CONCISE REPORTS

Absence of an association between HLA-DRB1*04 and rheumatoid arthritis in newly diagnosed cases from the community

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Abstract

***Objectives*—To determine HLA-DR4 and DR1 allele frequencies in a series of patients with newly diagnosed early inflammatory arthritis.**

***Methods*—HLA-DR1 and DR4 frequencies were determined by oligonucleotide typing of 208 patients classified as having either rheumatoid arthritis (RA) or undifferentiated inflammatory polyarthritis.**

***Results*—The frequency of occurrence of DR4 in these patients with RA did not differ significantly from that in controls in the United Kingdom (42 *v* 37%). HLA-DR1 was increased in the group with inflammatory polyarthritis (25 *v* 18%).**

***Conclusions*—The frequency of DR4 is not increased in newly diagnosed community based patients with RA. This supports the hypothesis that DR4 is less important as a marker for susceptibility to RA than it is for disease persistence or severity.**

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The aetiology of rheumatoid arthritis (RA) remains unknown; however, the strong association[1] between RA and HLA-DR4 that has been clearly shown in many groups[2] suggests that a genetic contribution to its development is important. The association between HLA and RA has been further refined and it is now clear that a shared amino acid sequence, or epitope, is common to the DRB1 chains of all DR specificities associated with RA (DRB1*0401, 0404, 0405, 0101, 1402, and 1001).[3] Precisely how this epitope is involved in the aetiopathogenesis of RA is unknown. Furthermore, it is unclear whether HLA is primarily associated with the initiation and triggering of disease (i.e. susceptibility) or with the progression and severity of the disease. Resolving this question is difficult. Most studies have investigated patients with RA attending hospital clinics, thus favouring the selection of patients with more severe or chronic disease.

There is considerable circumstantial evidence to support the existence of a relationship between DR4 status and severity of RA as measured indirectly by the number of American Rheumatism Association criteria satisfied,[4] the extent of erosive disease[4–6] and the presence of extra-articular manifestations.[7 8] Conversely, a lower DR4 frequency was seen in newly diagnosed patients with RA attending an early arthritis clinic.[9] No association between RA and DR4 was observed in a community based study of the prevalence of RA in The Netherlands.[10]

The Norfolk Arthritis Register was established to study the incidence and prognosis of RA in the Norwich Health Authority, United Kingdom[11] (population 478 000). This study provides an ideal opportunity to study further the relation between HLA and the initiation, progression, and severity of RA. We present our findings for cases referred during the first year of the study.

Subjects and methods

PATIENTS AND CONTROLS

All 273 general practitioners in the Norwich Health Authority refer to the Norwich Arthritis Register all new cases of inflammatory arthritis affecting at least two peripheral joints and persisting for at least four weeks. A parallel system of notification occurs from hospital clinics within the health authority. The patients are then examined by trained metrologists and a detailed clinical history obtained. Blood samples are taken for the measurement of rheumatoid factor and HLA typing. Rheumatoid factors are determined by a tube latex test and a titre $\geq 1/40$ is regarded as positive. Patients are classified as having RA using the 1987 American College of Rheumatology (ACR) criteria.[12] Those patients who neither satisfied the ACR criteria nor had another diagnosis were categorised as having undifferentiated inflammatory polyarthritis. Two hundred and eight patients recruited during 1990 were available for immunogenetic analysis. Of these, 89 had RA and 119 inflammatory polyarthritis. One hundred and thirty six healthy volunteers from the United Kingdom (members of staff from within the medical school/St Marys Hospital, Manchester) were available as controls.

PCR AMPLIFICATION AND OLIGONUCLEOTIDE TYPING

Genomic DNA was extracted from whole blood using a standard phenol/chloroform

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extraction procedure. The second exon of DRB1 genes for each sample was amplified in the polymerase chain reaction using generic DRB1 primers (British Society for Histocompatibility and Immunogenetics (BSHI) oligotyping kit)

Primer 1 CCC CAC AGC ACG TTT CTT G
Primer 2 CCG CTG CAC TGT GAA GCT CT

The polymerase chain reaction product was monitored for amplification and contamination on a 1·2% agarose gel, stained with ethidium bromide, then dot blotted onto nylon membrane (Amersham Hybond-N) and hybridised with oligonucleotide probes specific for DRB1*01 and DRB1*04:

1001 (DRB*01) TAA GTT TGA ATG TCA TTT
1004 (DRB1*04) GAG CAG GTT AAA CAT GAG
1008 (DRB1*10) GAG GAG GTT AAG TTT GAG

All probes were 3' end labelled with digoxigenin. Washes were all performed in 3 M tetramethylammonium chloride solution at 53°C and detection was via an alkaline phosphatase antibody to digoxigenin and 3-(2'-spirodamantane)-4-methoxy-4-(3'-phosphoryloxy)-phenyl-1,2-dioxetane.[13]

DNA from patients positive for DR1 was also amplified with DR1 specific primers from the XIth International Histocompatibility Workshop

Primer 1 TTC TTG TGG CAG CTT AAG TT
Primer 2 CCG CTG CAC TGT GAA GCT CT

and hybridised with probes recognising the three different variants of DR1:

7001 (DRB1*0101/0102) TCC TGG AGC AGA GGC GGG
7007 (DRB1*0103) ACA TCC TGG AAG ACG AGC
8601 (DRB1*0101/0103) AAC TAC GGG GTT GGT GAG
8602 (DRB1*0102) AAC TAC GGG GCT GTG GAG

STATISTICAL ANALYSIS

Results are expressed as antigen frequencies and compared with frequencies determined for a panel of 136 United Kingdom controls. Odds ratios were calculated with 95% confidence intervals.

