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HLA IN BRAZILIAN ASHKENAZIC JEWS WITH CHRONIC DERMATOPHYTOSIS CAUSED BY *TRICHOPHYTON RUBRUM*

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ABSTRACT

The frequency of HLA (Human Leucocyte Antigens) was analyzed in 25 non-consanguineous Brazilian Ashkenazic Jews, resident in the city of São Paulo, Brazil, suffering from chronic dermatophytosis caused by *T. rubrum*, and in 25 non-infected individuals belonging to the same ethnic group. Statistically significant values ($p<0.05$) were observed for HLA-B14 associated with resistance to chronic dermatophytosis and HLA-DQB1*06 ($p=0.05$) possibly related to susceptibility. These findings suggest that genes on the chromosome 6, in the region of the major histocompatibility complex, may influence the development of chronic dermatophytosis.

Key words: chronic dermatophytosis, *Trichophyton rubrum*, (HLA) human leucocyte antigens

INTRODUCTION

Dermatophytosis is a superficial skin infection and appendage caused by fungi of the genera *Trichophyton*, *Microsporum* and *Epidermophyton*.

The results of dermatophytosis prevalence studies in the population of different countries are of the most heterogeneous possible. However, in the majority of recent reports, *Trichophyton rubrum* and *Trichophyton mentagrophytes* are the more frequent etiologic agents (3,4,10,17,24,26).

According to the latest studies carried out in Brazil, the most isolated species in chronic infections of *tinea ungueum* and *pedis* has been the *T. rubrum* (6,9,16,23). Although this disease is not serious in terms of mortality or physical sequelae, it has significant clinical consequences, mainly regarding aesthetic and chronic aspects of the infection, besides therapeutics difficulties (17). A considerable number of patients fail to respond satisfactorily to instituted medical treatment, thus presenting remissions and relapses (15).

Several factors may be involved in the progression of the chronic form of the disease, from keratinase and mannans production by fungi, to the existence of alterations in the immunological response of the host.

Studies carried out with the purpose of verifying the susceptibility or resistance to chronic infection by *T. rubrum* are still very controversial. In humans, a relationship between the histocompatibility antigens, HLA (Human Leucocyte Antigens) and the incidence of the disease has been reported. HLA is a fundamental component of the immune system, playing an important role in the antigens presentation process to the lymphocytes T, resulting or not in an effective immunological response. HLA can be divided into: a) HLA-class I (A, B and C), virtually expressed in all cells with nucleus and involved in peptide presentation to lymphocytes TCD8+; b) HLA-class II

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(DR, DQ and DP), expressed in antigens presenting cells (APC) and taking part in peptides presentation to the lymphocytes TCD4+; and c) HLA-class III, that codify important molecules for the immune system, as some components of the Complement system and cytokine (21).

In the first study of HLA system and chronic dermatophytosis caused by *T. rubrum*, no association between this infection and histocompatibility antigens (22) was found. But later, high frequency of HLA-A26 and HLA-A33 was observed in patients with chronic dermatophytosis of the foot (1). Moreover, in individuals with onychomycosis, the higher frequency was HLA-DR52, and in the control group it was HLA-DR53, suggesting an important role of these antigens in the modulation of the immune response in chronic dermatophytosis for *T. rubrum* (27).

In this context, the present study analyzed the frequency of HLA in a group of Ashkenazic Jews with chronic dermatophytosis of the foot and nails by *T. rubrum*, directing the study towards a possible association of the disease to histocompatibility antigens.

MATERIALS AND METHODS

Patients

Fifty non consanguineous Brazilian Ashkenazic Jews, living in the city of São Paulo – SP, Brazil were selected, of which 25 were bearers of chronic dermatophytosis in the sole or nails, caused by *T. rubrum*, and 25 were healthy individuals (control group). The afflicted and the control groups were typed for HLA I class (A, B and C) and class II (DRB1* and DQB1*). This study was carried out on Brazilian Ashkenazic Jews of Central European ancestry, who were considered a homogeneous group (2,5) and presented a well-known HLA's profile, important for the preliminary identification of some HLA associate to the disease.

