQA1/B1 heterodimer. We found a significantly higher incidence of DR4 associated DQw7 in patients with moderate to severe RA.[15 16] Similarly, a higher prevalence of DQw7 has been found in patients with severe RA and in Felty's syndrome.[17–19] In contrast, some studies did not find an increase in the prevalence of DR4 associated DQw7 in patients with RA.[20–24] The clinical status of the patients in these studies, however, was not defined. The results in our study show that the prevalence of DR4 associated DQw7 was significantly higher in patients with severe RA (groups IIa and IIb) than in normal controls and patients with mild disease. These data together with those published earlier suggest that DR4 associated DQw7 may be related to the severity of the disease.

In summary, the prevalence of DR4 was significantly higher in all groups of patients with RA. In addition, the prevalence of DR1 and of DR1 in DR4 negative subjects was increased only in patients with mild RA, but not in patients with severe disease. On the other hand, the prevalence of DR4 associated DQw7 was significantly higher only in patients with severe disease, but not in patients with mild RA. These data suggest that HLA-D region genes which cause susceptibility to mild RA may be different from those causing susceptibility to severe RA. The classification of mild and severe disease was made by us on the basis of response to non-steroidal anti-inflammatory analgesic treatment; the mild disease being controlled by such treatment, whereas patients with severe disease required second line drugs. These results suggest that both DR and DQ (A, B) genes may be important in conferring susceptibility to RA; the former (DR) to mild disease, the latter (DQA, DQB) to severe RA.[49–51]

This work was supported by research grants from the Arthritis Society and the Medical Research Council of Canada. We thank Drs P A Peterson, Uppsala, Sweden and K Tsuji, Kanagawa, Japan for supplying the cDNA probe.

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