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HLA, blood groups and secretor status in patients with established rheumatic fever and rheumatic heart disease

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The distribution of HLA-A, -B and -DR antigens as well as blood groups and secretor status was studied in sporadic, North Indian patients of rheumatic fever and rheumatic heart disease. While HLA-Aw33 occurred with an increased frequency in the patient group ($X^2=4.01$), no statistically significant differences were observed in the frequency of B-locus antigens. In the DR locus, HLA-DR3 was found to be significantly increased (50% vs 26.1%, $X^2=13.8$) and DR2 significantly reduced (21.8% vs 47.0%, $X^2=15.6$). Also, there was a preponderance of non-'O' blood group individuals in the patient group as compared to controls. The DR3 association was significant only in those patients of RHD who did not have any previous history of rheumatic fever. These results indicate that susceptibility to rheumatic heart disease is HLA-class II mediated, with HLA-DR3 influencing susceptibility and DR2 conferring protection.

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Recent data indicates that an abnormal immune response in rheumatic fever (RF) and rheumatic heart disease (RHD) may arise from a genetically determined immunopathological response to streptococcal cell wall antigens (Sasazuki et al. 1983). Cheadle (1889) for the first time pointed out that rheumatic fever frequently occurred in more than one member of an affected family. Since then, several studies have recorded an increased familial incidence of this disease suggesting a possible role of heredity in governing susceptibility to RHD. However, neither the mode of inheritance nor the method of its expression have been satisfactorily defined.

Several groups of investigators have looked for a possible association between HLA-class I antigens and RF/RHD (Read & Zabriskie 1981). An increased or decreased incidence of HLA-A or -B antigens has been reported by some investigators (Falk et al. 1973, Caughey et al. 1975, Leirisalo et al. 1977, Joysey et al. 1977), while others failed to find any meaningful association (Matsumori et al. 1979, Haffejee et al. 1982). However, the associated antigen differed among various popu-

lations and in most cases did not reach statistical significance when correction for the number of antigens studied was made.

Recently, considerable interest has been generated by the report of a unique B-cell alloantigen (designated as 883) present in a large majority of patients with RF (Patarroyo et al. 1979). However, no large scale study has so far been conducted to find out an association of HLA-DR antigens in rheumatic heart patients, which could provide information on the involvement of immune response (Ir) genes in governing susceptibility to RHD. The present study was undertaken to investigate the distribution of HLA-A, -B and -DR antigens in a large, sporadic North Indian population. An effort has also been made to find out whether other genetic factors such as ABO and Rhesus blood groups and secretor status are also operative in the disease process.

Material and methods

Patients: The study was conducted on 134 patients of established rheumatic heart disease of both sexes admitted to the cardiology wards of the All-India Institute of Medical Sciences Hospital, New Delhi. All patients were taken from a single ethnic group representing North Indian Hindus, belonging to the states of Punjab, Bihar, Haryana, Uttar Pradesh and Delhi. Out of a total of 134 patients, 78 presented a positive history of rheumatic fever. The age at onset of symptoms of RHD was below 20 years in 87 patients and above 20 years in 47 patients. HLA-DR typing could be performed on 110 patients.

Controls: 400 normal healthy, unrelated individuals matched for age and sex and representing the same ethnic group as that of patients constituted controls for the study. The majority of these individuals belonged to the same socio-economic status as that of the patients. Immediate kin of both patients and controls were specifically excluded from the study. The HLA-DR data was compared with that of 134 healthy controls.

Techniques: HLA-A and -B antigens were determined by the standard two-stage microlymphocytotoxicity technique (Terasaki & McClelland 1964) using a panel of 118 allo-antisera defining 13 specificities of locus A and 15 of locus B with a minimum of 3–4 sera for each specificity. For the HLA-DR analysis, B-cells were isolated on nylon wool columns (Danilovs et al. 1980). A two-step method was used in accordance with the 7th International Histocompatibility Workshop recommendations employing 30 antisera defining specificities DR1 to DRw9.

ABO and Rhesus blood grouping was performed on 112 random patients and 160 controls by the standard agglutination method. The secretor status assignment of 112 patients and 145 healthy controls was done using the inhibition of the agglutination method.

Statistical analysis: Differences between the frequencies of various HLA antigens in patients and control groups were calculated using the standard Chi-square method with Yates' correction. P values were corrected (Pc) by multiplying them by 37, i.e. the total number of HLA antigens studied. Relative risk (RR) for measuring the strength of association with each antigen was calculated by the method of Woolf (Svejgaard et al. 1975).

