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ARTIGO ORIGINAL

HLA class II alleles as markers of tuberculosis susceptib and resistance

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KEYWORDS
HLA;
Tuberculosis;
Susceptibility;
Resistance;
Healthcare workers

Abstract
Background: Not every individual exposed to *Mycobacterium tubercu* One host genetic factor, involved in modulating the immune response many ethnic groups is the association of human leukocyte antigens (HL tuberculosis (TB).
Objective: To investigate the association between TB, HLA-DRB1 an Portuguese population.
Methods: HLA-DRB1 and HLA-DQB1 gene polymorphisms were analyz

PALAVRAS-CHAVE
HLA;
Tuberculose;
Susceptibilidade;
Resistência;
Profissionais de saúde

O papel do HLA classe II na susceptibilidade/resistência à tuberc

Resumo
Introdução: Nem todos os indivíduos expostos ao *Mycobacterium tube*
Um dos factores genéticos envolvidos na modulação da resposta imu
grupos étnicos é a associação entre moléculas HLA *(human le*
susceptibilidade à tuberculose (TB).
Objectivo: Investigar a relação entre TB e os alelos HLA-DRB1, DQB1 nu
Métodos: Os polimorfismos dos genes HLA-DRB1 e HLA-DQB1 foram a
92 doentes com TB e 82 profissionais de saúde saudáveis, exposto
baciliferos por um período superior a 2 anos (expostos saudáveis: ES). N
tuberculínico foi positivo (TST ≥ 10 mm) em 69 indivíduos (todos com
(ES+) e negativo (TST < 10 mm) em 13 (ES−). Resultados: A frequência
superior no grupo de doentes com tuberculose em relação ao grupo de
Conclusões: Não foi identificado neste estudo, nenhum marcador gené
resistência à doença. No entanto, o alelo HLA-DRB1*14 foi mais fre
tuberculose, sugerindo que possa estar envolvido na evolução da in
activa na nossa população.

Introduction

Infection with *Mycobacterium tuberculosis* (MT) results in a variety of conditions ranging from asymptomatic infection to active tuberculosis (TB) with pulmonary or extrapulmonary involvement. In extreme cases MT infection may be fatal. One third of the World's population is infected with MT;[1] however, only a minority ever develop clinical disease. In 90% of infected individuals, bacilli remain under control in a latent state[2] (latent TB infection).

The various clinical features of TB result from cell-cell interactions that are promoted by cytokines produced by immune cells in response to MT infection. Studies of the diverse consequences of infection in twins[3] and under similar exposure conditions in a familial context[4] suggest the importance of genetics in susceptibility and/or resistance to TB. Several case-control studies have identified associations between TB disease and gene polymorphisms. Among the candidate genes potentially involved in the immune response to TB are the murine natural resistance-associated macrophage protein 1 (NRAMP1) gene,[5] the vitamin D

Some hospitals were once TB sana
years maintained an important tra
treatment. Until recently, there were
prevent against nosocomial transmis
isolation rooms have only become
decade. However, after having w
setting with high TB exposure and wi
during the sanatorium phase, most
uninfected.

The aim of this study was to evalu
with outcome of TB exposure, par
professionals heavily exposed to T
on the importance of HLA-DRB1 an

Methods

Subjects

All individuals were vaccinated with b
(BCG) at birth, according to the nat

measures against nosocomial TB) for more than two years prior to 2000 were recruited (healthy exposed - HE). All were asymptomatic and presented normal pulmonary radiographies. Based on the results of tuberculin skin test (TST), 69 were found positive (HE+) and thirteen negative (HE−). HE+ positive results ranged from 15 to 20 mm.

Inclusion and exclusion criteria

Individuals belonging to any high-risk groups (e.g., drug users, sex workers, homeless) and persons with co-morbidities that would interfere with TST results or increased risk for TB, such as those with immunosuppressive disease (e.g., HIV infection, rheumatoid arthritis or cancer) or taking any immunosuppressive drug (e.g. corticosteroid) were excluded from the study.

Tuberculin skin test

Trained nursing staff from CDC administered TSTs (2-TU dose of purified protein derivative RT23) according to the guidelines established by the Portuguese Pneumologic Society (SPP).[25] Briefly, 0.1 ml of the tuberculin purified protein derivative containing five tuberculin units was injected intradermally on the volar surface of individuals' forearms. After 72 h, the diameter of the indurated area surrounding the injection site was measured and reported in millimeters. We considered 10 mm as the positive cut-off value.

