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HLA class I and class II profiles of patients presenting with Sydenham's chorea

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Abstract Sydenham's chorea (SC) may occur in rheumatic fever (RF) patients without arthritis and carditis. In this study we typed HLA antigens and alleles in patients presenting with the distinct major clinical manifestations of RF, i.e., chorea, carditis, or arthritis, in population and family studies. We evaluated 91 patients with RF for HLA-A, HLA-B, and HLA-DR antigens; of these, 33 had pure chorea, 26 pure carditis, 16 pure arthritis, and 16 carditis plus arthritis. We also typed 24 SC patients and their unaffected siblings for HLA-DRB1 and HLA-DQB1 alleles using molecular methods. HLA-B49 and HLA-DR1 antigens were overrepresented in the total group of patients with RF and in all the subgroups studied, excluding the SC subgroup in which the frequency of HLA-DR1 antigen was not increased. The frequencies of the HLA-DRB1 and HLA-DQB1 alleles in patients with pure chorea were not significantly different from those observed in controls. Similarly, the frequencies of HLA class II alleles in SC patients did not differ significantly from those observed in unaffected siblings. These findings show that immunogenetic susceptibility to RF varies according to the major clinical manifestation presented by the patient.

Key words HLA · Rheumatic fever · Sydenham's chorea · Carditis · Arthritis

Introduction

Sydenham's chorea (SC), also termed rheumatic chorea, represents one of the major clinical manifestations of acute rheumatic fever (RF) and is considered to be the most common form of acquired chorea in childhood [6]. Although the frequency of SC is declining in developed countries, it is increasing in underdeveloped countries [6, 10, 23]. The pathogenesis of SC is not completely understood; however, the participation of antibodies which cross-react with the cytoplasm of subthalamic and caudate nuclei neurons, and the presence of antiphospholipid antibodies have been reported [8, 13]. HLA studies, particularly in rheumatic heart disease, have been conducted on several ethnic groups [2–4, 12, 14, 16–18, 22, 27, 32, 34, 35]; however, the participation of HLA molecules in the pathogenesis of SC has not yet been investigated as a separate form of RF.

It has been suggested that RF is a reactive disorder caused by immune responses against human pathogens which cross-react with self antigens, producing autoimmune disease by the molecular mimicry mechanism [1]. Since cross-reactions between various group A streptococcal antigens and autologous human tissues, including myocardium, myocardial cell membranes, cardiac myosin, heart sarcolemmal membranes, heart valves, cytoplasm components of caudate and subthalamic neurons and articular cartilage structures have been documented [13, 36], it is possible that different HLA molecules may be involved in the presentation of peculiar streptococcal antigens, producing distinct clinical manifestations such as

carditis, chorea, and arthritis. In this study we evaluated HLA antigens in a large group of patients with RF presenting only with chorea, only carditis, only arthritis, or a combination of arthritis and carditis. In addition, we performed a family study of HLA alleles and haplotypes on a selected group of patients who had chorea as the only manifestation of RF.

Patients and methods

Subjects

A total of 91 (31 men, 60 women; aged 10–55 years, median 21) with RF, seen at the University Hospital of the School of Medicine of Ribeirão Preto, São Paulo, Brazil, were typed for HLA antigens. Of these, 33 (9 men, 24 women; aged 11–35 years, median 18) had rheumatic chorea as the only manifestation of the disease, 26 (10 men, 16 women; aged 12–55 years, median 28.5) had only carditis, 16 (8 men, 8 women; aged 10–39 years, median 21) had only arthritis, and 16 (4 men, 12 women; aged 13–51, median 36) had carditis and arthritis. All patients fulfilled the revised Jones criteria [30] for the diagnosis of RF at any time during their follow-up of at least 5 years. Patients with carditis were studied during the chronic phase of their disease, and those presenting with SC or arthritis were regularly submitted to cardiac auscultation, electrocardiography, and echocardiography to ensure that cardiac manifestations were not superimposed. SC was diagnosed on the basis of clinical features of choreic movements, emotional lability, and hypotonia. Other causes of chorea including congenital, hereditary, drug-induced, metabolic, endocrinological, infectious, and autoimmune conditions were excluded on the basis of clinical and laboratory features.

