DISEASE MARKERS, VOL. 11, 37–44 (1993)

HLA-DPA1 AND HLA-DPB1 IN RHEUMATOID ARTHRITIS AND ITS SUBSETS

C. J. HUTCHINGS*, M. C. HILLARBY, M. J. MCMAHON, W. E. R. OLLIER† AND D. M. GRENNAN

University of Manchester Rheumatic Diseases Centre, Clinical Sciences Building, Hope Hospital, Eccles Old Road, Salford M6 8HD

†ARC Epidemiology Research Unit, Stopford Building, Oxford Road, University of Manchester, Manchester M13 9PT

SUMMARY

The aim of this study was to examine the relationship between HLA-DP and susceptibility to articular and extra-articular features (Felty's syndrome and vasculitis) of rheumatoid arthritis (RA). The possible association of DP types with severity of articular disease was also analysed. No statistically significant associations were observed between HLA-DP alleles and articular or extra-articular features of RA, or to the severity of the arthritis when p was corrected for the number of alleles tested.

KEY WORDS HLA-DP Rheumatoid arthritis (RA) RA-vasculitis RA-Felty's syndrome

INTRODUCTION

Rheumatoid arthritis is a chronic inflammatory disease of unknown aetiology and a genetic predisposition. The strongest genetic association in rheumatoid arthritis has been with HLA-DR4. It has been suggested that the molecular basis for this HLA association is a particular sequence in the third hypervariable region of the DRB1 gene (Gregersen *et al.*, 1987). In previous studies we and others have found associations between HLA variants outside the DRB1 locus with certain extra-articular features of rheumatoid disease. Thus Felty's syndrome, which is found in less than 1 per cent of rheumatoid arthritis patients attending rheumatology clinics, has an association at the DQB locus with DQB1*0301 (So *et al.*, 1988; Clarkson *et al.*, 1990), whereas rheumatoid vasculitis shows associations with unusual DQ-DR haplotypic combinations (Hillarby *et al.*, 1993). These associations, which were not accounted for by a primary association at the HLA-DRB1 locus with HLA-DR4, suggested that HLA variants outside the DRB1 locus influence susceptibility to particular rheumatoid disease features. HLA-DQA and HLA-DQB loci map centromeric to HLA-DR loci and it would now be of interest to see whether similar associations may be demonstrated at other HLA loci centromeric to DR.

Few investigations into RA have demonstrated a disease association with HLA-DP (Pawelec *et al.*, 1988; Stephens *et al.*, 1989; Begovich *et al.*, 1989; Perdriger *et al.*, 1992). Until recently this was due to the restrictions of the primed lymphocyte typing (PLT) system. Allelic polymorphism of the DP region has now been studied

*Addresee for correspondence.

Received 30 September 1992
Accepted 18 November 1992

by RFLP analysis (Stephens *et al.*, 1989) and PCR-SSO DNA dot blot hybridizations (Perdriger *et al.*, 1992) with DPA1 and DPB1 probes. However, using the method of cellular typing it has been reported that there is a significant decrease in the frequency of DPw3 and a tendential decrease of DPw1 in RA (Pawelec *et al.*, 1988), but these decreases do not reach significance when corrected for the number of alleles tested. In Stephens' study DPw4 was increased in RA (87·5 per cent in RA vs 80·7 per cent in controls), but this difference was not significant when p was corrected. A similar increase was reported in Pawelec's study (79 per cent in RA vs 71 per cent in controls). These methods did not take into account the heterogeneity of the DPw4 specificity. More recently, a DP study using PCR-SSO typing with a panel of 14 probes (Perdriger *et al.*, 1992) revealed an increase of DPB1*0401 in RA (77·46 per cent in RA vs 55·4 per cent in controls). Gao *et al.* (1991) have also demonstrated an increase in DPB1*0401, but Begovich *et al.* (1989) found no association with this DP polymorphism in their adult patient group. However, the relative risk values in both studies (Perdriger *et al.*, 1992; Gao *et al.*, 1991) were relatively low (2·74 and 3·1, respectively). In this report we have examined by PCR-SSO typing, allele frequencies of DPB1 and DPA1 genes in RA patients with or without extra-articular disease features. In a previous study we demonstrated different HLA associations between rheumatoid subjects with mild and severe articular disease (McMahon *et al.*, 1992). We have therefore examined such groups in this study.

