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### Research Article

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# Association of Class II Human Histocompatibility Leukocyte Antigens with Rheumatic Fever

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## Abstract

The association of class I and II HLA antigens with rheumatic fever and its manifestations was examined in 72 patients, including 48 blacks and 24 Caucasians. No significant association was found between class I antigens and rheumatic fever. In contrast, HLA-DR2 and HLA-DR4 phenotypes were encountered in a significantly higher frequency in black and Caucasian patients with rheumatic fever, respectively, compared with the control populations ( $P < 0.005$ ). The most significant association ( $P < 0.005$ ) of these DR antigens with a major manifestation of rheumatic fever was found for mitral insufficiency. In addition, a significant association was encountered between persistent elevation of antibody to the group A streptococcal carbohydrate and HLA-DR4 in Caucasian patients ( $P < 0.04$ ) or HLA-DR2 in the black patients ( $P < 0.001$ ). The frequency of HLA-DR2/4 heterozygotes among patients with rheumatic fever did not differ significantly from controls. These findings support the concept of a genetically determined susceptibility to rheumatic fever and, particularly, to rheumatic heart disease. The association of the clinical manifestations of rheumatic fever and the immune hyperresponsiveness to a streptococcal antigen could be ascribed to a disease-associated immune-response gene which is in linkage disequilibrium with the DR2 and DR4 alleles of HLA-DR locus on chromosome six.

## Introduction

Numerous epidemiological studies indicating a high familial incidence suggest that hereditary factors may determine susceptibility to rheumatic fever (1–3). Early but unconfirmed studies suggested that susceptibility to rheumatic fever followed a simple autosomal recessive pattern of inheritance (4–6), or paralleled the inheritance of ABO blood group or ABO secretor status (7–17). The pathogenesis of rheumatic fever is also thought to involve an aberrant immunological reaction, either humoral or cellular, or both, triggered by an antecedent group A streptococcal infection (18–25). Since cell surface antigens encoded by the genes of the major histocompatibility complex are known to be important in controlling immunological responsiveness, recent genetic studies have examined the association between rheumatic fever and HLA antigens. Initial studies examined the frequency of class I (HLA-A, B) antigens in patients with rheumatic fever, but no consistent association between these antigens and rheumatic fever was found (26–33).

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To test for possible associations between the class II HLA antigens and rheumatic fever, we performed HLA-DR typing of patients with rheumatic fever and normal individuals. A significant association between rheumatic fever and the expression of HLA-DR2 in blacks and HLA-DR4 in Caucasians was found. Furthermore, the expression of these two DR antigens was associated with serological hyperresponsiveness to the group A streptococcal polysaccharide antigen. Our results are consistent with the hypothesis that susceptibility to rheumatic fever is genetically linked and may be associated with an HLA-associated immune hyperresponsiveness to a streptococcal antigen.

## Methods

**Patient selection.** Patients attending the rheumatic fever clinics of the University of Florida Health Center and of the Children's Medical Services were asked to participate in the study. All patients presented initially with an illness which fulfilled the modified Jones criteria for the diagnosis of acute rheumatic fever (34). All of the patients were studied during the chronic phase of their illness, from 1 to 15 yr after initial diagnosis. A total of 72 patients were studied, including 24 Caucasians and 48 blacks. The demographic data and the major manifestations with which these patients presented are outlined in Table I. Cardiac involvement was classified as being persistent or transient, based on the clinical findings at the time of the patient's acute episode and the status of the cardiac findings at the time of enrollment in the study.

Controls for HLA typing consisted of healthy young adults and adult volunteers who resided in the same geographic area. The controls comprised 285 Caucasians and 64 blacks.

Written informed consent was obtained either from the patient or his guardian before enrollment in the study.