Mycological Procedures

Feet skin scales and nail scrapings were examined directly after preparation with 10% potassium hydroxide (KOH) and dimethyl sulfoxide (DMSO) treatment for visualization of hyphal elements by optical microscopy. For culture, skin scales and nail scrapings were inoculated in a Sabouraud agar medium, supplemented with cycloheximide and chloramphenicol. The isolated fungi were identified by: a) slide culture (microslide) by the Riddell method (19), for observation of the structures characteristic of the fungus; b) Urease test by the Philpot method (18), which was negative; c) nutritional tests for dermatophytes, using 7 medium types (agar-trichophyton 1-7), which was positive for the base medium, containing only casaminic acid, free vitamins and medium containing tiamin; and d) pigmentation test in potato agar, *T. rubrum* produces a red-purple pigment (13).

HLA class I (A, B and C) Typing

Antigens were determined by a standard microlymphocytotoxicity test. Well-characterized antisera obtained commercially were used for HLA typing (Biotest Diagnostics Corporation, U.S.A. and One Lambda, U.S.A.). In brief, 10 mL of whole blood was collected with heparin. The mononuclear cells of the peripheric blood (PBMC) were obtained by density gradient in ficoll-hypaque. The cells were adjusted to $2x10^6$ cells/mL concentration and 1uL of this suspension was added in a Terasaki microplate of 96 wells, containing antibodies for different HLA types. After a 30 minute incubation, rabbit complement was added and incubated for an additional 60 minutes. Afterwards, eosin was added, and finally the reaction was stopped with buffered formaldehyde. After letting it stand for an hour, the reaction was visualized by phase contrast optical microscope. The reactions in the wells with 75% of non-viable cells were considered positive.

HLA class II, DRB1 and DQB1 Typing

The HLA class II typing was performed by DNA-based techniques (high resolution) of specific allele oligonucleotide hybridization using the Inno-LiPA kit (Innogenetics N. V., Belgium). In brief, the DNA was extracted from 5 mL of whole blood using the "Salting out" technique. The DNA was amplified by PCR with biotin-labeled primers for DRB1 and DQB1. The amplified products were hybridized with probes which were immobilized in parallel lines on a nitrocellulose strip, specifically for identification of the different alleles. After hybridization, streptavidin alkaline phosphatase conjugate was added followed by cromogenic substratum. The interpretation was performed by analyzing the hybridization profile with each probe, indicating the HLA genes detected.

Each individual expresses two antigens for *locus*, in other words, the "n" used for the calculations of frequency and percentage of these antigens was 50, indicating the number of alleles observed.

Statistical Analysis

The frequency of antigens and HLA genes was calculated using standard methods and analysis of association by a qui-square test with Yates correction, establishing a significance level in $p<0.05$.

RESULTS

The HLA-A and C frequencies observed in both the patients and the control group showed no significant values (Tables 1 and 3). However, in *locus* B, the HLA-B14 was more frequent in the control group ($p<0.05$) compared to the group of patients, as shown in Table 2. This indicates a possible association of HLA-B14 for resistance to infection and development of the disease by *T. rubrum*.

Table 1. Frequency of HLA-A patients with chronic dermatophytosis and controls in Brazilian Ashkenazic Jews.

HLA-A	Patient		Control		*p*
	n	%	n	%	
A1	8	16.0	4	8.0	NS
A2	8	16.0	7	14.0	NS
A3	7	14.0	4	8.0	NS
A11	5	10.0	1	2.0	NS
A23	0	0.0	1	2.0	NS
A24	5	10.0	2	4.0	NS
A25	1	2.0	3	6.0	NS
A26	5	10.0	7	14.0	NS
A28	2	4.0	5	10.0	NS
A29	1	2.0	6	12.0	NS
A30	2	4.0	1	2.0	NS
A31	1	2.0	3	6.0	NS
A32	0	0.0	1	2.0	NS
A33	0	0.0	1	2.0	NS
A34	0	0.0	1	2.0	NS
A66	1	2.0	0	0.0	NS
Blank	4	8.0	3	6.0	NS
Total	50	100.0	50	100.0	

NS = Not Significant.

Table 2. Frequency of HLA-B patients with chronic dermatophytosis and controls in Brazilian Ashkenazic Jews.

HLA-B	Patient		Control		*p*
	n	%	n	%	
B7	1	2.0	1	2.0	NS
B8	4	8.0	2	4.0	NS
B13	2	4.0	2	4.0	NS
B14	2	4.0	10	20.0	$p<0.05$
B18	0	0.0	3	6.0	NS
B27	1	2.0	2	4.0	NS
B35	15	30.0	9	18.0	NS
B37	1	2.0	1	2.0	NS
B38	6	12.0	7	14.0	NS
B41	1	2.0	2	4.0	NS
B44	2	4.0	2	4.0	NS
B50	0	0.0	2	4.0	NS
B51	2	4.0	0	0.0	NS
B52	3	6.0	0	0.0	NS
B35	0	0.0	1	2.0	NS
B57	1	2.0	1	2.0	NS
B60	1	2.0	2	4.0	NS
B61	0	0.0	1	2.0	NS
Blank	8	16.0	2	4.0	NS
Total	50	100.0	50	100.0	

NS = Not Significant.