A subgroup of 24 patients (5 men, 19 women, aged 11–35, median 17 years) had only SC retrieved from the major group of 33 patients was also typed for HLA specificities at the molecular level, using the following procedures: restriction fragment length polymorphism (RFLP), sequence-specific primers (SSP), sequence-specific oligonucleotide probes (SSOP), and DNA sequence analysis.

To compare the frequencies of HLA specificities observed in SC patients with those of their siblings, and to confirm HLA haplotypes, we also studied 20 SC patients and 59 unaffected siblings.

As controls, we evaluated 219 normal individuals (100 for serological studies and 119 for molecular studies) from the same geographical area and of similar ethnic background.

The study protocol was performed in accordance with the guidelines of the Ethics Committee of the University Hospital of Ribeirão Preto, and informed consent was obtained from all subjects prior to their inclusion in the study.

Serological analysis

Mononuclear peripheral cells were isolated using a Ficoll-Hypaque gradient density. B-lymphocytes were obtained by adherence to nylon wool. HLA typing was performed by a standard complement-dependent micro-lympho-cytotoxicity assay [33], using a panel of 97 antisera which defined 50 HLA-A, HLA-B and HLA-DR specificities.

RFLP analysis

DNA was prepared from peripheral blood mononuclear cells. Cell lysates were digested with proteinase K followed by phenol-chloroform extraction and ethanol precipitation. Purified DNA was digested with 2–5 U/mg DNA of the restriction endonuclease *Taq*I (BRL, USA) for 3 h, electrophoresed on 1% agarose gel in Tris-acetate, edetic acid buffer for 16 h, and stained with ethidium bromide. After electrophoresis the gels were denatured, neutralized, and dried on Whatman 3MM paper, and soaked in water to remove the backing paper. For HLA-DQB analysis, the dried gels were prehybridized for 2 h at 50 °C in 6 × NET (0.6 M NaCl, 0.18 M Tris, pH 8.0, 6 mM edetic acid), containing 250 μg/ml tRNA, then hybridized 3 h at 50 °C in 6 × NET, 5 × Denhardt's solution, 10% dextran sulfate, 5 mM EDTA, 0.1% dodecyl sulfate, 0.05% NP-40, 250 μg/ml tRNA and 10^7 cpm/ml of the oligonucleotide probe DQβ 1–26 end-labeled to yield 10^9 cpm/μg DNA with γ^{32}P-ATP, using T4 polynucleotide kinase. After hybridization the gels were washed in 6 × standard sodium citrate (SSC) four times: once at room temperature for 10 min and three times for 30 min at 54 °C. Finally, gels were wrapped in plastic and exposed to XAR-5 films for 5–7 days at –70 °C. After HLA-DQB analysis, the old probe was stripped in an 85 °C distilled water wash. For HLA-DRB analysis, the gels were prehybridized in 5 × SSC, 5 × Denhardt's solution, 50 mM $NaPO_4$, pH 6.5, 0.5 mg/ml salmon sperm DNA, and 50% formamide for 16 h at 42 °C, then hybridized in 5 × SSC, 1 × Denhardt's solution, 20 mM $NaPO_4$ pH 6.5, 0.2 mg/ml salmon sperm DNA, 50% formamide, and the cDNA probe was labeled by nick translation with [α^{32}P]dCTP to yield approximately 10^8 cpm/μg DNA, for 16 h at 42 °C. Gels were washed once at room temperature with 2 × SSC containing 0.1% sodium dodecyl sulfate (SDS) and then three times with 0.1% SSC, containing 0.1% SDS, for 30 min at 50 °C. Gels were wrapped in plastic and exposed to XAR-5 films for 1–3 days at –70 °C.

PCR/SSP/SSOP and DNA sequence analysis

DNA from peripheral blood mononuclear cells was extracted using a salting out procedure [20]. HLA-DRB and HLA-DQB generic typing, and HLA-DRB*04 subtyping were carried out by DNA amplification using sets of SSP which were able to identify 21 HLA-DRB, 9 DQB1, and 10 DRB1*04 specificities. The mixture of oligonucleotides and the dNTPs were previously prepared by the Collaborative Transplant Study (Ruprecht-Karls-Universität Heidelberg, Germany) and the procedure used was that described by Olerup and Zetterquist [26]. All polymerase chain reaction (PCR) amplifications were performed in a Perkin-Elmer model 9600 (USA) apparatus with the following cycling profile: 94 °C for 30 s, 55 °C for 1 min, and then 72 °C for 1 min for 35 cycles.