**HLA typing.** Typing for HLA was performed as described previously (Rotter, J. I., W. J. Riley, C. M. Vadheim, R. Spillar, and M. C. Mengel, unpublished data presented in part to the American Diabetes Association Annual Scientific Meeting, Las Vegas, June 1984). Heparinized blood was collected and processed for typing of leukocyte antigens within 24 h on all samples. In the majority of cases (80–90%), typing was performed on the day of blood sampling. The microcytotoxicity methods of Amos (35) and Terasaki (36) were used for identification of class I antigens (A, B, C alleles). Class I antisera were obtained from the National Institutes of Health (HLA Typing Bank), local sources, and by serum exchange with Dr. Rene Duquesnoy of the Milwaukee Blood Bank (Milwaukee, WI). The double microfluorescent procedure of van Rood (37) and Terasaki (Terasaki, P. I., 1980, histocompatibility testing, University of California at Los Angeles Tissue Typing Laboratory, unpublished data) were used to detect class II antigens (DR and MT alleles). Approximately 90% of the patients' samples were studied using Terasaki trays to detect B cell or DR alloreactivity. Others were studied using trays with maternally derived alloantisera developed at the University of Florida and antisera kindly provided by Dr. Duquesnoy. Multiple antisera for specificities DR1 through DR8 were available for typing all subjects throughout the study, whereas antisera for specificities DRW9 and DRW10 were available for most, but not all, of the patients and controls. Sera identified as type-specific by the Eighth International Histocompatibility Workshop (Los Angeles, CA, 4–10 February 1980) were included as controls throughout the study. Six patients were studied on two occasions and identical results for the HLA typing were obtained for each patient.

**Streptococcal antibody tests.** Antibody assays for the antistreptolysin

**Table I. Clinical Manifestations of Rheumatic Fever and their Frequency in 24 Caucasian and 48 Black Patients**

Clinical manifestation	Caucasian (24)		Black (48)		Total (72)	
	No.	(%)	No.	(%)	No.	(%)
Arthritis	6	(25.0)	22	(45.8)	28	(38.9)
Sydenham's chorea	9	(37.5)	4	(8.3)	13	(18.1)
Cardiac involvement:						
Mitral insufficiency and/or aortic insufficiency	19	(79.2)	43	(89.6)	62	(86.1)
Mitral insufficiency	19		41		60	
Persistent mitral insufficiency	15		30		45	
Aortic insufficiency	2		12		14	

O, anti-deoxyribonuclease B, and the group A streptococcal carbohydrate (anti-ACHO)<sup>1</sup> were performed as described previously (22). Persistence of the anti-ACHO was defined as presence of elevated levels of this antibody for 5 yr or longer from the date of the acute rheumatic illness (38).

**Statistical analysis.** Relative risk (RR) or the number of times risk of disease is increased or decreased in individuals with a certain HLA marker in comparison to individuals without the marker, was calculated according to the method of Woolf as expanded by Svejgaard (39, 40). When the frequencies were sufficiently large (>10), the classical chi-square statistic (41) was used to analyze contingency tables. Otherwise, an exact test based on the chi-square was used (42). In some instances, the strength of associations (*P* value) was tested by multiplying by the number of antigens at an HLA locus (e.g., DR) (43). The etiologic fraction (EF) or proportions of disease due to the HLA marker associated with the disease was calculated according to the method of Green (44).

## Results

### Patients

Rheumatic heart disease was the most common clinical manifestation in the patients studied, being present in 86% of the study patients. This high frequency of cardiac involvement reflects the predominant clinical feature of the patients that return for follow-up to the rheumatic fever clinics. All but two of the patients with cardiac involvement had mitral insufficiency. 12 patients had both mitral and aortic insufficiency, while two black patients had only aortic insufficiency. 45 of the 60 (75%) patients with mitral valve disease had persistent mitral insufficiency, as defined by clinical evidence for the presence of that valvular lesion, both initially and throughout the course of their follow-up. Of these 45 patients, 40 had had persistent mitral insufficiency for at least 5 yr before the time of the HLA study.

Of the 72 black or Caucasian patients studied, 50 had serial blood samplings during a follow-up period of at least 5 yr beyond their initial episode. Of these 50 patients, 32 patients (23 black and 9 Caucasian) had persistence of the anti-ACHO.