Results

The frequency distribution of various HLA antigens is presented in Table 1 and 2, respectively. No significant deviation was observed for any of the HLA-A and -B antigens except Aw33 which gave a χ^2 of 4.81, P<0.05. How

Table 1.
Percent phenotype frequency of HLA-A and -B antigens in rheumatic heart disease and healthy controls.

Antigen	Patients (n=134)	Controls (n=400)	X^2	Relative risk
HLA-A1	26.12	27.00	0.007	0.95
A2	23.88	22.50	0.044	1.08
A3	17.91	17.00	0.119	1.06
A9	35.82	27.00	3.362	1.50
A10	8.21	10.50	0.360	0.76
A11	17.91	25.75	2.984	0.63
A28	11.19	15.25	1.040	0.70
Aw19	34.33	33.5	0.004	1.04
A29	0.74	4.25	2.783	1.16
A30	6.71	7.00	0.007	0.96
A31	4.48	4.25	0.017	1.05
A32	13.43	9.75	1.067	1.43
Aw33	8.95	4.00	4.013*	2.36
B5	29.85	29.50	0.000	0.98
B7	6.72	12.75	3.083	0.49
B8	8.20	8.75	0.000	0.93
B12	16.42	17.25	0.007	0.94
B13	16.72	8.00	0.087	0.82
B15	8.20	13.50	2.156	0.57
B16	1.49	2.25	0.033	0.65
B17	20.89	15.00	2.124	1.49
B18	3.73	4.50	0.017	0.82
B21	10.44	6.50	1.723	1.68
Bw22	3.73	5.75	1.074	0.50
B27	6.72	6.00	0.390	0.99
B35	28.36	27.00	0.006	0.99
B37	5.22	4.75	0.000	1.10
B40	20.89	23.00	0.042	0.92

* $P<0.05$, Pc = not significant.

Table 2.
Percent phenotype frequency of HLA-DR antigens in rheumatic heart disease.

Antigen	Patients (n=110)	Controls (n=134)	X^2	P-value	Relative risk
HLA-DR1	21.82(24)	14.17(19)	1.93	NS	1.96
DR2*	21.82(24)	47.02(63)	15.63	<0.0001	0.31
DR3*	50.00(55)	26.12(35)	13.79	<0.0001	2.83
DR4	18.18(20)	26.12(35)	1.75	NS	0.63
DR5	17.27(19)	23.13(31)	0.94	NS	0.69
DRw6	13.63(15)	17.91(24)	0.53	NS	0.74
DR7	25.45(28)	22.38(30)	0.17	NS	1.18
DRw8	1.32(2)	0.74(1)	0.03	NS	2.46
DRw9	2.73(3)	2.78(4)	0.07	NS	0.91

* Corrected P value significant for these antigens. Numbers in parenthesis indicate the number of subjects positive for the antigen.

ever, this did not reach statistical significance when Pc correction was applied. In the DR locus, the frequency of HLA-DR3 antigen was found to be significantly higher in the patient group as compared to controls (50% vs 26.1%, $X^2=13.8$, $P<0.0001$.). A striking finding observed was an appreciable decrease in the antigen frequency of DR2 in patients as compared with controls (22% vs 47%, $X^2=15.6$, $P<0.0001$). No significant differences in the HLA antigen distribution were noted between patients with mitral valve disease and those with combined mitral and aortic disease.

Significant differences were obtained when the data on RHD patients was analyzed with regard to the history of rheumatic fever. In the HLA-class I antigens (Table 3), the increase in Aw33 was confined to patients having a positive history of rheumatic fever. The other two antigens which showed moderate deviation in the total patient group, viz B7 and B17, were also observed with a significantly deviated frequency in the RF + ve group only. Whereas the former showed a decreased frequency in the patient group (3.4% vs 12.7%, $X^2=4.3$), the latter was raised (25.6% vs 15%, $X^2=4.5$). All these antigens, however, did not reach statistical significance when P correction was applied.

The positive association with DR3 was found almost entirely in the group without any history of rheumatic fever, i.e. a frequency of 63.5% as compared to 26% in con-

Table 3.
Percent phenotype frequency of some HLA-A,-B antigens in RHD patients with or without the history of rheumatic fever (RF).

Antigen	Controls (n=400)	RF+ve patients (n=78)	X^2	RF+ve patients (n=56)	X^2
A9	27.0	34.6	1.51	37.5	2.15
A19	33.5	37.2	0.25	32.1	0.00
Aw33	4.0	10.2	4.12*	8.9	1.71
B5	29.5	23.0	1.02	89.3	1.77
B7	12.7	3.5	4.31*	10.7	0.46
B17	15.0	25.6	4.56*	16.0	0.00

* $P<0.05$, P corrected not significant.