All control and SC patient samples were also tested for intermediate and high resolution HLA-DRB and HLA-DQB typing by SSOP, using a panel of HLA-DRB and HLA-DQB primers as previously described [19, 29]. PCR amplification was carried out by standard methods using 25 pmol of each primer, 12.5 pmol of each dNTP, 1.25 mM $MgCl_2$, 5 μl 10 × Stoffel buffer, and 5 U AmpliTaq DNA polymerase–Stoffel fragment (Perkin-Elmer, USA). HLA-DRB and HLA-DQB PCR conditions included a previous cycle at 94 °C for 4 min, followed by 30 cycles at 94 °C for 30 s, 53 °C for 30 s, 72 °C for 60 s, and a final 10 min extension cycle at 72 °C. Probes end-labeled with 11-dUTP digoxygenin were hybridized to denatured DNA and blotted onto nylon membranes in a chemoluminescent dot blot assay, as previously described [19].

DNA sequence analysis was performed using the chain-termination procedure after gene cloning of PCR amplified/digested DNA [28]. Briefly, PCR amplification was performed by standard procedures using 100 pmol DRB (GH 46 and GH 50) and DQB (GH 28 and GH 29) primers in a total volume of 100 μl, and the following cycling conditions: 96 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, for 30 cycles. The presence of the appropriate amplified DNA product was verified by submarine gel electrophoresis. Amplified DNA was precipitated with 1 vol. 4 M ammonium acetate and 2.5 vol. ethanol on dry ice for 30 min, vacuum dried and resuspended in 50 μl distilled water. Amplified DNA and phage

DNA (M-13; Pharmacia, Sweden) were digested with the restriction endonucleases *Bam*HI (BRL, USA) and *Pst*I (Boehringer-Mannheim, Germany) at 37 °C for 2 h (phage) or overnight (insert). Digested DNA was electrophoresed on a 1.4% low-melting agarose gel containing ethidium bromide, extracted with Tris-HCl-saturated phenol and chloroform, and then precipitated with 3 M ammonium acetate and ethanol. The ligation reaction was performed at the ratio of 1 pmol end of the vector to 3 pmol ends of the insert, using T4 DNA ligase (BRL, USA). Recombinant DNA molecules were introduced into DH5 αF' cells (BRL, USA) and the identification of recombinant colonies was based on phenotypic changes detected with chromogenic substrates. Single-stranded DNA was obtained by centrifugation of the recombinant colonies. PEG 6000 was used to precipitate proteins, and DNA was extracted using phenol-chloroform, then precipitated with ammonium acetatate and ethanol. HLA-DRB and HLA-DQB gene sequencing was performed by the dideoxy method using the Sequenase version 2 kit (United States Biochemical, USA).

Statistical analysis

HLA frequencies observed in patients and controls, and in affected and unaffected siblings were compared using the two-tailed Fisher's exact test, correcting the *P* value (*P*c) according to the number of specificities tested and the number of comparisons performed. Differences were considered significant at *P*c < 0.05. The relative risk (RR), which indicates how many times more often the disease occurs in individuals with the HLA marker compared to those without it, and the etiological fraction (EF), which indicates the attributable risk at the population level, were also calculated [31].

Results

Among the HLA class I antigens, only HLA-B49 was significantly increased in the RF patients as a whole (12%) and in the subsets presenting with chorea (15%), arthritis (19%), or carditis (8%), compared to none of the controls. Although the RR for HLA-B49 antigen was correspondingly high (9.7–26.0), the generally low frequency of this antigen in the patient group yielded a uniformly low EF (0.06–0.18) for all groups studied (Tables 1, 2).