### Association of HLA phenotypes with susceptibility to rheumatic fever and its major clinical manifestations

**Class I antigens.** An initial survey of the frequency of the class I antigens previously reported to be positively associated with rheumatic fever (A3, A8, B5, BW15, BW17, and BW35), did

not reveal significantly increased frequencies of any of these antigens for our patients as a whole group, or when analyzed on the basis of race. Further analysis however, revealed that the class I antigen A23 was present at a significantly higher frequency and A2 at a significantly lower frequency in black patients than in controls (Table II). However, when the strengths of the associations were reduced by a factor dependent on the number of antigens at the two loci, as suggested by Grumet et al. (43), these findings became nonsignificant. HLA-B5 was more frequent in Caucasian patients than in controls, but not significantly so.

**Class II antigens.** A survey of the distribution of the class II (HLA-DR) antigen frequencies yielded an evident association between the DR2 and DR4 antigens and rheumatic fever in the black and Caucasian patients, respectively. The frequencies of the distribution of the class II antigens are shown in Tables III-VI.

In black patients with rheumatic fever the frequency of HLA-DR2 was significantly higher ( $P < 0.001$ ) than its frequency in controls (Tables III and IV), even when the number of DR antigens tested (10) was considered (43). A highly significant positive association ( $P < 0.002$ ) between DR2 was present for black patients with cardiac involvement and, in particular, those with persistent mitral insufficiency. An association of borderline significance was present between HLA-DR2 and arthritis ( $P < 0.05$ , Fisher's Exact Test). Chorea showed a significant ( $P < 0.02$ ) association with DR-1 antigen, and the presence of two major manifestations of rheumatic fever showed an association with DR-1 that is of borderline significance ( $P = 0.05$ ), albeit the actual number of observations was small. A negative association ( $P < 0.05$ ) with the DR5 or DR8 antigens was present in black patients with rheumatic fever and some of its major manifestations.

In Caucasian patients (Tables V and VI), the frequency of the association of HLA-DR4 with rheumatic fever was significant ( $P < 0.002$ ). This association was also found for Caucasian patients with rheumatic carditis and persistent mitral insufficiency ( $P < 0.003$ ), and for patients with two major manifestations of rheumatic fever. The presence of Sydenham's chorea showed a significant association ( $P < 0.04$ ) with the same allele. A significant association ( $P < 0.001$ ) was also found between rheumatic fever and HLA-DR9.

### Association of HLA-DR antigens with persistence of the anti-ACHO

In the 32 patients who had persistence of the anti-ACHO for 5 yr or longer, there was a significant association with HLA-DR2

**Table II. Class I HLA Phenotypes Showing Increased Frequency in Patients with Rheumatic Fever**

HLA	Race	Frequency of phenotype		$\chi^2$	<i>P</i>	RR	EF
		Patients	Controls				
HLA-A2	Black	16/48	38/68	5.63	<0.02	0.39	(-).051
HLA-A23	Black	12/48	6/68	5.2	<0.02	3.44	(+).018
HLA-B5*	Caucasian	3/24	15/366	3.23	0.09	3.34	(+).009

\* HLA-B5 includes B51, B52, and B53. Data expressed in above fashion for comparison with previous studies in literature.

1. Abbreviations used in this paper: Anti-ACHO, antibody to group A streptococcal carbohydrate; EF, etiologic fraction; RR, relative risk.

Table III. Frequency of HLA-DR Alleles in 48 Black Patients with Rheumatic Fever and 64 Black Controls

HLA allele	Controls (64)		All rheumatics (48)		Major manifestation						Two major manifestations (19)		Persistent mitral insufficiency (30)		Persistent anti-ACHO (23)	
	No.	%	No.	%	Arthritis (22)		Chorea (4)		Carditis (43)		No.	%	No.	%	No.	%
					No.	%	No.	%	No.	%						
DR1	10	15.6	11	22.9	6	27.3	3	75.0	10	23.3	7	36.8	6	20.0	6	26.1
DR2	15	23.4	26	54.2	10	45.5	2	50.0	23	53.5	8	42.1	17	56.7	16	69.6
DR3	19	29.7	12	25.0	6	27.3	2	50.0	10	23.3	5	26.3	5	16.7	8	34.8
DR4	5	7.8	7	14.6	4	18.2	0	—	6	14.0	3	15.8	4	13.3	2	8.7
DR5	26	40.6	10	20.8	3	13.6	0	—	10	23.3	3	15.8	8	26.7	2	8.7
DR6	14	21.9	12	25.0	5	22.7	0	—	12	27.9	5	26.3	8	26.7	4	17.4
DR7	13	20.3	5	10.4	2	9.1	0	—	5	11.6	2	10.5	4	13.3	4	17.4
DR8	11	17.2	3	6.3	2	9.1	0	—	2	4.7	1	5.3	2	6.7	1	4.3
DR9	4	6.3	1	2.1	1	4.5	0	—	0	—	0	—	0	—	0	—

