RHEUMATIC FEVER AND HLA ANTIGENS

ANTÍGENOS HLA NA FEBRE REUMÁTICA

Eduardo A Donadi[1]; Paula PC Lamparelli[2]; Lídia Z Figueiredo[2]; Cássia M Paula-Santos[3] & Julio C Voltarelli[1]

[1]Docentes. [2]Monitoras. Divisão de Imunologia Clínica. Departamento de Clínica Médica - Faculdade de Medicina de Ribeirão Preto-USP.
[3]Técnica Especializada da Faculdade de Medicina de Ribeirão Preto-USP.
CORRESPONDENCE: Eduardo A. Donadi. Division of Clinical Immunology. Department of Medicine. Faculty of Medicine of Ribeirão Preto - University of São Paulo. 14049-900 Ribeirão Preto, SP, Brazil. Tel: +55 16 602 2566 - Fax: +55 16 633 6695. E-mail: eadonadi@fmrp.usp.br

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ABSTRACT: In this study we typed HLA class I and II antigens in a series of patients presenting with the distinct major clinical manifestations of rheumatic fever (RF), i.e, chorea, carditis or arthritis. Ninety-one patients with RF were evaluated for HLA-A, -B and -DR antigens. Thirty- three had pure chorea, 26 pure carditis, 16 pure arthritis, and 16 carditis plus arthritis. HLA antigens were typed by a complement-dependent microlymphocytotoxicity assay. HLA-B49 and HLA-DR1 antigens were overrepresented in the total group of patients with RF and in all the subgroups studied, excluding the chorea subgroup in which the frequency of HLA-DR1 antigen was not increased. The results reported here indicate that immunogenetic susceptibility to RF may vary according to the major clinical manifestation presented by the patient.

UNITERMS: HLA Antigens. Rheumatic Fever. Chorea. Myocarditis. Arthritis.

1. INTRODUCTION

It is suggested that rheumatic fever (RF) is a reactive disorder caused by immune responses against streptococcal antigens 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[(2,3)], 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, arthritis and so forth. In this study, we evaluated HLA antigens in a large group of patients with RF presenting only chorea or only carditis or only arthritis or a combination of arthritis and carditis.

2. PATIENTS AND METHODS

2.1. Patients

Ninety-one patients (31 men) aged 10 to 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. Thirty-three patients (24 women) aged 11-35 years (median= 18) presented rheumatic chorea as the sole manifestation of the disease, 26 patients (10 men) aged 12-55 years (median= 28.5) presented only carditis, 16 patients (8 men) aged 10-39 years (median= 21) presented only arthritis, and 16 patients (12 women) aged 13-51 (median= 36) presented carditis and arthritis. Although the patients were studied during the chronic phase of their disease, all of them fulfilled the revised Jones criteria[(4)] for the diagnosis of rheumatic fever at any time during their follow-up of at least 5 years. All patients were regularly submitted to cardiac auscultation,

electrocardiograph and echocardiograph. Chorea was diagnosed on the basis of clinical features of choreic movements, emotional lability and hypotonia, after excluding other causes of chorea.

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

2.2. Controls

We evaluated 100 normal individuals from the same geographical area and presenting a similar ethnic background in relation to patients.

2.3. HLA typing

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 microlymphocytotoxicity assay [5], using a panel of 97 antisera which defined 50 HLA-A, -B and -DR specificities.

2.4. Statistical analysis

HLA frequencies observed in patients and controls were compared using the two-tailed Fisher's Exact test, correcting the p value (pc) according to the number of specificities tested and the number of comparisons performed. Differences were considered significant at $pc < 0.05$. 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 etiologic fraction (EF), which indicates the attributable risk at the population level, were also estimated [6].

3. 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 (9.72 to 26.05), the generally low frequency of this antigen in the patient group yielded a uniformly low EF (0.06 to 0.18) for all groups studied (Tables I and II).

Table I - Frequency of relevant HLA -B and -DR antigens in the total group of patients with rheumatic fever, 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 II - Uncorrected and corrected p value (pc), relative risk (RR) and etiologic fraction (EF) for serologically defined HLA specificities in patients with rheumatic fever (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
Pc	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
Pc	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

Fifty percent of the RF patients carried the HLA-DR1 specificity, compared to 26% of controls (pc = 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 carditis or arthritis or both, and even higher (75%) in the carditis plus arthritis subset. Interestingly, only 27% of the patients with chorea carried the HLA-DR1 antigen, a value which is identical to controls. With the exception of the chorea 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 the chorea subgroup, no other HLA class II specificity was significantly associated with RF as a whole or RF subsets (Tables I and II).