METHODS

Subjects

All individuals examined were Caucasoid and living in the northwest of England. Seventy unrelated normal subjects were included as controls for DPA1 typing and 65 were included as controls for DPB1 typing. One hundred and forty-three unrelated patients with classical or definite RA (Ropes, 1959) were DPA1 typed and 96 were DPB1 typed. None of these patients had Felty's syndrome or major vasculitis. Thirty RA patients with Felty's syndrome were typed for DPA1 and 29 for DPB1; Felty's syndrome was defined as RA plus the following; splenomegaly documented by a clinician or ultrasound; white blood cell counts less than $3{\cdot}5 \times 10^9\ l^{-1}$ and granulocyte counts less than $2{\cdot}0 \times 10^9\ l^{-1}$ over periods of at least 6 months and which were not attributable to drug toxicity. Forty-one subjects with RA and major vasculitis were typed for DPA1 and 24 for DPB1. Criteria for major vasculitis were one or more of the following: major cutaneous ulceration, non-arteriosclerotic arterial involvement, scleritis or mononeuritis multiplex.

Articular disease severity in the rheumatoid subjects was assessed by radiological scoring of the hands and feet (Larsen *et al.*, 1975). The relationship between radiographic scores and disease duration has been investigated previously by regression analysis (McMahon *et al.*, 1991), and confidence intervals around a similarly developed regression equation allowed identification of those rheumatoid subjects whose radiographic score and disease duration were in the upper 20 per cent and lower 20 per cent of severity assessments. These patients were classed as 'severe' and 'mild', respectively. Twenty-two severe RA subjects and 29 mild RA subjects were included in the study in order to investigate the relationship of DP with severity

of disease. The control group and the RA only group were divided into 'epitope positive' (those who type for the DRB1 shared epitope) and 'epitope negative' (those who do not type for this DR sequence). The RA group was divided into seronegative (seronegative was classified as the rheumatoid factor titre being consistently lower than 1 in 32) and seropositive subsets.

DNA samples

Genomic DNA was extracted from circulating white cells as previously described (Kunkel *et al.*, 1977).

PCR amplification

Standard PCR procedure was followed. The second DPB1 exon and the second DPA1 exon were amplified using the oligonucleotide primers from the BSHI-Class II oligo typing kit. The primer sequences were as follows:

DPB I = 5′-GAG AGT GGC GGC TCC GCT CAT-3′;
DPB II = 5′-GCC GGC CCA AAG CCC TCA CTC-3′;
DPA I = 5′-GTG TCA ACT TAT GCC GCG-3′;
DPA II = 5′AGT GTG GTT GGA ACG CTG-3′.

PCR was carried out on 1 μg of genomic DNA by using 30 cycles of amplification. Each cycle comprised three steps: denaturation at 95° C for 1 min, primer annealing at 63° C for 2 min, and finally synthesis of complementary sequences at 72° C for 1 min. In each reaction mix 2·5 units of Taq polymerase was used. Amplified DNA was denatured at 95° C for 5 min, cooled on ice, diluted with an equal volume of 20 × SSC and transferred to positively charged nylon membrane (Boehringer Mannheim) using an ATTO dot blotter. Filters were hybridized to one of the 19 DPB specific oligonucleotide probes, or to one of the two DPA specific oligonucleotide probes (Table 1). Prehybridization was carried out for 30 min in 5 × SSC, 2 per cent (w/v) blocking reagent (Boehringer Mannheim), 0·1 per cent (w/v) *N*-lauroylsarcosine, and 0·02 per cent (w/v) sodium dodecyl sulphate (SDS) at 42° C. Hybridization was in fresh buffer plus probe at 42° C for 18 h in a shaking water bath. DPB1 oligonucleotide probes were labelled with digoxigenin and detected with AMPPD according to the manufacturer's instructions (Boehringer Mannheim).