Frequencies are also shown for the various clinical manifestations of rheumatic fever (see text). Total number of patients in each category is shown in brackets under the respective heading.

Table IV. Significance of the Frequencies of DR Alleles in Black Patients With Rheumatic Fever and Its Manifestations Compared with Controls Represented in Table III

Clinical manifestation		HLA-allele								
		DR1	DR2	DR3	DR4	DR5	DR6	DR7	DR8	DR9
Rheumatic fever	$\chi^2$	0.95	10.67	0.3	1.28	4.78	0.15	1.93	2.77	1.01
	<i>P</i>		<0.001			<0.03*				
	RR	1.61	3.86	0.79	2.02	0.39	1.19	0.46	0.32	0.32
	EF	0.09	(+)0.40	0.07	0.07	(-)0.33	0.04	0.12	0.13	0.04
Arthritis	$\chi^2$	1.43	3.71	0.05	1.78	4.77	0.007	1.35	0.81	0.09
	<i>P</i>		<0.05			<0.02*				
	RR	2.03	2.72	0.89	2.62	0.23	1.05	0.39	0.48	0.71
	EF	0.14	(+)0.29	0.03	0.11	(-)0.45	0.01	0.14	0.10	0.02
Chorea	$\chi^2$	5.34	1.29	0.69	—	—	—	—	—	—
	<i>P</i>	0.02								
	RR	16.2	3.27	2.37						
	EF	(+)0.7	(+)0.35	0.29						
Carditis	$\chi^2$	0.97	9.7	0.54	1.03	3.4	0.51	1.35	3.3	—
	<i>P</i>		<0.002			<0.05*			<0.05*	
	RR	1.64	3.76	0.72	1.91	0.44	1.38	0.52	0.24	
	EF	0.09	(+)0.39	0.09	0.07	(-)0.29	0.08	0.11	(-)0.15	
Two major manifestations	$\chi^2$	3.82	2.47	0.08	1.03	3.64	0.16	0.9	1.49	—
	<i>P</i>	0.05				<0.04*				
	RR	3.15	2.38	0.85	2.13	0.27	1.28	0.46	0.27	
	EF	(+)0.25	(+)0.24	0.05	0.09	(-)0.42	0.06	0.12	0.14	
Persistent mitral insufficiency	$\chi^2$	0.28	9.46	1.78	0.7	1.7	0.26	0.66	1.76	—
	<i>P</i>		<0.002							
	RR	1.35	4.27	0.47	1.82	0.53	1.30	0.60	0.34	
	EF	0.05	(+)0.43	0.19	0.06	(-)0.24	0.06	0.09	0.13	
Persistent anti-ACHO	$\chi^2$	1.21	13.82	0.20	0.02	6.35	0.21	0.09	2.00	—
	<i>P</i>		<0.001			<0.005*			<0.02*	
	RR	1.91	7.47	1.26	1.12	0.14	0.75	0.83	0.22	
	EF	0.12	(+)0.60	0.07	0.01	(-)0.54	0.06	0.04	(-)0.16	

Statistical analysis calculated as described in Methods.  $\chi^2$ , Chi-square value. *P*, probability level. \* Negative association.