Of the RF patients 55% carried the HLA-DR1 specificity, compared to 26% of controls (*P*c = 0.015). Although statistical significance was lost after correction of the *P* value among the subsets of RF, HLA-DR1 antigen frequency varied in subgroups, being 64% in patients with

Table 1 Frequency of relevant HLA-B and HLA-DR antigens in the total group of patients with RF, and in those presenting the following features: only chorea, only carditis, only arthritis, carditis plus arthritis, and normal individuals. Subgroups encompassing patients with carditis or arthritis or both, and carditis or arthritis are also shown

HLA	Total group (*n* = 91)		Only chorea (*n* = 33)		Only carditis (*n* = 26)		Only arthritis (*n* = 16)		Carditis plus arthritis (*n* = 16)		Carditis or arthritis or both (*n* = 58)		Carditis or arthritis (*n* = 42)		Normal individuals (*n* = 100)	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
B49	11	12	5	15	2	8	3	19	1	6	6	10	5	12	0	0
DR1	46	50	9	27	15	58	10	62	12	75	37	64	25	59	26	26
DR2	30	33	11	33	8	31	6	37	5	31	19	33	14	33	31	31
DR3	39	43	18	54	10	38	6	37	5	31	21	36	16	38	28	28
DR4	22	24	6	18	4	15	5	31	7	43	16	27	9	21	23	23
DR5	23	25	10	30	9	35	2	12	2	12	13	22	11	26	20	20
DR6	3	3	0	0	1	4	1	6	1	6	3	5	2	5	6	6
DR7	6	7	2	6	2	8	2	12	0	0	4	7	4	9	21	21
DR8	4	4	4	12	0	0	0	0	0	0	0	0	0	0	2	2
DR9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2
DR10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2 Uncorrected and corrected *P* value (*P*c), relative risk (RR) and etiological fraction (EF) for serologically defined HLA specificities in patients with RF (total group and other clinically defined subsets)

	Total group (*n* = 91)	Only chorea (*n* = 33)	Only carditis (*n* = 26)	Only arthritis (*n* = 16)	Carditis plus arthritis (*n* = 16)	Carditis or arthritis or both (*n* = 58)	Carditis or arthritis (*n* = 42)
HLA-B49							
P	< 0.001	< 0.001	0.04	0.002	< 0.001	0.002	< 0.001
*P*c	0.01	< 0.001	NS	NS	< 0.001	NS	< 0.001
RR	14.35	19.39	10.2	26.05	9.72	12.44	14.74
EF	0.11	0.14	0.07	0.18	0.06	0.09	0.11
HLA-DR1							
P	< 0.001	0.6	0.002	0.005	< 0.001	< 0.001	< 0.001
*P*c	0.015	NS	NS	NS	NS	0.001	NS
RR	2.91	1.06	3.18	4.74	8.54	5.01	4.18
EF	0.33	0.015	0.39	0.49	0.66	0.51	0.45

Table 3 Frequency (%) of HLA class II (DRB1 and DQB1) specificities in patients with rheumatic chorea and in controls, as defined by sequence-specific oligonucleotide probe analysis or DNA sequencing

	Patients (n = 24)	Controls (n = 119)
HLA-DRB1		
DRB1*0101	20.8	7.2
DRB1*0102	4.2	5.0
DRB1*1501	12.5	16.8
DRB1*1503	12.5	0.0
DRB1*1602	8.3	2.5
DRB1*0301	20.8	19.3
DRB1*0401	8.3	3.4
DRB1*0402	4.2	1.7
DRB1*0404	4.2	5.0
DRB1*0405	4.2	2.5
DRB1*0410	4.2	0.0
DRB1*1101	16.7	11.0
DRB1*1103	4.2	1.7
DRB1*1104	4.2	6.7
DRB1*1301	20.8	13.4
DRB1*1302	4.2	11.8
DRB1*1402	4.2	1.7
DRB1*0701	12.5	14.3
DRB1*0803	8.3	0.0
DRB1*0804	4.2	3.4
DRB1*0807	4.2	3.4
DRB1*0901	4.2	4.2
DRB1*1001	4.2	0.8
HLA-DQB1		
DRB1*0501	33.3	25.2
DRB1*0502	8.3	5.0
DRB1*0602	16.7	29.4
DRB1*0603	20.8	15.1
DRB1*0604	4.2	5.0
DRB1*0607	4.2	0.0
DRB1*0201	29.1	32.8
DRB1*0301	29.1	33.6
DRB1*0302	16.7	15.1
DRB1*0303	8.3	5.0
DRB1*0401	20.8	11.8

carditis or arthritis or both, and even higher (75%) in the carditis plus arthritis subset. Interestingly, only 27% of the patients with SC carried the HLA-DR1 antigen, a value identical to that in controls. With the exception of the SC subset in which the RR was 1.06, the RR conferred by HLA-DR1 antigen ranged from 2.9 to 8.5, yielding distinct values of EF for each subset of the disease. Although the frequency of HLA-DR3 and HLA-DR8 antigens tended to be increased in SC, no other HLA class II specificity was significantly associated with RF as a whole or RF subsets (Tables 1, 2).