DPA1 oligonucleotide probes were radiolabelled with ^{32}P by polynucleotide kinase. Post-hybridization washes were carried out in 6 × SSC for 5 min at room temperature, followed by a further wash in 2 × SSC/0·2 per cent (w/v) SDS at the specified wash temperature (refer to Table 1). To validate the specificity of the hybridization and post-hybridization wash conditions reference cell lines were included in each analysis.

Statistics

Differences between DPA1 and DPB1 frequencies in patients and controls were analysed by (2×2) χ^2 (with Yates' correction) and Fisher's exact tests. The p values obtained were multiplied by the number of comparisons made to correct for that

Table 1. DPA and DPB SSO probe sequences and wash temperatures

Probe	Wash temperature (° C)
DPA1	
3101	43
3102	43
DPB1	
0901	53
0902	54
0903	55
0904	53
3503	57
5501	55
5502	51
5503	55
5504	51
6502	55
6901	52
6902	53
6903	56
6905	56
6906	56
7601	51
7602	51
8501	57
8503	55

number of alleles tested (p^c). Odds ratios (O.R.) and 95% confidence limits were calculated for each comparison (according to the Mantel-Haenzel method), in order to give a measure of the strength of the association.

RESULTS

The frequencies for DPA1 alleles are given in Table 2 and those for DPB1 alleles in Tables 3 and 4.

DPA1*01 is the most frequent DPA1 allele in all the subject groups studied (90 per cent in the control group and 99 per cent in the RA group), whereas the frequency of DPA1*0201 is lower (40 per cent in controls and 34 per cent in RA; Table 2). Among the different DPB1 alleles, DPB1*0401 has the highest frequency (54 per cent in controls, 58 per cent in RA, 38 per cent in RA-vasculitis, and 69 per cent in RA-Felty's), followed by DPB1*0402 (23 per cent in controls, 27 per cent in RA, 25 per cent in RA-vasculitis, and 35 per cent in RA-Felty's; Table 3). DPB1*0101 and 0201 were moderately frequent (25 per cent and 20 per cent in controls and 15 per cent and 13 per cent in RA, respectively). Particular alleles were infrequently present; these being DPB1*0202, 0601, 0901, and 1101.

No association between DPA1 or DPB1 alleles and severity of disease was detected (Table 3) or with the epitope negative or epitope positive patients (Table 4).

Table 2. Frequencies (%) of DPA1 alleles in patients and controls

Subject Group	*n*	DPA*01	DPA*0201
Controls	70	90	40
RA	143	99	34
Mild	35	100	29
Severe	26	100	42
RA-FEL	30	100	13*
RA-VAS	41	100	20

Mild = mild erosive disease; Severe = severe erosive disease; RA-FEL = RA with Felty's syndrome; RA-VAS = RA with major vasculitis.
*RA vs RA-FEL DPA1*0201, $p = 0{\cdot}04$ ($\chi^2 = 3{\cdot}9$) O.R. = 0·3.

Results for DPB1 typing in seronegative subjects are presented in Table 5; as only seven seronegatives had been typed no comparisons were made with seropositive subjects.

No statistically significant associations between DPA1 or DPB1 alleles and disease groups were found in this study when p was corrected for the number of alleles tested. However, a number of non-significant differences were observed as follows. DPA1*0201 was decreased in RA-Felty's patients ($\chi^2 = 3{\cdot}9, p = 0{\cdot}04$) vs RA only patients. The odds ratio indicates that DPA1*0201 positive RA patients are 3·3 times less likely to develop Felty's syndrome than others. No other DPA associations were apparent. DPB1*0101 was decreased amongst RA only patients ($\chi^2 = 2{\cdot}6, p = 0{\cdot}1$) vs controls and decreased in RA-Felty's patients ($\chi^2 = 4{\cdot}7, p = 0{\cdot}03$) vs controls. The odds ratios indicate that DPB1*0101 positive people are 1·9 times less likely to develop RA and 9·1 times less likely to develop Felty's syndrome than those who are DPB1*0101 negative. DPB1*0401 was decreased in RA-vasculitis ($\chi^2 = 2{\cdot}6, p = 0{\cdot}1$) vs RA only patients. The odds ratio suggests that DPB1*0401 positive RA patients are 2·3 times less likely to develop rheumatoid vasculitis than those who are DPB1*0401 negative. DPB1*0801 was increased in RA-vasculitis ($\chi^2 = 5{\cdot}4, p = 0{\cdot}02$) vs controls and in RA-vasculitis ($\chi^2 = 3{\cdot}3, p = 0{\cdot}07$) vs RA only patients, as was DPB1*1001 in RA-vasculitis ($\chi^2 = 2{\cdot}7, p = 0{\cdot}09$) vs controls and in RA-vasculitis ($\chi^2 = 2{\cdot}9, p = 0{\cdot}08$) vs RA only patients. A similar non-significant increase was found with DPB1*1801 in RA-vasculitis ($\chi^2 = 3{\cdot}2, p = 0{\cdot}07$) vs controls and in RA-vasculitis ($\chi^2 = 4{\cdot}2, p = 0{\cdot}04$) vs RA only patients.