Table V. Frequency of HLA-DR Alleles in 24 Caucasian Patients with Rheumatic Fever and 285 Caucasian Controls

HLA allele	Controls (285)		All rheumatics (24)		Major manifestation						Two major manifestations (10)		Persistent mitral insufficiency (15)		Persistent anti-ACHO (9)	
					Arthritis (6)		Chorea (9)		Carditis (19)							
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
DR1	60	21.1	2	8.3	0	—	0	—	2	10.5	0	—	2	13.3	1	11.0
DR2	83	29.7	8	33.3	1	6.7	5	55.6	5	26.3	3	30.0	2	13.3	2	22.0
DR3	56	19.7	4	16.7	2	33.3	1	11.9	2	10.5	1	10.0	2	13.3	2	22.0
DR4	91	31.9	15	62.5	4	66.7	6	66.7	13	68.4	8	80.0	11	73.3	6	67.0
DR5	55	19.3	6	25.0	3	50.0	1	11.1	6	31.6	4	40.0	4	26.7	2	22.0
DR6	60	21.1	3	12.5	0	—	0	—	3	15.8	0	—	2	13.3	1	11.0
DR7	68	23.9	4	16.7	1	16.7	1	11.1	3	15.8	1	10.0	3	20.0	3	33.0
DR8	20	7.0	1	4.2	0	—	1	11.1	1	5.3	1	10.0	1	6.7	0	—
DR9	3	1.1	4	4.2	1	16.7	0	—	1	5.3	1	10.0	1	6.7	0	—

Frequencies are also shown for the various clinical manifestations of rheumatic fever (see text). Total number of patients in each category is shown in brackets under the respective heading.

Table VI. Significance of Frequencies of DR Alleles in Caucasian Patients with Rheumatic Fever and Its Manifestations Represented in Table V

Clinical manifestations		HLA allele								
		DR1	DR2	DR3	DR4	DR5	DR6	DR7	DR8	DR9
Rheumatic fever	$\chi^2$	2.0	0.19	0.13	8.29	0.45	0.97	0.63	0.28	13.5
	<i>P</i>				0.002					<0.001
	RR	0.34	1.22	0.82	3.55	1.39	0.54	0.64	0.58	18.8
	EF	0.16	0.06	0.04	(+)0.45	0.07	0.11	0.09	0.03	(+)0.16
Arthritis	$\chi^2$	—	0.43	0.66	2.74	2.97	—	0.17	—	5.6
	<i>P</i>									
	RR	—	0.49	2.05	4.26	4.18	—	0.64	—	18.8
	EF	—	0.18	0.17	(+)0.51	0.38	—	0.09	—	0.16
Chorea	$\chi^2$	—	2.65	0.39	4.07	0.37	—	0.74	0.22	—
	<i>P</i>				<0.04					
	RR	—	3.04	0.51	4.26	0.52	—	0.40	1.66	—
	EF	—	0.37	0.11	(+)0.51	0.10	—	0.17	0.004	—
Carditis	$\chi^2$	1.15	0.07	0.92	9.01	1.62	0.3	0.64	0.08	1.96
	<i>P</i>				<0.002					
	RR	0.44	0.87	0.48	4.62	1.93	0.70	0.60	0.74	5.22
	EF	0.13	0.40	0.11	(+)0.54	0.15	0.07	0.11	0.019	0.04
Two major manifestations	$\chi^2$	—	0.004	0.55	7.16	2.39	—	0.95	0.13	3.8
	<i>P</i>				<0.003					
	RR	—	1.04	0.45	8.53	2.79	—	0.36	1.47	10.44
	EF	—	0.01	0.12	(+)0.71	0.26	—	0.18	0.03	0.09
Persistent mitral insufficiency	$\chi^2$	0.5	1.62	0.36	8.76	0.48	0.51	0.11	0.003	2.57
	<i>P</i>				<0.002					
	RR	0.58	0.37	0.63	5.86	1.52	0.58	0.80	0.95	6.71
	EF	0.10	0.22	0.08	(+)0.61	0.09	0.10	0.05	0.01	0.06
Persistent anti-ACHO	$\chi^2$	0.5	0.2	0.04	4.07	0.047	0.50	0.42	—	—
	<i>P</i>				<0.04					
	RR	0.46	0.70	1.17	4.26	1.19	0.47	1.60	—	—
	EF	0.13	0.10	0.03	(+)0.5	0.04	0.13	0.12	—	—

Statistical analysis calculated as described under Methods.  $\chi^2$ , Chi square value. *P*, probability level.

in the black patients ( $P < 0.001$ ) and with HLA-DR4 in the Caucasian patients ( $P < 0.04$ ) (Tables III–VI).

