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D. Glass, ... , C. B. Carpenter, P. H. Schur

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Research Article

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45 unrelated Caucasian patients (41 female) with early-onset pauciarticular JRA were human leukocyte antigen (HLA) types. 28 of the study group were found to be HLA-DRw5 compared with 16 of 84 controls (X^2 , 24.3, $P = <0.001$). 9 patients were HLA-DRw8 compared with 4 of 84 controls (X^2 , 7.51, $P = <0.01$). Iritis developed in 24 of the 45 children studied, 17 of whom were typed as HLA-DRw5 when compared to controls (X^2 , 26.76, $P = <0.001$) and 6 with iritis typed as HLA-DRw8 (X^2 , 9.10, $P = <0.01$). Antinuclear antibody was found in the serum of 17 of the 28 patients typing as HLA-DRw5 compared with 4 of the 17 who did not have this antigen (X^2 , 5.88, $P = <0.02$). No such association was seen in patients with HLA-DRw8.

In a study of linked genes, a delta value of 0.090 was found for HLA-DRw5 with HLA-B12, of 0.070 for DRw5 with HLA-Cw4, and a value of 0.050 for DRw5 and HLA-Bw35. This suggests a linkage disequilibrium between HLA-DRw5 and these two B series alleles, a conclusion which was supported by haplotype analysis in families of 11 of the disease [...]

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Early-Onset Pauciarticular Juvenile Rheumatoid Arthritis Associated with Human Leukocyte Antigen-DRw5, Iritis, and Antinuclear Antibody

D. GLASS, D. LITVIN, K. WALLACE, L. CHYLACK, M. GAROVOY, C. B. CARPENTER, and P. H. SCHUR, *Departments of Medicine and Ophthalmology, Robert B. Brigham and Peter Bent Brigham Divisions of the Affiliated Hospitals Center, and Harvard Medical School, Boston, Massachusetts 02120*

ABSTRACT Evidence has been sought for a genetically determined predisposition among children with juvenile rheumatoid arthritis (JRA) who are also at particular risk for the development of inflammatory eye disease.

45 unrelated Caucasian patients (41 female) with early-onset pauciarticular JRA were human leukocyte antigen (HLA) typed. 28 of the study group were found to be HLA-DRw5 compared with 16 of 84 controls (X^2 , 24.3, $P = <0.001$). 9 patients were HLA-DRw8 compared with 4 of 84 controls (X^2 , 7.51, $P = <0.01$). Iritis developed in 24 of the 45 children studied, 17 of whom were typed as HLA-DRw5 when compared to controls (X^2 , 26.76, $P = <0.001$) and 6 with iritis typed as HLA-DRw8 (X^2 , 9.10, $P = <0.01$). Antinuclear antibody was found in the serum of 17 of the 28 patients typing as HLA-DRw5 compared with 4 of the 17 who did not have this antigen (X^2 , 5.88, $P = <0.02$). No such association was seen in patients with HLA-DRw8.

In a study of linked genes, a delta value of 0.090 was found for HLA-DRw5 with HLA-B12, of 0.070 for DRw5 with HLA-Cw4, and a value of 0.050 for DRw5 and HLA-Bw35. This suggests a linkage disequilibrium between HLA-DRw5 and these two B series alleles, a conclusion which was supported by haplotype analysis in families of 11 of the disease probands. HLA-DRw5 has not previously been reported to be increased in any rheumatic disease group. It is proposed that HLA-DRw5 is a genetic marker defining those at risk for early-onset pauciarticular JRA with iritis.

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INTRODUCTION

Long-term evaluation of children with juvenile rheumatoid arthritis (JRA)¹ suggests that patients with this diagnosis comprise several groups with clinically important distinctions (1, 2). The variables commonly used to define assignments to these clinical divisions include: sex, age of onset, extent of joint involvement, and the presence or absence of systemic features. The existence of several groups within the JRA population is to some extent reinforced by recent reports suggesting a complexity of human leukocyte antigen (HLA) associations in JRA populations (3, 4, 5).