DISCUSSION

In this study we found DPA1*01 and DPA1*0201 control allele frequencies are in agreement with previous observations (Al-Daccak *et al.*, 1991) and there are no significant associations between DPA1 and any of the disease groups studied.

Cellular typing of DP alleles has previously demonstrated a decrease in the DPw3 allele in RA subjects versus controls (Pawelec *et al.*, 1988), however our study shows

Table 3. Frequency (%) of DPB1 alleles in patients and controls

DPB1*	Control	RA	Severe	Mild	RA-FEL	RA-VAS
0101	25	15*	14	10	3**	21
0201	20	13	23	10	28[1]	8
0202	2	5	9	0	0	0
0301	5	4	5	7	0	4
0401	54	58	55	59	69[2]	38[3]
0402	23	27	41[4]	41[5]	35	25
0501	5	1	0	3	0	4
0601	2	1	0	0	0	0
0801	3	6	5	14	7	21[6]
0901	0	0	0	0	0	8
1001	3	2	0	7	0	13[7]
1101	3	0	0	0	0	0
1301	2	5	5	3	0	4
1401	5	8	9	7	0	8
1501	6	1	0	3	0	0
1601	6	4	5	7	0	0
1701	3	1	5	0	0	4
1801	0	3	5	3	0	17[8]
1901	6	1	5	0	0	0
n	65	96	22	29	29	24

*RA v C *0101 $p=0{\cdot}1$ ($\chi^2=2{\cdot}6$) O.R. $=0{\cdot}53$
**RA-FEL v C *0101 $p=0{\cdot}03$ ($\chi^2=4{\cdot}7$) O.R. $=0{\cdot}11$
RA-FEL v RA *0101 $p=0{\cdot}2$ ($\chi^2=1{\cdot}7$) O.R. $=0{\cdot}21$
[1]RA-FEL v C *0201 $p=0{\cdot}6$ ($\chi^2=0{\cdot}3$) O.R. $=1{\cdot}5$ (95% CI 0·6–4·2)
[2]RA-FEL v RA *0401 $p=0{\cdot}6$ ($\chi^2=0{\cdot}7$) O.R. $=1{\cdot}6$ (95% CI 0·7–3·8)
[3]RA-VAS v RA *0401 $p=0{\cdot}1$ ($\chi^2=2{\cdot}6$) O.R. $=0{\cdot}43$
[4]Severe v RA *0402 $p=0{\cdot}3$ ($\chi^2=1{\cdot}0$) O.R. $=1{\cdot}9$ (95% CI 0·7–4·5)
[5]MILD v RA *0402 $p=0{\cdot}1$ ($\chi^2=2{\cdot}2$) O.R. $=1{\cdot}9$ (95% CI 0·8–4·5)
[6]RA-VAS v C *0801 $p=0{\cdot}02$ ($\chi^2=5{\cdot}4$) O.R. 8·3 (95% CI 1·8–37·5)
RA-VAS v RA *0801 $p=0{\cdot}07$ ($\chi^2=3{\cdot}3$) O.R. $=4{\cdot}0$ (95% CI 1·2–13·4)
[7]RA-VAS v C *1001 $p=0{\cdot}09$ ($\chi^2=2{\cdot}7$) O.R. $=9{\cdot}3$ (95% CI 1·3–66·2)
RA-VAS v RA *1001 $p=0{\cdot}08$ ($\chi^2=2{\cdot}9$) O.R. $=6{\cdot}7$ (95% CI 1·3–34·6)
[8]RA-VAS v C *1801 $p=0{\cdot}07$ ($\chi^2=3{\cdot}2$) O.R. $=6{\cdot}3$ (95% CI 1·3–31·2)
RA-VAS v RA *1801 $p=0{\cdot}04$ ($\chi^2=4{\cdot}2$) O.R. $=6{\cdot}2$ (95% CI 1·5–25·6)