An alternative analysis of the association of DR2 and DR4 with persistence of the anti-ACHO revealed that of the 21 black patients with the DR-2 phenotype, 16 (76%) had persistence of the anti-ACHO, while only 7 of the 14 patients with other DR phenotypes had persistence of this antibody. Six of the nine (67%) Caucasian patients with DR4 phenotype manifested persistence of the anti-ACHO compared with three of six Caucasian patients with other DR phenotypes. Neither of these differences, in this relatively small number of patients, were statistically significant. Similarly, the association of persistence of the anti-ACHO was examined in all 54 patients with either DR2 or DR4 and compared with the 21 patients negative for these phenotypes. Persistence of anti-ACHO was found in 28 of the 54 patients positive for DR2 or DR4, and in 7 of the 21 patients negative for DR2 or DR4. Analysis of these frequencies yielded a chi-square value of 2.04, RR = 2.15, EF of 0.28 with a  $P$  value of 0.15.

*Association of HLA-DR2 and DR4 phenotypes with persistence of the anti-ACHO in patients with or without persistent mitral insufficiency.* The above findings raised the possibility that the observed association between the DR2 or DR4 antigens and persistence of the anti-ACHO may reflect the previously established association of persistence of this antibody with chronic mitral valve disease. To address this question, the frequencies of these alleles were determined separately on patients who had persistent anti-ACHO with or without mitral insufficiency. The results of this analysis (Table VII) revealed that, in black patients, the frequencies of DR2 in the two patient categories did not differ significantly, but that the frequency of this allele in either of the two patient categories was significantly higher ( $P = 0.0004$ – $0.007$ ) than its frequency in the normal control population. The availability of only two Caucasian patients with no mitral insufficiency precluded any conclusions regarding the significance of the frequency of DR4 in these patients.

## Discussion

Previous studies by Falk and co-workers (26) on the association of class I HLA antigens with rheumatic fever, reported a decreased frequency of HLA-A3 in Caucasians with rheumatic

Table VII. Frequency of DR2 and DR4 Alleles in Patients With Persistent Anti-ACHO, With or Without Persistent Mitral Insufficiency

Category	Frequency of allele	$P$ value*
Black patients	DR2	
With mitral insufficiency	10/17	]NS ] = 0.0004
No mitral insufficiency	6/6	
Controls‡	15/64	
Caucasian patients	DR4	
With mitral insufficiency	5/7	]NS ]NS
No mitral insufficiency	1/2	
Controls‡	91/285	

\* Fisher's Exact Test.  $P > 0.05$ .

‡ Refers to normal population in Tables III and V, for which frequency of the allele was determined in this study.

fever. Caughey et al. (27) found increased frequencies of HLA-A3 and HLA-B8 and decreased frequency of HLA-A10 antigens in Maoris, as well as a decreased frequency of HLA-A28 with increased HLA-B17 antigens in Europeans with this disease. Leirisalo et al. (29) reported an increased frequency of HLA-B35 in his initial study for Finnish patients with rheumatic fever, but could not confirm that finding in a subsequent study (45). Murray's study revealed an increase in HLA-B5 phenotype in his patients with rheumatic fever, but this finding proved to be not statistically significant (30). It is notable that Greenberg et al. (23) reported that HLA-B5 (currently designated B51, B52, B53) was associated with an increased response in vitro to streptococcal antigens, while Yoshinoya and Pope (33) found that patients with rheumatic fever who were positive for the HLA-B5 antigen cluster demonstrated a significantly more pronounced immune response as measured by circulating immune complexes.

The results of our studies, however, did not reveal a significant association of the previously reported class I antigens with rheumatic fever in Caucasian or black patients. The frequency of HLA-23 was increased while that of HLA-A2 was decreased in the black patients. These associations become nonsignificant when reduced by the number of antigens tested for (43). Interestingly though, the association of the HLA-B5 cluster with rheumatic fever demonstrated the same trend observed in previous studies (23, 30, 33). The frequency of this antigen group was also higher in our Caucasian patients than in their respective controls, but the differences, as in the preceding studies, did not reach statistical significance.

