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IDENTIFICATION OF HLA-DQB1*01 ALLELES IN MITSUDA NEGATIVE LEPROSY PATIENTS

ABSTRACT

The purpose of this study was to identify the gene encoding HLA-DQ1 involved in the absence of cellular immune response in 60 Mitsuda negative leprosy patients (50LL and 10BL). The results showed the presence of HLA-DQB1*0501 in 48.30% of patients, followed by HLA-DQB1*0602 in 31.66%, both subtypes of the phenotype HLA-DQB1*01. Despite the prevalence of these alleles, we can not say that they are responsible for the lack of response to the Mitsuda antigen. We suggest further studies to confirm the results.

Keywords: Leprosy. HLA. Lepromin. *Mycobacterium leprae*.

INTRODUCTION:

Leprosy is manifested in different clinical forms, which express the degree of specific cellular immunity of the host against *Mycobacterium leprae*, the etiologic agent of the disease¹. This variation in the immune response can be assessed by intradermal Mitsuda reaction, a test officially accepted as a criterion for classification and prognosis of disease²⁻⁴.

According to the classification proposed by Ridley & Jopling⁵, tuberculoid (TT) are defined as Mitsuda positive, lepromatous (LL) are Mitsuda negative. Interpolar groups - borderline tuberculoid (BT), borderline-borderline (BB), borderline-lepromatous (BL) - show Mitsuda reactions that vary according to the degree of immunity of the patient⁶.

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After 1982, OMS⁷ established a classification appropriate for multidrugtherapy (MDT), in which the clinical forms were divided into paucibacillary (PB), individuals with up to five skin lesions, and multibacillary (MB), with more than five lesions⁸. For operational purposes, therefore, the PB include TT and BT patients – Mitsuda positive, while MB patients included LL, BB and BL - Mitsuda negative or weakly positive patients.

A positive Mitsuda reaction is usually found in contacts of patients and also in individuals who were never exposed to *M. leprae*^{4,9}.

In order to explain the influence of genetic background on response to Mitsuda antigen, Rotberg in 1937¹⁰ suggested the existence of a natural factor of resistance to leprosy. According to the author, 90% of the population would be resistant to infection by *M. leprae* and only 10% of the individuals would be genetically incapable of mounting an immune response to the bacillus, therefore, they would be permanently Mitsuda negative.

The immunological mechanisms involved in cellular response in Mitsuda negative patients still remain un-

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clear, but studies show that the anergy is specific to *M. leprae*, so the response remains unchanged or slightly reduced compared to other antigens^{11, 12, 13}.

The major histocompatibility complex (HLA), encoded on the short arm of chromosome 6 (6p.21.3), is responsible for macrophagic antigen presentation to T lymphocytes, triggering a specific immune response¹⁴. The HLA is extremely polymorphic, gives a wide variety of responses, which vary from one individual to another, so that the HLA complex exerts a great attraction for study of diseases.

Due to the dichotomy between the host and *M. leprae* immunity, and the variety of immune responses originated, studies have suggested that participation of HLA in the modulation and manifestation of different clinical forms of leprosy and not with susceptibility to infection itself^{15, 16, 17}.

The frequency of HLA antigens and haplotypes varies considerably among different ethnic groups. There are also differences in the frequencies, when considering different populations in the same racial group¹⁸.

In leprosy, the HLA complex began to be studied in an attempt to elucidate the mechanisms of susceptibility or resistance to disease due to its involvement in immune response. Positive associations between TT and HLA-DR2, HLA-DR3 and between LL and HLA-DQ1 - DQB1*05 and DQB1*06 subtypes, have been described in different populations around the world¹⁹⁻²⁴.

A positive Mitsuda reaction indicates the existence of specific cellular immune response to the bacillus, directing the clinical manifestation of the disease for the TT and negative reaction to the LL pole, in the same way, HLA-DR2, HLA-DR3 and allele HLA-DQ1 suggest, respectively, disease prognosis for the TT or LL leprosy forms.

Thus, Souza and cols.²⁵ evaluated the agreement between Mitsuda reaction results, and HLA class II (loci DRB1 * and DQB1*) in different clinical forms of leprosy. The results revealed no association between the Mitsuda reaction, HLA alleles and TT, BB and LL clinical forms, however, when patients were selected according to the response to the Mitsuda test, regardless of clinical form, a significant association was found between the Mitsuda negative response and HLA-DQ1 (p=0.002).

The HLA-DQ1 phenotype consists of the subtypes HLA-DQB1*05 and HLA-DQB1*06 and these are encoded by different alleles. There are, so far, 44 different genotypes described which encode the same phenotype²⁶.

In face of these findings, the purpose of this study was to identify HLA-DQ1 in high resolution and their respective frequencies in the same group of patients in the study mentioned above, by Souza and cols.²⁵, in order to verify the distribution of these alleles in that specific population.