Table 3. Frequency of HLA-C patients with chronic dermatophytosis and controls in Brazilian Ashkenazic Jews.

HLA-C	Patient		Control		*p*
	n	%	n	%	
Cw1	0	0.0	2	4.0	NS
Cw2	1	2.0	1	2.0	NS
Cw3	0	0.0	2	4.0	NS
Cw4	13	26.0	9	18.0	NS
Cw5	0	0.0	1	2.0	NS
Cw6	4	8.0	8	16.0	NS
Cw7	9	18.0	10	20.0	NS
Blank	23	46.0	17	34.0	NS
Total	50	100.0	50	100.0	

NS = Not Significant.

Analyzing the frequency of the antigens HLA-class II, no significant value for HLA-DR was observed (Table 4). However, when the percentages of HLA-DQB1*0602, 0603, 0607, 0609 and 06011 in the patients group were totaled, values proximate to the significant (p=0.05) were detected, possibly related to susceptibility (Table 5).

In the typings by serology (HLA class I), relatively high percentages of "blanks" were detected, mainly in the *locus* C, with 46% in the patients and 34% in the controls (Table 3). These blanks may represent a homozygosis or commercial available antisera that were not able to identify the expressed antigen. Blanks went less frequent in typing by molecular biology (HLA class II), and in these cases are probably only reflecting a homozygosis.

DISCUSSION

Chronic infections caused by dermatophytes usually involve anthropophilic fungi, such as *Trichophyton rubrum*. This fungus is capable of invading the corneous stratum and nails. The infections are typically asymptomatic, demonstrating the successful adaptation of this fungus to the human organism (7).

The cellular immunity has an important role for dermatophyte eradication in the skin. Individuals, who have this immunity impaired, frequently develop widespread and recurring fungi infections (8,20).

McGregor *et al.* (14) suggested that the lack of cutaneous inflammation in the infection site by *T. rubrum*, may reflect a local effect of dermatophyte-derived factors and inhibitory to lymphocytes. This would be in contrast to previous hypothesis that patients predisposed to chronic dermatophytosis might exhibit a selective anergy to *T. rubrum* (14).

Table 4. Frequency of HLA-DRB1 patients with chronic dermatophytosis and controls in Brazilian Ashkenazic Jews.

HLA-DRB1	Patient		Control		*p*
	n	%	n	%	
DRB1*0101	1	2.0	2	4.0	NS
DRB1*0102	3	6.0	5	10.0	NS
DRB1*0301	4	8.0	3	6.0	NS
DRB1*03011	1	2.0	0	0.0	NS
DRB1*0401	1	2.0	0	0.0	NS
DRB1*0402	5	10.0	7	14.0	NS
DRB1*0403	3	6.0	0	0.0	NS
DRB1*0404	0	0.0	1	2.0	NS
DRB1*0408	1	2.0	0	0.0	NS
DRB1*0701	5	10.0	12	24.0	NS
DRB1*08031	1	2.0	0	0.0	NS
DRB1*1001	0	0.0	2	4.0	NS
DRB1*1101	7	14.0	5	10.0	NS
DRB1*1104	3	6.0	2	4.0	NS
DRB1*1201	1	2.0	1	2.0	NS
DRB1*1301	4	8.0	1	2.0	NS
DRB1*1302	1	2.0	1	2.0	NS
DRB1*1303	2	4.0	0	0.0	NS
DRB1*1305	1	2.0	1	2.0	NS
DRB1*1306	1	2.0	0	0.0	NS
DRB1*1408	0	0.0	1	2.0	NS
DRB1*1501	1	2.0	1	2.0	NS
DRB1*1502	4	8.0	0	0.0	NS
Blank	0	0.0	5	10.0	NS
Total	50	100.0	50	100.0	

NS = Not Significant.

Table 5. Frequency of HLA-DQB1* patients with chronic dermatophytosis and controls in Brazilian Ashkenazic Jews.