Results

Fifty five (62%) of the 89 patients with RA and 16 (13%) of the 119 patients with inflammatory polyarthritis were positive for rheumatoid factor. Tables 1 and 2 give the frequencies of HLA-DR4 and DR1 in the controls and patient subgroups respectively; HLA-DR10 has not been included as only one patient and one of the controls was positive for this antigen. The frequency of HLA-DR4 was not significantly different between controls (37%) and either the patients with RA as a whole (42%) or the subgroup who were positive for rheumatoid factor (46%). There was also no increase in HLA-DR4 frequency (33%) in the patients with inflammatory polyarthritis compared with controls. There was no significant difference in HLA-DR1 frequency between controls (20%) and patients with RA (20%), though the frequency of DR1 was increased in the inflammatory polyarthritis group as a whole (25%) and this increase was significant in the rheumatoid factor negative inflammatory polyarthritis group (28%). In the controls and the patients with inflammatory polyarthritis, HLA-DR1 alleles were largely accounted for by HLA-DRB1*0101.

Table 1 Frequency of HLA-DR4 in controls and subgroups of patients with rheumatoid arthritis (RA), inflammatory polyarthritis (IP), and patients positive for rheumatoid factor (RF)

	Frequency of HLA-DR4 (%)	*Odds ratio (95% confidence interval)**
Controls (n=136)	36·7	
All RA (n=89)	41·6	1·22 (0·8 to 2·1)
RF+RA (n=55)	45·6	1·43 (0·8 to 2·7)
IP (n=119)	32·8	0·84 (0·5 to 1·4)

*Patients *v* controls.

Table 2 Frequency of HLA-DR1 in controls and subgroups of patients with rheumatoid arthritis (RA), inflammatory polyarthritis (IP), and patients positive for rheumatoid factor (RF)

	Frequency of HLA-DR1 (%)	*Odds ratio (95% confidence interval)**
Controls (n=136)	19·9	
All RA (n=89)	20·2	1·2 (0·6 to 2·5)
RF+RA (n=55)	25·2	1·9 (1·0 to 3·4)
IP (n=119)	28·2	2·2 (1·1 to 4·0)

*Patients *v* controls.

Of the patients with RA, 47 (53%) had been referred to hospital. The frequency of DR4 in these patients (40%) was similar to that in the patients with RA not referred to hospital (43%).

Discussion

These results show that the frequencies of DR4 and DR1 are not increased in patients with newly diagnosed RA in the community. This is in contrast with the HLA associations seen in hospital based RA studies and supports the suggestion that HLA-DR4 and DR1 are not markers for RA disease susceptibility. The possibility remains that HLA-DR4 and perhaps other DR alleles carrying the shared RA epitope are associated with disease severity and chronicity. As many measures of disease severity are largely dependent on disease duration, it will be difficult to resolve this distinction. A relation between HLA-DR4 and RA persistence would explain the high frequency of HLA-DR4 (82·3%) reported in patients with RA who had been followed up for 25 years.[5 14] If HLA is associated with long term RA, this would indicate a direct involvement of DR4 in driving the disease process of RA.

All general practitioners in the health authority were recruited into the study. It is too early to be sure whether those general practitioners who have not yet reported a

patient are not complying with the study. The general practitioners referred approximately half of the patients with RA to hospital. A number of factors may prompt referral, but it might be expected that patients with a more severe disease onset would be more likely to be referred. It is perhaps surprising that no difference in DR4 frequency was seen between patients with RA referred to hospital and those not referred. An acute disease onset may, however, be associated with a good long term prognosis. The patients with RA recruited into the Norwich Arthritis Register study are followed up annually and their disease outcome is documented. It will therefore be possible to explore the relation between the possession of the shared epitope and disease severity and duration.

HLA-DR1 has been shown to be associated with RA in a number of population studies[2] and this has been explained by the shared epitope sequence. The observation that HLA-DR1 is also associated with early inflammatory polyarthritis may indicate that DR1 is also a marker for a range of disorders. A previous study has shown an association between DR1 and seronegative RA.[15] In addition, we have observed an increased frequency of DR1 in patients with late onset pauciarticular juvenile chronic arthritis (Thomson W, Donn R, Robertson L, Carthy D, Ollier WER, Holt L, unpublished data). Adult onset RA is known to be a heterogeneous disease and some of this heterogeneity may be due to distinct persistent arthropathies which are associated with DR1 but not DR4.

These questions will be addressed as the patients identified in this community based population study are followed up longitudinally.

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