Table 4.
Percent phenotype frequency of prominent HLA-DR antigens in RHD patients with or without rheumatic fever (RF).

Antigen	Controls (n=134)	RF+ve patients (n=58)	X^2	RF-ve patients (n=52)	X^2
DR2	47.0	20.7	10.70**	25.0	6.63***
DR3	26.1	37.9	2.16	63.5	20.95*
DR4	26.1	22.4	0.13	17.3	1.16

* $P<0.0001$, ** $P<0.0005$, *** $P<0.01$.

Table 5.
Status of ABO blood groups in RHD patients as compared to controls.

Blood Groups	Patients % phenotype frequency (n=112)	Controls % phenoype frequency (n=160)	X^2	P value
A	19.64(22)	15.62(25)	1.45	NS
B	43.75(49)	31.25(50)	4.23	<0.05
O	25.00(28)	48.75(78)	15.62	<0.0001
AB	11.61(13)	4.38(7)	5.05	<0.05

Numbers in parentheses indicate the number of subjects carrying the allele.

Table 6.
Secretor status in RHD patients as compared to controls.

Secretor	Patients % Phenotype frequency (n=112)	Controls % Phenotype frequency (=145)	X^2	P value
Secretor	81.25(91)	87.59(127)	1.970	NS
Non-Secretor	18.75(21)	12.41(18)	1.970	NS

Numbers in parentheses indicate the number of positive subjects.

trols, $p<0.0001$ and Pc significant (Table 4). The DR2 revealed negative correlation in both the RF+ve as well as RF−ve groups, though more significantly in the former.

The results of the ABO blood groups and secretor status in RHD are shown in Table 5 and 6, respectively. While blood group 'O' is significantly decreased in the patient group (25% versus 48.7% in controls, $X^2=15.62$, $P=0.001$), no deviation in the secretor status is observed.

Discussion

It is known that patients with RF respond by higher titers of streptococcal antibodies than those who have suffered streptococcal infection without any complications. Indeed, RF never develops in individuals who have not responded immunologically to streptococcal infection, i.e. in whom an elevation of antibody titer could not be demonstrated. (Rotta 1983). Although an abnormal immune response following streptococcal infection has been proposed to be a potential cause of RHD (Read & Zabriskie 1981), the exact mechanism of this damage has not been clear. Several investigators have attempted to establish an association of HLA antigens with rheumatic fever and rheumatic heart disease (Read & Zabriskie 1981). However, most of these investigators took into consideration only the HLA-A,-B,-C antigens while ignoring the HLA-D region products.

Recently, Patarroyo and colleagues (1979)

reported that a B-cell alloantigen designated as 883 was present in at least 75% of patients with RHD as compared to 16% in control subjects. While the relationship of this alloantigen with the conventional class II antigens is not clear, the investigators have subsequently identified two monoclonal antibodies, 83S19.23 (identical to 883) and 256S10 which together identified 95% of the patients of RF (personal communication). Furthermore, in a preliminary investigation on 10 RHD patients of Caucasian origin, HLA-DR4 was reported to occur in 8 of them (Ayoub 1984). The present study is the first large scale attempt employing DR-locus antigens in RHD. Although no significant association was observed for class I antigens, the increased occurrence of HLA-DR3 and an appreciable decrease in the frequency of DR2 observed by us indicates that susceptibility to RHD may be HLA-class II mediated. Also, the lack of DR3 associations in RHD subjects with a positive history of rheumatic fever suggests a possible involvement of other genetic factors as well.

The deviation on the class II molecules (HLA-DR antigens) observed in this study is indeed expected in a disease such as this where the primary defect may be due to an abnormality at the antigen presentation level. Recently, a genetically controlled antigen presentation function has been proposed to explain the induction phase of streptococcal induced heart tissue damage (Dos Reis & Barcinski 1980). Following streptococcal infection, the pathogenic bacteria are ingested and processed by the macrophages (antigen presenting cell or APC). Several antigenic determinants may get expressed on the surface of APC for presentation to the immune T-Cell. Out of these, one cardiac-like determinant (rheumatogenic) from group A streptococcus may indeed be crucial for bringing about carditis and rheumatic fever. These investigators have also suggested the possible existence of susceptible and resistant human phenotypes in RHD. The present study provides further evidence in support of this hypothesis.

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