HLA-DQB1	Patient		Control		*p*
	n	%	n	%	
DQB1*0201	9	18.0	13	26.0	NS
DQB1*0301	14	28.0	9	18.0	NS
DQB1*0302	6	12.0	7	14.0	NS
DQB1*03032	1	2.0	1	2.0	NS
DQB1*0304	1	2.0	0	0.0	NS
DQB1*0305	1	2.0	0	0.0	NS
DQB1*0501	5	10.0	8	16.0	NS
DQB1*0502	1	2.0	0	0.0	NS
DQB1*05031	0	0.0	1	2.0	NS
DQB1*0602	1	2.0	1	2.0	NS
DQB1*0603	6	12.0	2	4.0	NS
DQB1*0607	1	2.0	0	0.0	NS
DQB1*0609	1	2.0	1	2.0	NS
DQB1*06011	3	6.0	0	0.0	NS
Blank	0	0.0	7	14.0	NS
Total	50	100.0	50	100.0	

NS = Not Significant.

In this context, antigens derived from *T. rubrum* was described as exhibiting immunological properties in the capacity of inducing immediate or delayed-type hypersensitivity reactions in intradermic tests in different individuals. The immediate response is associated to the chronic recurring infections and the presence of IgE type antibodies in the serum. In contrast, the acute dermatophytosis, with spontaneous resolution, has been associated with delayed-type hypersensitivity response. Thus, this study proposes that the response mediated by cells (Th1), results in resistance to fungi, while the immediate hypersensitivity response (Th2) is not protective. In this same study a peptide synthetic, P5 of the antigen (Tri r2) of *T. rubrum* was observed, which induced strong cellular response. The individuals who responded to this peptide had diverse HLA class II haplotypes, suggesting that P5 is a promiscuous epitope. The identification of peptides that are protective and promiscuous is relevant to the vaccine development for chronic dermatophytosis (25).

Immunogenetic mechanisms also seem to execute an important role in pathogenesis of the mycosis. Studies about associations of HLA and mycosis have been carried out, and some HLA class II alleles, DRB1*11 and DQB1*03 were significant in North American Caucasian patients with mycosis fungoides (12). Similar results were observed in another study with Ashkenazic Jews in which allele specifics of HLA-DRB1*1104 and DQB1*0301 were associated to susceptibility (11). Although association with HLA-class I have not been observed in these more recent studies with mycosis fungoides, in our investigation, the HLA-B14 presented a significant value for resistance to chronic dermatophytosis, in other words, the individuals who express this antigen may be more resistant to the disease. In this case, it is possible that expression of HLA-B14 together with peptides of *T. rubrum* can modulate positively the performance of the immune cells of the host against the fungus.

Regarding HLA class II, in our investigation no allele associate to disease was found. However, gathering all the different subtypes of DQB1*06 (DQB1*0602, 0603, 0607, 0609 and 06011), values close to the significant (p=0.05) for susceptibility was observed. Probably one or more common regions of the molecule of HLA-DQB1*06, but not the molecule in its totality, can bind itself to antigens of *T. rubrum*, modulating negatively the immune response to fungus by T cell, in other words, by presenting fungal peptides that do not induce a protective cellular response (Th1), limiting the action on the fungus, and consequently allowing it to remain in the human organism.

Our results suggest that in this population, the system HLA certainly plays an important role in the immunological response of cells T for fungi antigens, with the HLA-B14 controlling the resistance for chronic dermatophytosis by *T. rubrum* and HLA-DQB1*06, possibly for susceptibility.

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RESUMO

Antígenos Leucocitários Humanos (HLA) em Judeus Ashkenazitas Brasileiros portadores de dermatofitose crônica causada por *Trichophyton rubrum*

A freqüência dos HLA foi analisada em 25 Judeus Ashkenazitas, não consangüíneos, residentes em São Paulo, Brasil, com dermatofitose crônica causada por *T. rubrum* e em 25 indivíduos sadios, pertencentes ao mesmo grupo étnico dos pacientes. Observou-se valor estatisticamente significante ($p<0,05$) para HLA-B14 associado a resistência à dermatofitose crônica enquanto HLA-DQB1*06 ($p=0,05$) possivelmente relacionado a susceptibilidade. Estes achados indicam que o desenvolvimento da dermatofitose crônica pode ser influenciado por genes localizados no cromossomo 6, na região do complexo principal de histocompatibilidade.

Palavras-chave: dermatofitose crônica, *Trichophyton rubrum*, HLA (Antígeno Leucocitário Humano)

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