that the DPB1*0301 gene frequency is practically the same in both groups (5 per cent in controls and 4 per cent in RA). It has previously been reported that DPB1*0201 is significantly increased in juvenile RA patients over controls, but not increased in patients with adult RA (Begovich *et al.*, 1989) and our results are concordant with this adult RA study. This study did not detect the reported increase of DPB1*0301 in seronegative patients (Gao *et al.*, 1991), however only seven seronegative subjects were included in our RA population. No significant associations were detected with the severity of erosive disease in RA.

Cellular typing of DP alleles has previously shown a decrease of the DPw1 allele in Felty's syndrome (Pawelec *et al.*, 1988). Our study also demonstrated a decrease in DPB1*0101 in Felty's syndrome. This was not statistically significant when p was corrected for the number of alleles tested and it should be taken into consideration that only 29 cases of Felty's syndrome were DPB typed in our study.

Table 4. Frequency (%) of DPB1 alleles in epitope negative (epi −ve) and epitope positive (epi +ve) controls and in RA patients

DPB1*	Controls epi −ve	RA epi −ve	Controls epi +ve	RA epi +ve
0101	29	19*	18	13
0201	12	10**	29	13
0202	6	0	0	7
0301	6	0	0	6
0401	59	52	53	58
0402	18	38[1]	29	26
0501	0	5	0	1
0601	0	5	6	0
0801	0	5	6	6
0901	0	0	0	0
1001	0	0	6	3
1101	0	0	6	0
1301	6	5	0	4
1401	0	5	0	10
1501	0	0	6	1
1601	0	0	6	6
1701	6	0	0	1
1801	0	0	0	4
1901	12[2]	0	0	1
n	17	21	17	71

*RA epi −ve vs controls epi −ve *0101 $p=0{\cdot}7$ ($\chi^2=0{\cdot}1$) O.R. $=0{\cdot}56$.
**RA epi −ve vs controls epi −ve *0201 $p=0{\cdot}8$ ($\chi^2=0{\cdot}1$) O.R. $=0{\cdot}77$.
[1]RA epi −ve vs controls epi −ve *0402 $p=0{\cdot}3$ ($\chi^2=1{\cdot}0$) O.R. $=2{\cdot}9$ (95% CI 0·6–13·1).
[2]Controls epi −ve v RA epi −ve *1901 $p=0{\cdot}6$ ($\chi^2=0{\cdot}8$) O.R. = indeterminate.

Table 5. DPB1 typing of rheumatoid factor negative RA patients

Patient	DPB1
1	*0402/0402
2	*0401/0401
3	*0202/0402
4	*0101/0201
5	*0101/1901
6	*0402/0402
7	*0101/0201

This study has demonstrated that there is a lack of association between DPA1 or DPB1 alleles and disease. Hence, there is no evidence to suggest that HLA-DP alleles have a primary effect on susceptibility to RA. This includes subjects that do not type for the known DR susceptibility gene. These results also suggest that DP alleles do not increase the risk of extra-articular disease features (except for

RA-vasculitis, where statistically non-significant increases for alleles DPB1*0801, 1001, and 1801 and decrease for allele DPB1*0401 were observed). Additionally, there is no indication that either DPB1 or DPA1 alleles are associated with severity of arthritis in rheumatoid disease. However, larger rheumatoid disease subset groups will be necessary (to overcome the difficulties and limitations encountered when small numbers of patients are involved) in order to provide 'significant' evidence.

ACKNOWLEDGEMENT

We are grateful to the Arthritis and Rheumatism Council for financial support.