In contrast to the above, definite and highly significant associations were found between rheumatic fever and the class II HLA-DR antigens. As with previously described associations between HLA-DR antigens and a variety of human diseases, particularly collagen vascular diseases, the association appeared to be race-specific (46). HLA-DR2 and HLA-DR4 were present in a significantly higher frequency in black patients and in Caucasian patients with rheumatic fever, respectively, when compared with race-matched controls. The positive DR9 association with rheumatic fever in Caucasian patients may be more apparent than real, since the control frequencies listed give minimal estimates for this allele. Previously, some early results listed as DR4 may have been confirmed as DR9 on retesting. Nevertheless, the finding of 4 of 24 patients (17%) positive for the DR9 allele is striking, albeit requiring confirmation in an expanded study.

Cardiac involvement, particularly mitral insufficiency, and Sydenham's chorea are considered the most specific of the clinical manifestations of rheumatic fever, while arthritis is the least specific (34). Therefore, it was not unexpected to find a highly significant association of mitral insufficiency with these two DR antigens in black and Caucasian patients, and of Sydenham's chorea in the Caucasian patients. The suggestion of a significant association between HLA-DR1, and the absence of an association with HLA-DR2, in the black patients with chorea should be interpreted with some reservation because of the small number of black patients (four) with this manifestation. The lack of a significant association between arthritis and the DR2 or DR4 alleles in both races reemphasizes the low specificity of this clinical manifestation for rheumatic fever. In addition, the number of Caucasian patients with arthritis was small.

The presence of two major clinical manifestations of rheumatic fever is a basis for the diagnosis of acute rheumatic fever

according to the Jones criteria (34). While the presence of two major manifestations shows a highly significant association with DR4 in Caucasian patients ( $P < 0.008$ ), this was not so for the DR2 antigen in black patients. Although the frequency of HLA-DR2 in these patients was relatively high (42%) and approached that of the patients with carditis (53.5%), the lack of a statistically significant association of the former again reflects the smaller number of patients involved.

Other investigators have examined the association of class II antigens with rheumatic fever. Patarroyo et al. (32) identified an alloantiserum (alloantiserum 883), derived from among 200 human sera surveyed, which reacted with B cells from 71–75% of patients with rheumatic fever. In recent studies, Zabriskie et al. (47) confirmed the above findings using monoclonal antibodies with similar specificity as the 883 alloantiserum. Both the human alloantiserum and the monoclonal antibodies showed no reactivity with cell lines derived from individuals homozygous for the various MLC D-locus alleles (32), or with panels of cells with HLA-A, B, C and DR antigen specificity (47). The fact that the alloantiserum and the monoclonal antibodies did not react with known B cell antigens suggests that they possess specificity for an as yet undefined B cell antigen(s). The relationship of this B cell antigen(s) to the DR alleles remains to be clarified.

Additionally, Patarroyo et al. (32) tested six antisera with known DR specificity (DR1, 2, 3, 5, 4/7, 4/7/10) for reactivity with leukocytes from normal controls, and patients with rheumatic fever from New York and Bogota, Colombia. The frequency of alloantigens detected by these six sera among the patients was not different from their controls. Our finding of the association of DR antigens with rheumatic fever appears to conflict with the results obtained by Patarroyo et al. (32). Possible reasons for the different results include both of the following. The first may relate to differences in the specificity of the DR antisera used. As listed above, only four of the sera used by Patarroyo (32) were monospecific, while the DR4 antisera appear to be heterospecific. The heterologous antigenic reactivity of the DR4 antiserum may have increased the frequency of reactivity with normal controls, thus reducing the significance of the differences between controls and patients. It is of note that in the data presented by Patarroyo et al. (32), the frequencies of DR reactivity in the patients, and the RR values for associations are highest for DR2- and DR4-associated antisera, albeit the differences from controls are far lower than those obtained with the 883 alloantiserum and are not statistically significant. The second possible reason for the observed discrepancy may be related to geographical or racial differences in genetic background. Patarroyo et al. (32) do not present data related to the racial background of the subjects studied. It has been clearly established that DR antigen expression is disparate between races, both in frequencies as well as in patterns of reactivity to alloantisera (27, 30, 46, 48).