The HLA-DR1, HLA-DR3 and HLA-DR8 specificities include several related alleles which can be distinguished by genotyping methods. Since SC patients differed from all other subsets in terms of serology typing, a combination of RFLP, SSP, and SSOP analysis was performed on SC patients, SC unaffected siblings, and controls. The frequencies of HLA-DRB1 and HLA-DQB1 alleles between affected and unaffected siblings, and between SC patients and controls were not significantly different. Group and allele-specific genotyping indicated an increased frequency of DRB1*0803 and DQB1*0401, consistent with serological HLA-DR8 haplotype, relative to controls (Table 3). However, as was also the case for serological studies, after correction for the number of independent determinations, none of the differences between SC patients and controls was significant, and the previous suggestion of an increased HLA-DR3 or HLA-DR8 frequency was not confirmed by genotyping. Interestingly, an unusual DRB1*1602/DQB1*0502 haplotype, confirmed by sequence analysis, was observed in two of the SC patients and in none of the controls.

Discussion

It is reported that a considerable proportion of children in Egypt present primarily with recurrent bouts of chorea without the clinical manifestations of carditis and arthritis [13]. A recent survey of a large group of SC patients in Brazil found that 53% had isolated rheumatic chorea [10]. Based on our experience with all the distinct forms of presentation of RF patients, we addressed some questions regarding the possible role of HLA markers in the suscepti-

Table 4 Associations between RF clinical manifestation and HLA antigens/alleles

Clinical features	HLA antigens/alleles	Population	References
Carditis	HLA-A17, HLA-B18, HLA-DRB1*0701, HLA-DQA1*0201	European, North American whites	4, 17, 11
	HLA-B7, HLA-DQA1*0104, DQB1*05031	Japanese	22, 16
	HLA-DR4, HLA-DRB1*0403	North American whites, Mexicans	2, 3, 9
	HLA-DR7, HLA-DR53	Brazilians, Egyptians	12, 9
	HLA-DR2	North American blacks	3
	HLA-DR3	Indians, Saudi Arabians	14, 27
	HLA-DR1, HLA-DR6	South African blacks	18
Chorea	HLA-DR1, HLA-DR4	North American blacks, whites	3
Whole group[a]	HLA-DR1	Central American blacks	21
	HLA-DR9	North American whites	3

[a] Irrespective of clinical features

bility to SC: Does the immunogenetic susceptibility to SC differ from that observed for other forms of presentation of RF? Is the frequency of HLA specificities in siblings different from that observed in SC probands? Are there susceptibility/protective HLA-DRB1 and HLA-DQB1 alleles or haplotypes associated with SC? To answer these questions we stratified the analysis of HLA markers using several methods for HLA typing at the cellular and molecular levels.

At the cellular level we observed that HLA-B49 antigen was increased in the total group and subgroups of RF patients. Because of the lack of HLA-B49 antigen in controls and the relatively low frequency of this antigen in patients, the calculation of EF for this antigen in the total group and in the subgroups showed values of about 10%, which means that the susceptibility conferred by this antigen at the population level is low. Other HLA class I associations with rheumatic heart disease [4, 17, 22] are shown in Table 4. There is only one study evaluating HLA antigens in a small subgroup (nine white and four black North Americans) of patients with SC in which no association with HLA class I antigens was found [3].