MATERIAL AND METHODS

Patients: The study included 60 Caucasian leprosy patients, unrelated, from the region of Bauru (state of São Paulo). They were classified according to criteria established by OMS⁷ as multibacillary, Mitsuda negative and presenting HLA-DQ1 phenotype, diagnosed at the Dermatology Service, Lauro de Souza Lima Institute (ILSL), Bauru-SP.

Ethical Aspects: All participants were informed about the purpose of the study and only those who agreed to participate signed an informed consent, in accordance with resolution 196 of the National Board of Health. The project was approved by the Scientific Research and Ethics Committee of ILSL, protocol No. 040/2007.

Typing of HLA-DQ1 HLA class II DQB1* locus (DQB1*02:01 / 02:02 / 03:01 / 03:02 / 03:03 / 03:08 / 04:02 / 05:01 / 05 : 02/05: 03/06: 01/06: 02/06: 03 / 6:04 / 6:09) was performed by extracting DNA from peripheral venous blood by the salting out²⁷ technique and the high resolution PCR-SSP using the kit *Micro SSP™ Allele Specific HLA Class II DNA Typing Tray-DQB1* (One-Lambda/USA).

Statistical Analysis: The frequency of alleles was obtained by direct counting. The analysis of the frequencies distribution of genotypes was assessed using the Arlequin software, version 31²⁸.

RESULTS:

The frequency distribution of HLA-DQB1* is described in Table 1.

Table 1 Frequency distribution of alleles in 60 HLA-DQB1*01 positive and Mitsuda negative leprosy patients.

HLA-DQB1*	Number of the antigen	Frequency (%)
02:01	6	10,00
02:02	7	11,67
03:01	16	26,67
03:02	2	3,33
03:03	5	8,33
03:08	1	1,67
04:02	4	6,67
05:01	29	48,33
05:02	9	15,00
05:03	2	3,33
06:01	3	5,00
06:02	19	31,66
06:03	7	11,67
06:04	4	6,67
06:09	6	10,00

Table 2 shows the frequencies of HLA-DQB1*05 and HLA-DQB1*06 alleles. The most frequent allele found

in the sample was HLA-DQB1*05:01 (48.30%), followed by HLA-DQB1*06:02 (31.66%). Both alleles are subtypes of the phenotype HLA-DQB1*01, as expected. The frequency distribution of genotypes is found in Hardy-Weinberg equilibrium.

Table 2 Frequency of HLA-DQB1*05:01 and HLA-DQB1*06:02 in 60 Mitsuda negative leprosy patients.

HLA-DQB1*	Number of the antigen	Frequency (%)
05:01	29	48,33
06:02	19	31,66

DISCUSSION

Considering that similar antigens are recognized by both, sick individuals and individuals who will never become sick, and that there are differences in the response of these individuals to the same antigens, it can be inferred that the antigenic peptides presented by HLA molecule are responsible for the absence of cellular immunity in Mitsuda negative patients²³.

Although the literature describes the association of HLA-DQB1*01 as a factor predisposing individuals to the LL form of the leprosy, Souza and cols. in a previous work observed that this association occurs in MB patients (LL/BL), with Mitsuda negative reaction, suggesting that this association is related to the Mitsuda reaction and not the clinical form itself.

The present study follows the descriptive genetic-epidemiological model. Using this model the genotypes of the allelic group HLA-DQB1*01 (HLA-DQB1*05 and HLA-DQB1*06) were identified in 60 MB leprosy patients with Mitsuda negative reaction.

Currently, five (5) distinct alleles encoding the DQB1*05 (05:01 / 05:02 / 05:03 / 05:04 / 05:05) and thirty-three (39) that encode different alleles DQB1*06 (6:01 to 6:39) are described by the Nomenclature Committee for HLA system factors²⁶. The presently studied sample identified three (3) DQB1*05 (05:01 / 05:02 / 05:03) and five (5) DQB1*06 (06:01 / 06:02 / 06:03 / 06:04/06:09).

Among all alleles identified, the most frequent were HLA-DQB1*5:01 (48.30%) and HLA-DQB1*6:02 (31.66%), as expected, since the sample consisted of HLA-DQB1*01 positive patients (DQB1*05 and DQB1*06).

The distribution of allele frequencies is in equilibrium in the population, as observed by Hardy-Weinberg analysis, behaving similarly to the published data on the Caucasian population of southeastern Brazil²⁹.

Despite the predominance of HLA-DQB1*05:01 and HLA-DQB1*06:02, it can not be proved that they are the alleles responsible for the lack of response to the Mitsuda test and thus directing the manifestation to the multibacillary spectrum of leprosy.

However, the results show that the frequencies of

alleles (genotype) HLA-DQB1*01 were predominant in multibacillary leprosy patients in the geographic region of Bauru, State of São Paulo. Information about HLA profiles in different populations contributes to a better understanding of the natural course of infection and, therefore, they may improve actions for prevention and treatment of leprosy. Therefore, similar studies should be carried out in order to replicate and confirm the present findings.

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