One group of children with JRA, comprising some 15% of the whole, share features sufficient to regard them as a relatively homogeneous population. These patients are predominantly female; have onset of their disease in the first 5 yr of life and whose joint involvement, at onset, is described as pauciarticular; that is, four or fewer joints are involved in the first 6 mo of the disease. Antinuclear antibodies are frequently found in the serum of these pauciarticular patients who are at particular risk for an inflammatory ocular complication, namely, iritis (6). The iritis may be chronic with a significant risk of impairment in vision (7).

We report a study of HLA-A, -B, -C, and -DR polymorphisms in children with early-onset pauciarticular JRA and iritis, a search for evidence of an inherited predisposition.

METHODS

Subjects. 45 Caucasian patients (41 female and 4 male) with early-onset pauciarticular JRA were studied, 24 of whom

¹ *Abbreviations used in this paper:* ANA, antibody nuclear antigen; CDC, complement-dependent cytotoxicity; JRA, juvenile rheumatoid arthritis; MNL, mononuclear leukocyte.

developed iritis in addition to JRA. The majority of patients were involved in earlier reports (2, 8) and were only studied if meeting the definition of JRA as outlined by the JRA Criteria Committee of the American Rheumatism Association (9). Pauciarticular onset arthritis was defined as disease involvement of four or less joints within the first 6 mo of the disease (9). Age of onset was 5 yr or younger in 41 of the 45 patients. Length of follow-up at the Robert Breck Brigham Hospital JRA clinic ranged from 3.5 to 38 yr, with 28 patients having been followed for 10 yr or more. All patients were evaluated on an annual basis, on which occasion their work-up included an ophthalmological consultation and an assay for antinuclear antibody. Additional eye examinations were carried out as demanded by the clinical situation. Members of families of 11 of the 45 disease probands were studied.

Control data for HLA typing was obtained from healthy hospital personnel, 283 of whom were HLA-A, -B, and -C typed and 84 HLA-DRw typed.

Cells and sera. Peripheral blood mononuclear leukocytes (MNL) were obtained by density gradient centrifugation through Ficoll-Hypaque (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) and, if not used immediately, stored under liquid nitrogen after controlled rate freezing (Cryo-Med, Weld Metal Inc., Clemens, Mich.). Sera were stored at -70°C .

HLA-A, -B, and -C typing. HLA-A, -B, and -C typing for 39 specificities was carried out by microdroplet complement-dependent cytotoxicity (CDC) (10). HLA-A, -B, and -C antisera were kindly supplied by the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md.

HLA-DRw typing. HLA-D-related (DRw) specificities were typed by CDC on a B cell-enriched mononuclear leukocyte population (MNL). Enrichment of MNL for B lymphocytes was achieved by nylon wool column separation (nylon fiber, 30 denier type 200, Fenwal Laboratories, Deerfield, Ill.) (11).

The nylon wool-adherent MNL obtained after 1 h 37°C incubation have been shown to contain 60–84% surface IgM-bearing lymphocytes. All preparations studied were evaluated

in CDC with a polyvalent rabbit anti-human Ia serum, kindly supplied by Dr. A. Fuks and Dr. J. Strominger (Biological Laboratories, Harvard University), and were found to contain $>70\%$ Ia-bearing MNL.

The CDC assays were performed as follows: MNL with antisera were incubated at room temperature for 1 h and then for a further 2 h with rabbit complement (Pel Freeze Biologicals, Inc., Rogers, Ark.) (12).

Typing antisera induced sera defined by the Seventh and Eighth International Histocompatibility Workshops (13, 14) covering eight DR specificities.

Antinuclear antibody (ANA). ANA in serum was detected by immunofluorescence as described (15).

Statistical methods. Frequencies of HLA in patients and control groups were compared with the X^2 test. The relative risk was calculated with the formula hK/Hk , where h and k represent the number of patients and controls, respectively, with a particular antigen and H and K the number without (16).

Δ represents an estimate of linkage disequilibrium for a given pair of genes and was derived as follows: $\Delta = pq - p'q'$, where pq is the observed frequency of a pair of genes p and q in the same individual and $p'q'$ is the expected frequency of the combination of genes based on their individual frequencies in the population (17).