The literature findings regarding HLA class II antigens vary with the population studied and to the form of disease presentation. We found that the frequency of HLA-DR1 antigen was closely similar in patients presenting with carditis, arthritis, or both, suggesting a shared marker for these clinical manifestations, in contrast to SC in which the HLA-DR1 frequency was closely related to that of controls. Similarly, Monplaisir et al. [21] reported that HLA-DR1 is increased in patients with RF from Martinique, regardless of the clinical manifestation. Rheumatic heart disease has been associated with several HLA class II antigens and alleles [2, 3, 9, 11, 14, 16, 18, 27] (Table 4). A previous study by Guilherme et al. [12] reported an increased frequency of the HLA-DR7 antigen in Brazilian patients with RF as a whole from several regions of the country; however, further studies encompassing a larger group of patients have not confirmed such an association [9]. Regarding the HLA class II associations with rheumatic chorea, we have found no reports focusing solely on this manifestation. However, Ayoub et al. [3] studying a large group of RF patients, of whom only nine blacks and four whites presented pure SC, showed increased frequency of HLA-DR1 (blacks) and HLA-DR4 (whites) antigens, but no significant association was observed after correction of *P* values.

To further investigate the association of HLA markers with SC we analyzed the frequency of RFLP-defined HLA specificities in 20 probands and their unaffected siblings. As in population studies, the frequencies of HLA specificities observed in SC patients were closely similar to those observed in unaffected siblings. Although we did not find similar molecular studies on SC patients, a cosegregation serological study conducted on Brazilian families with multiplex cases of RF, irrespective of clinical manifestation, also reported no significant difference in the frequency of HLA class I or class II antigens between patients and family members [5]. Although most of the haplotypes that we identified were usual HLA-DRB1/DQB1 associations, we found an unusual HLA-DRB1*1602/DQB1*0502 haplotype in SC patients which was not seen in controls. This haplotype was observed only in two SC patients; however, we believe that its occurrence may be related to the extensive polymorphism of the Brazilian population more than to a specific marker for SC.

Although serological and RFLP typing suggested an association between SC and HLA-DR3 antigen in this study, the evaluation of HLA-DRB1 and HLA-DQB1 alleles by SSP or SSOP analysis did not confirm it. Serology is a low-resolution method for defining HLA class II specificities because of the dependence on the HLA molecule expression on the cell surface, the unavailability of specific antibodies for certain DRB1 specificities, and the ambiguity in antigen assignment [19]. It appears likely that serology and RFLP did not fully discriminate HLA-DR3 from other antigens, apparently overestimating the frequency of the HLA-DR3 antigen in our patient series. The assignment of HLA-DRB1*03 alleles using high-resolution methods showed closely similar frequency (approximately 20%) in SC patients and controls. Although we did not define any novel HLA class II association with chorea, other studies report different markers for RF. A B cell alloantigen, identified by the monoclonal antibody D8/17, which did not appear to be associated with any known MHC antigen, has been observed in rheumatic heart disease patients and their families [15] and in some patients with SC [7]. A relationship between this B cell antigen and the immunogenetic susceptibility to RF has not been established. A RFLP study conducted on Brazilian patients with RF, most of them with heart disease, reported a significant association of a 13.83-kb fragment upon *Taq*I digestion and hybridization with a cDNA probe for the DRB gene, which was correlated with the HLA-DR53 and HLA-DR16 specificities [35].

Rheumatic fever has not been studied as extensively as insulin-dependent diabetes mellitus (IDDM) or adult rheumatoid arthritis in terms of immunogenetic susceptibility. However, the findings reported for these diseases may help us to understand those reported for RF. IDDM is highly associated with HLA-DQB1*0302 in several white and nonwhite populations [24], and the molecule per se may play a role in selecting the “diabetogenic peptide” which is presented to autoreactive lymphocytes [25]. In contrast, in rheumatoid arthritis a unique stretch of amino acids shared by some HLA molecules known as “shared epitope” is thought to be associated with susceptibility to the disease. It has also been suggested that for IDDM this association lies at the peptide contact site of the HLA class II molecule, whereas for rheumatoid arthritis the shared epitope region of the HLA class II molecule may be involved in direct T-cell–HLA interactions [24]. Re-

garding RF, none of these models of association of HLA molecules and disease appears to be adequate. Rheumatic heart disease is associated with a vast array of HLA class II antigens in patients of distinct ethnic backgrounds. No HLA class II association was found for SC patients; however, a false-negative result cannot be excluded considering the extensive polymorphism of these antigens. Although much remains to be learned about susceptibility to RF, the findings reported here suggest that the immunogenetic susceptibility to RF may differ according to the form of disease presentation.

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