4. DISCUSSION

Regarding HLA class I specificities, only HLA-B49 antigen was increased in the total group and in the subgroups of RF patients studied here. Because of the lack of HLA-B49 antigen in controls and the relatively low frequency of this antigen in patients, the estimation of the 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 studies conducted on Caucasian, Maori, Mexican and Japanese patients with rheumatic heart disease included the class I specificities HLA-A29, -A30, -A31; HLA-A3 and -B8; HLA-B22; HLA-B5; and HLA-B18 and -B35 as susceptibility markers[7/10]. In addition, there is only one study evaluating HLA antigens in a small subgroup (9 Caucasians and 4 North-American Blacks) of patients with chorea in which no association with HLA class I antigens was found [11].

The literature findings regarding HLA class II antigens are heterogeneous according to the population studied and to the form of disease presentation. We showed 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 chorea in which the HLA-DR1 frequency was closely related to that of controls. Similarly, Monplaisir et al.[12] reported that HLA-DR1 antigen is increased in patients with RF from Martinique, regardless of the clinical manifestation. Rheumatic heart disease is associated with HLA-DR1 and HLA-DR6 antigens in South-African Blacks[13], HLA-DR2 antigen in North-American Blacks[11], HLA-DR3 antigen in the Indian population[14], HLA-DR4 antigen in North-American Caucasians[11], and Saudi Arabians[15]. In a recent collaborative study encompassing almost 300 patients with RF, irrespective

of the major clinical manifestations of the disease, showed association with HLA-DR4 antigen in Egyptians, and increased prevalence of HLA-DR4 and -DR7 in Indians and Mexicans[16]. In Brazilians, a previous study by Guilherme *et al*[17] reported an increased frequency of the HLA-DR7 antigen in patients with RF as a whole from several regions of Brazil; however, further studies encompassing a larger group of patients did not confirm such an association [16]. Discrepancies between their results and ours may be related to regional population differences and to the lack of patient selection according to clinical manifestations. Regarding the HLA class II associations with rheumatic chorea, we did not find reports solely focusing on this manifestation; however, Ayoub *et al* [11] studying a large group of RF patients, of whom only 9 Black and 4 Caucasian presented pure chorea, showed no association with any HLA class II antigens after correction of the p values.

Although we did not define any novel HLA class II association with chorea, other studies describe 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, is observed in rheumatic heart disease patients and their families[18], and in some patients with chorea [19]. The relationship between this B cell antigen and the immunogenetic susceptibility to RF has not been established. A study of HLA alleles using restriction fragment length polymorphism conducted on Brazilian patients with RF, most of them presenting 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 -DR16 specificities [20].

Susceptibility to rheumatic fever has not been extensively studied as other autoimmune disorders such as insulin-dependent diabetes mellitus (IDDM) or adult rheumatoid arthritis; however, the findings reported for these diseases may help us understand those reported for rheumatic fever. IDDM is highly associated with HLA-DQB1*0302 in several Caucasian and non-Caucasian populations [21], and the molecule per se may play a role in selecting the "diabetogenic peptide" which is presented to autoreactive lymphocytes [22]. In contrast, in rheumatoid arthritis, a unique stretch of amino acids shared by some HLA molecules known as "shared epitope" is suggested 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 [21]. Regarding 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; however, chorea is not associated with any of these antigens[23]. Although much has 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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RESUMO: Antígenos HLA de classe I (HLA-A e HLA-B) e II (HLA-DR) foram tipificados em um grupo de 91 pacientes com as principais formas de apresentação da febre reumática, ou seja, coréia, cardite ou artrite. Desses pacientes, 33 tinham apenas coréia, 26 apenas cardite, 16 apenas artrite e 16 cardite e artrite. Os antígenos HLA foram tipificados, utilizando-se o teste de microlinfocitotoxicidade dependente de complemento. As freqüências dos antígenos HLA-B49 e HLA-DR1 estavam significantemente aumentadas nos pacientes, quando considerados como um todo, e, ainda, em todos os subgrupos estudados, excetuando-se aquele com coréia, no qual a freqüência do antígeno HLA-DR1 não estava aumentada. Esses resultados indicam que a susceptibilidade imunogenética à febre reumática pode variar de acordo com as manifestações clínicas, apresentadas pelos pacientes.

UNITERMOS: Antígenos HLA. Febre Reumática. Coréia. Miocardite. Artrite.

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