RESULTS

HLA-D antigens. Two HLA-DRw antigens, DRw5 and DRw8 were found in increased frequency in the JRA population (Table I). HLA-DRw5 was found in 28 (62%) of the 45 patients as compared to 16 (19%) of the 84 controls (X^2 , 24.3, $P = <0.001$); of the controls, 45 were female and 39 male, 8 in each group typed as HLA-DRw5. Relative risk for HLA-DRw5 was 7.0. HLA-DRw5 was found in 17 (71%) of the 24 patients with iritis as compared with controls (X^2 , 23.59, $P = <0.001$) and in 11 (52%) of the 21 (X^2 , 9.77, $P = <0.01$) who did not develop this complication. Of the 45 patients, 9 (20%) were typed as HLA-DRw8 (X^2 , 7.51, $P = <0.01$) compared with 4 (5%) of 84 controls, including 6 (25%) of the 24 with iritis (X^2 , 9.10, $P = <0.01$). The relative risk for HLA-DRw8 was 5.0. HLA-DRw7 was found less commonly than expected in the patient population (Table I).

HLA-A, -B, and -C antigens. One HLA-B antigen, Bw35, was increased in the JRA population, being present in 14 of the 45 patients compared with 51 (18%) of 283 controls (X^2 , 4.19, $P = <0.05$) and was also present in 9 (37%) of 24 patients with iritis compared with 51 (18%) of the 283 controls (X^2 , 6.01, $P = <0.05$). No other HLA-A, -B, or -C antigens were found to an increased extent, and one patient only typed HLA-B27. Estimates of linkage disequilibrium (Δ) on the JRA population data gave a value of 0.090 between HLA-DRw5 and HLA-B12, a value of 0.070 between HLA-DRw5 and HLA-Cw4, and a value of 0.050 between HLA-DRw5 and HLA-Bw35.

HLA-A, -B, and -C haplotypes. Family studies allowed definition of 11 haplotypes carrying HLA-

TABLE I
Frequency of HLA-DRw in Patients with
Early-onset Pauciarticular JRA

HLA-DRw specificity	All patients (n = 45)	With iritis (n = 24)	Without iritis (n = 21)	Controls (n = 84)
	%			
DRw1	13	13	14	21
DRw2	20	21	19	29
DRw3	24	21	29	31
DRw4	16	17	14	25
DRw5	62 \S	71 \S	52 \S	19
DRw6	16	17	8	8*
DRw7	7 \ddagger	0	14	29
DRw8	20 \ddagger	25 \ddagger	14	5
DRw-	24	21	29	26

* 60 controls.

$\ddagger P = <0.01$.

$\S P = <0.001$.

DRw5; in 6 of the haplotypes HLA-B12 was linked to HLA-DRw5 and HLA-Bw35 in three.

ANA. ANA was found in the serum of 15 (63%) of the 24 patients with iritis compared with 6 (28.5%) of 21 without the ocular complication (X^2 , 5.18, $P = <0.05$). 17 (62%) of the 28 patients typed as HLA-DRw5 were ANA positive compared with 4 (24%) of the 17 not so typed (X^2 , 7.03, $P = <0.01$). 12 (70%) of the 17 patients with both iritis and HLA-DRw5 had a positive ANA compared with one of 10 patients without either HLA-DRw5 or iritis (X^2 , 9.26, $P = <0.01$) (Table II).

DISCUSSION

Three antigens, HLA-DRw5, HLA-DRw8, and HLA-Bw35, were found to be increased in frequency in these 45 patients with early-onset pauciarticular JRA. HLA-DRw5, an antigen without other reported rheumatic disease associations, was present in approximately two-thirds of the patient population. The increase in HLA-Bw35 in these 45 patients would appear secondary to that of HLA-DRw5 in view of the raised delta values for the linkage disequilibrium between these two antigens shown in this study. International Workshop studies (13) have shown that Cw4 to be in linkage disequilibrium with HLA-Bw35, so that the increase in delta for HLA-DRw5 and HLA-Cw4 reinforces the view that, in these pauciarticular JRA patients, HLA-DRw5, HLA-Bw35, and HLA-Cw4 form a group of genes in linkage disequilibrium. HLA-B12 was also shown to be in linkage with HLA-DRw5. This antigen, like HLA-Cw4, was present in the patients to a greater extent than the controls but did not prove to be a statistically significant increase. The analysis of haplotypes in the 11 families studied to date would allow

confirmation of the inferences on linkage disequilibrium obtained through study of the patient population; six haplotypes shared linkage between HLA-DRw5 and HLA-B12 and three between HLA-DRw5 and HLA-Bw35.