Despite the encountered differences, it is important to emphasize the similarity between our results and those reported by Patarroyo et al. (32). Both studies demonstrated a high incidence of expression of a B cell marker among patients with rheumatic fever. Our study extends this finding by demonstrating a race-specific association between certain major clinical manifestations of rheumatic fever and two of the conventional HLA-DR antigens. Furthermore, our study suggests an association between a DR antigen and the immune response of patients with rheumatic fever to a streptococcal antigen.

We have previously demonstrated a relationship between

persistence of mitral insufficiency and persistence of the anti-ACHO (22, 38). This relationship was also evident in the present study. As shown in Tables IV and VI, there was a significant association between HLA-DR2 in black patients and HLA-DR4 in Caucasian patients with persistence of the anti-ACHO. In black patients, this association carried a higher chi-square value than all the major manifestations of rheumatic fever. Because of the inclusion of a high proportion of patients with mitral insufficiency, it was important to ascertain that the observed association between the DR2 and DR4 alleles and persistence of the anti-ACHO was not biased by patient selection. The results of the separate analysis of the data based on the presence or absence of mitral insufficiency, shown in Table VII, supports the association of persistence of the anti-ACHO with the HLA-DR2 genetic marker in the black patients. This observation is of particular importance because of previous studies showing that both the antibody response to the group A streptococcal carbohydrate in laboratory animals and in vitro human lymphocyte reactivity to streptococcal cell wall antigen(s) are under genetic control (49–51). The lack of a significant association when persistence of the antibody to the streptococcal carbohydrate is compared in individuals who are positive for HLA-DR2 or -DR4 with those who are negative for these phenotypes may be due to the small numbers of subjects or to the possibility that the gene controlling the antibody response to this streptococcal antigen is in linkage disequilibrium with the genes of the HLA-DR region.

Although the HLA-DR association with rheumatic fever was not universal for all patients, the finding of an association with HLA-DR2 in blacks or HLA-DR4 in Caucasians could be a useful marker for susceptibility to rheumatic fever and/or predilection to rheumatic heart disease. The frequency of HLA-DR2/4 heterozygotes among patients with rheumatic fever was not different than in controls (7% vs. 5%, respectively; chi-square value = 0.69, R.R. = 1.55). Thus the HLA-DR association we have demonstrated could be due to the presence of a disease-associated immune-response gene which is in linkage disequilibrium with the HLA-DR2 allele in blacks and with the HLA-DR4 allele in Caucasians. The primary susceptibility gene could reside at another D locus, at the complement or class III locus on chromosome six, or at another as yet undefined region. Further studies to examine frequencies of complement allotypes, or of subtypes or supra types of HLA class II alleles by restriction endonuclease fragment length polymorphisms using DR $\alpha$ , DR $\beta$ , DP $\alpha$ , and DP $\beta$  complementary DNA probes could be most revealing in this regard.

Rheumatic fever is the only connective tissue disease in which the inciting factor is known. Preceding infection with the group A streptococcus is clearly required to trigger the acute rheumatic process. However, the precise immunopathogenic mechanisms leading to the clinical manifestations of rheumatic fever are not understood. The finding of a significant association between rheumatic fever and HLA-DR2 in blacks and HLA-DR4 in Caucasians, together with the finding of a high association of these HLA alleles with an immunological hyperresponsiveness to the streptococcal group-specific polysaccharide, are consistent with the hypothesis that rheumatic fever is the consequence of a genetically controlled immunological reaction to prior infection with group A streptococcus. However, it is necessary to emphasize again that our study was biased toward patients with rheumatic heart disease. Due to a decline in the incidence of new patients with acute rheumatic fever seen at our clinic, we were

unable to accumulate an adequate number of patients fulfilling the criteria for acute rheumatic fever who did not have cardiac involvement. Additional studies will be needed to determine whether the strong DR association with rheumatic heart disease and persistence of antibody to the group A streptococcal carbohydrate can be confirmed for other major manifestations of this disease.

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