REFERENCES

Al-Daccak, R., Fu Qing Wang, Theophille D., Lethielleux, P., Colombani, J., Loiseau, P. (1991). Gene polymorphism of HLA-DPB1 and -DPA1 loci in Caucasoid population: frequencies and DPB1-DPA1 associations. *Hum. Immunol.* **31,** 277–285.

Begovich, A.B., Bugawan, T.L., Nepom, B.S., Klitz, W., Nepom, C.T., Erlich, H.A. (1989). A specific HLA-DPB allele is associated with pauciarticular juvenile rheumatoid arthritis but not adult rheumatoid arthritis. *Proc. Natl. Acad. Sci. (USA),* **86,** 9489–9493.

Clarkson, R.W.E., Bate, A.S., Grennan, D.M., Chattopadhyay, C., Sanders, P., Davis, M., Kelly, C. (1990). DQw7 and C4B null allele in rheumatoid arthritis and Felty's syndrome. *Ann. Rheum. Dis.,* **49,** 976–979.

Gao, X., Fernandez-Vina, V., Olsen, N.J., Pincus, T., Stastny, P. (1991). HLA-DPB1*0301 is a major risk factor for rheumatoid factor-negative adult rheumatoid arthritis. *Arthritis Rheum.,* **34,** 1310–1312.

Gregersen, P.R., Silver, J., Winchester, R.J. (1987). The shared epitope hypothesis: an approach to understanding the molecular susceptibility to RA. *Arthritis Rheum.,* **30,** 1205–1213.

Hillarby, M.C., Ollier, W.E.R., Davis, M., Davidson, J., Sanders, P.A., Grennan, D.M. (1993). Unusual DQA-DR haplotypes in rheumatoid vasculitis. *Br. J. Rheum.*, in press.

Kunkel, L.M., Smith, K.D., Boyer, S.H., Borgaonkar, D.S., Wachtel, S.S., Miller, O.J., Breg, W.R., Jones, H.W., Rary, J.M. (1977). Analysis of human Y-chromosome specific reiterated DNA in chromosome variants. *Proc. Natl. Acad. Sci. (USA),* **24,** 1245–1249.

Larsen, A., Dale, K., Eck, M. (1975). *Standard Radiographs of Rheumatoid Arthritis*: Adopted by the EULAR standing committee on international studies including therapeutic trials. Dept. of Radiology, Oslo Sanitetsforenging Rheumatism Hospital. Published courtesy of Eli Lilly Co.

McMahon, M.J., Hollis, S., Sanders, P.A., Grennan, D.M. (1991). Articular disease severity in rheumatoid subjects with and without Felty's syndrome. *Br. J. Rheumatol.,* **30,** 217–219.

McMahon, M.J., Hillarby, M.C., Clarkson, R.W.E., Hollis, S., Grennan, D.M. (1992). HLA variants and rheumatoid disease severity. *Br. J. Rheumatol.,* **31,** Abstract suppl. 2.

Pawelec, G., Reekers, P., Brackertz, D., Sansom, D., Scheider, E., Blaurock, M., Muller, C., Rehbein, A., Balko, I., Wernet, P. (1988). HLA-DP in rheumatoid arthritis. *Tissue Antigens,* **31,** 83–89.

Perdriger, A., Semana, G., Quillivic, F., Chales, G., Chardevel, F., Legrand, E., Meadeb, J., Fauchet, R., Pawlotsky, Y. (1992). Evidence of an association with allele DPB1 0401. *Tissue Antigens,* **39,** 14–18.

Ropes, M.W. (1959). Diagnostic criteria for RA. 1958 revision. *Ann. Rheum. Dis.,* **18,** 49–53.

So, A.K.S., Warner, C.A., Sanson, D., Walport, M.J. (1988). DQB polymorphism and genetic susceptibility to Felty's syndrome. *Arthritis. Rheum.,* **31,** 990–994.

Stephens, H.A.F., Vaughan, R.W., Lazaros, I.S., Welsh, K.I., Panayi, G.S. (1989). Southern blot analysis of HLA-DP gene polymorphisms in Caucasoid rheumatoid arthritis (RA) patients and controls. *Immunogenetics,* **30,** 149–155.