HLA-DRw5 was present in increased frequency in patients with and without iritis; in 71% of those with iritis and 45% of those without. The difference between the two groups was not significant. When present in JRA patients, ANA is found in children with early onset disease with limited joint involvement. Children, mostly female, with a positive ANA and pauciarticular JRA have substantial risk for iritis, with ANA being regarded in this context as a significant marker for the eye disease (6). The significant association between HLA-DRw5 and ANA in serum, and the high incidence of ANA in those with both iritis and HLA-DRw5 suggests that the HLA-DRw5 association does determine a group at risk not just for pauciarticular JRA but also for those with iritis. Three other groups have recently reported data confirming an association between HLA-DRw5 and pauciarticular-onset JRA (18, 19, 20).

Stastny and Fink (5) have shown an increase in frequency of HLA-DRw8 in their JRA populations, but not of HLA-DRw5, as in this study. The reasons for differences in HLA typing in the two studies on patient populations, with ostensibly the same disease, may include differences in the racial and disease makeup of the patient series. These JRA populations could have some racially determined differences even though both are North American Caucasian. Some regional variation in distribution of HLA is anticipated. Additional differences between these two studies with respect to HLA-DRw5 also derive from selection of the JRA patient population. JRA is presently diagnosed largely by exclusion of other diseases, so that as follow-up continues, alternative diagnoses become evident. The 45 patients in this study were drawn from a group of over 370 patients with JRA followed for at least 5 yr, and the majority for at least twice that period of time (2). In the study by Stastny and Fink (5), a larger group of patients were investigated, 106 in all, but at least four different groups of patients were included, as judged clinically and by the HLA typing results, and duration of follow-up was not given.

There are methodological problems in evaluating the HLA-D locus; these we have tried to minimize by including among our antisera a number of antisera of known quality, defined in the Seventh and Eighth International Histocompatibility Workshops (13, 14). We also took the precaution of excluding sera known to react with the MB or MT antigens, which are believed to be additional specificities expressed on B lymphocytes, each one of which is common to several HLA-DR types (21).

TABLE II
ANA

Population	n	Positive %
Early-onset pauciarticular JRA	45	47
Iritis	24	63*
Noniritis	21	29
HLA-DRw5	28	62†
Non-HLA-DRw5	17	24
HLA-DRw8	9	33
Non-HLA-DRw8	36	50
HLA-DRw5 and iritis	17	71†
Non-HLA-DRw5, noniritis	10	10

* $P < 0.05$ iritis vs. noniritis.

† $P < 0.01$ HLA-DRw5 vs. non-HLA-DRw5.

Other HLA have been associated with different groups of children with JRA; HLA-B27 in a later onset, predominantly male group, who are at risk for the development of ankylosing spondylitis (3, 22, 23) and a more acute form of iritis than is typically found in the patients in the present study. HLA-Dw3 has been found to be increased in patients with polyarticular-onset JRA, whereas HRA-Dw7 has been reported to be increased in patients with systemic-onset JRA (4, 5). A HLA-Dw7 and -Dw11 cross-reacting antigen, Tmo (detected by mixed lymphocyte culture) has been detected in patients with either pauciarticular or polyarticular JRA (5). In this study we were not able to test for HLA-Dw11 and did not find an increase in HLA-B27, HLA-DRw3, or HLA-DRw7, reinforcing the view that the clinical criteria used in this study led to the selection of a relatively homogeneous group of patients.

It is concluded that an additional set of JRA patients, the predominantly female group, with early-onset pauciarticular disease, iritis, and ANA, be added to the other groups of children within a JRA population in which there is evidence of an inherited predisposition. The association with HLA-DRw5 provides an additional measure distinguishing between the groups of patients. The extent to which HLA-DRw5 will have clinical relevance as a predictor for disease along with ANA awaits further study, especially of those with iritis.

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