HLA genotyping

Peripheral blood samples (10 ml) were collected in EDTA tubes. Genomic DNA was obtained from Proteinase-K treated peripheral blood leukocytes using a salting-out procedure.[26]

DNA was amplified by polymerase chain reaction (PCR) using sequence-specific primers (PCR-SSP) for HLA-DRB1 and HLA-DQB1 genes, based on methods previously described.[27]

PCR products were electrophoresed on 1.5% agarose gels containing ethidium bromide, and visualized under ultraviolet light.

Statistical analysis

HLA-DRB1 and HLA-DQB1 phenotypic frequencies were determined by direct count. Comparisons of HLA frequencies between patients and controls were performed using the Pearson χ^2 Test with continuity correction or the Fisher's

Table 1 Characteristics of TB patien individuals and healthy controls

Parameter	TB patients (n = 92)
Age	
Mean ± SD (years)	45,47 ± 15,40
Range	21-82
Gender	
Female	33 (36%)
Male	59 (64%)
Positive TST	92 (100%)

TB patients and the HE group (data no group was stratified for the presence we found that the HLA-DRB1*14 all HE+ group (0/6 cases) (7% vs. 0; p =

HLA-DQB1 alleles

There were no statistically signific frequencies of the distribution of H patients and the HE group.

The allele frequencies for normal h were not substantially different from same region.[28]

Discussion

TB pathogenesis is very complex influencing disease development outcome of infection. MT usually the respiratory route. Phagocyto macrophages is the first event i interaction and may determine the

Table 2 HLA-DRB1* alleles phenotyp patients and HE+ individuals

Alleles HLA-DRB1*	TB patients (n = 92)	HE+ (n = 6

Within 2 to 6 weeks of infection, cell-mediated immunity is developed and an influx of lymphocytes and activated macrophages appears in the lesion, resulting in granuloma formation. The bacilli are contained in the granuloma, where they may remain forever (latent TB infection), or become re-activated at a later date to proliferate and ultimately evolve to an active disease state.

Not all individuals exposed to MT become infected. Of those who do become infected, the course and duration of progression to active disease are highly variable. This lack of uniformity in disease manifestation among infected individuals may reflect a complex interaction between genetic and environmental factors. The relative weight of some risk factors, such as AIDS, diabetes, family income and nutritional status, are known, but host genetic factors may also influence susceptibility to infection and disease pathogenesis. Despite the compelling rationale for the involvement of host genetic influences, evidence for a genetic basis for TB susceptibility has been difficult to establish.

Some candidate genes have been reported,[15-18] and a role for HLA, which is important in the immune response, has been recognized. In particular, a number of studies have reported an association between HLA Class II alleles and TB, but these associations are not consistent across different populations[15-18] and the results remain controversial. A study by Ruggiero et al showed an increase in HLA-DR4 frequency in an Italian population.[29] A significant increase in the frequency of HLA-DRB1*14 has been reported in Iranian patients,[30] an association that was confirmed by Matrashkin et al in a Russian population.[31] A case-control study published by Dubaniewicz et al reported a strong association of HLA-DRB1*16 with TB in a Polish population.[32] More recently, this group confirmed and extended these results using a "high resolution" method, showing a high frequency of the HLA-DRB1*1601 allele[33] and a low frequency of HLA-DQB1*0201 in Polish TB patients; this latter result suggests that the HLA-DQB1*0201 allele may be linked to TB resistance. A high frequency of the HLA-DQB1*0501 allele has been reported among North American Indian[34] and Mexican[35] TB patients, whereas the frequency of the HLA-DQB1*0502 was increased among TB patients in a Thai population.[16] In a report from Cambodia, the HLA-DQB1*0503 allele was found to be associated with TB whereas the HLA-DQB1*0501 allele was not.[15] These differences are likely attributable to the differing ethnic backgrounds of the studied populations, but it should be

There are two tests used in clini
individuals with latent TB. These a
test, and the interferon-gamma r
which identify a memory of an ada
against mycobacterial antigens. At t
run, IGRAs were not widely availab
could not use it.

The sensitivity of the tuberculin s
in individuals with immunosuppres
treatment, but those were not stu
of TST cutpoint influences the pro
TST reaction is a true positive d
versus a false positive, often due t
infection with nontuberculous m
BCG vaccination. But on the oth
TST reactions are generally in the
All exposed group who tested posi
15 mm which is very likely MT- induc

We found that the HLA-DRB1*14 al
among TB patients compared to HE-
HLA-DRB1*14 could be a susceptibili
Although the difference is relatively
with previous reports from Iran and
was found to be increased among TB
of these observations will require fu
groups of patients and healthy, TB-e

Conflict of interest

Authors state that they don't have a

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