SHORT PAPER

Analysis of DQB1 allele frequencies in pulmonary tuberculosis: preliminary report

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Thorax 2003;**58**:890–891

Revised version received 8 January 2003
Accepted for publication 10 June 2003

Background: The human leucocyte antigen (HLA) system plays an important role in the modulation of the immune response. An association between HLA and pulmonary tuberculosis (TB) has been examined in several populations but the results have been inconsistent. The aim of this study was to evaluate the correlation of DQB1 alleles with TB patients and healthy controls in the same ethnic group in Poland.
Method: The DQB1 alleles of 38 patients with TB and 58 healthy university staff volunteers were determined by a PCR-SSP low resolution method.
Results: The DQB1*05 allele occurred more frequently (p adjusted for multiple comparison = 0.002, OR = 2.84, 95% CI 1.57 to 5.15) and the DQB1*02 allele occurred less frequently (p = 0.01, OR = 0.39, 95% CI 0.21 to 0.71) in patients with TB than in controls. The occurrence of DQB1*03,*04,*06 alleles was similar in the two populations.
Conclusions: The occurrence of specific DQB1 alleles may be linked to susceptibility/resistance to tuberculosis.

Tuberculosis (TB) is still an important world health problem, and it is estimated that about one third of the earth's population has been infected with *Mycobacterium tuberculosis*. Each year there are ~2 million deaths from TB. It is still not clear why only approximately one in 10 of those infected progress to active disease during their lifetime when only a minority have a risk factor.[1]

Tuberculosis develops by a complex of environmental factors and genetic susceptibility. The observations of the familial occurrence of TB and the description of the disease in monozygotic twins suggest that genetic elements might contribute to determining the course of the infection. It has recently been reported that mutations in genes encoding natural resistance associated macrophage protein 1 (NRAMP-1), interferon-γ receptor, or one component of the interleukin-12 receptor might affect the susceptibility to TB.[1]

The main stages of cell reactivity—presentation of antigen, phagocytosis, cooperation with T and B lymphocytes, or bactericidal activity—depend on the specificity of the HLA system.[1] Since the DR alleles play an important role in the modulation of the immune response, a possible association between DQB alleles and TB has been examined in Asian and American ethnic groups, but the results have been inconsistent.[2–7] Despite the rising incidence of TB in Europe, no such analysis has yet been carried out in European white populations. A study was therefore undertaken to evaluate the occurrence of DQB1 alleles in TB patients and healthy controls in the same ethnic group in Poland.

METHODS

Patients

With the approval of the Independent Bioethics Committee for Scientific Researches, Medical University of Gdańsk, Poland, 38 unrelated patients (23 men) of mean age 40 years (range 22–68) with newly detected active pulmonary TB were studied at the Pulmonological Hospital, Sopot between July and December 2002. The diagnosis of TB was confirmed in all patients by the presence of acid-fast bacilli in sputum smears and by positive sputum culture of *M tuberculosis* strains. Patients were classified according to clinical stage and appearance on the initial chest radiograph (infiltrates with cavitation in one or two lung zones). A positive PPD skin test was an additional diagnostic criterion. Patients who did not respond to first line treatment (rifampin, isoniazid, ethambutol, pyrazinamide) were excluded from the study.

Controls

Fifty eight unrelated individuals (32 men) of mean age 42 years (range 27–60) without clinical, physical, or laboratory evidence of TB formed the control group. A negative PPD skin test was used to confirm that they did not have TB.

Patients and controls were excluded from the study if they had a family history of TB or other related diseases. Those of different socioeconomic status and ethnic background were also excluded from the study. The Polish population is a relatively homogenous white ethnic group. All patients and controls had been vaccinated with BCG (bacillus Calmette-Guerin).

HLA typing

Genomic DNA was extracted from 10 ml peripheral blood from each individual using the salt extraction method. DQB1 typing was performed using sequence specific amplification polymerase chain reaction with sequence specific primer (PCR-SSP) according to the method described by Olerup and coworkers.[8] The DQB1 primers were supplied by DYNAL in the DYNAL DQ ''low resolution'' SSP standard kit.

Analysis of data

Data were analysed with STATISTICA for Windows Version 6.0 (StatSoft Inc,USA). Group comparisons were made using the χ^2 test after the Bonferroni correction ($p \leqslant 0.05$). The odds ratio (OR) was calculated with 95% confidence intervals (CI).

RESULTS

A comparison of the frequencies of the DQB1 alleles in the patients with TB and in the control population is shown in table 1.

The results indicate that the DQB1*05 allele was detected more frequently in patients with TB than in control subjects

Table 1 Frequency of DQB1 alleles in patients with pulmonary tuberculosis (TB) and controls

DQB1 alleles	No (%) allele positive individuals: Controls (n = 58)	TB patients (n = 38)	Odds ratio (95% CI)
DQB1*02†	27 (47%)	10 (26%)	0.39 (0.21 to 0.71)
DQB1*03	39 (67%)	28 (73%)	1.33 (0.72 to 2.44)
DQB1*04	0	2 (3%)	0
DQB1*05‡	15 (26%)	19 (50%)	2.84 (1.57 to 5.15)
DQB1*06	28 (48%)	18 (47%)	0.96 (0.55 to 1.67)

†p = 0.002, pc (p adjusted for multiple comparison) = 0.01; ‡p = 0.0005, pc = 0.002.

(50% *v* 26%; $\chi^2 = 12.12$; p = 0.0005; pc = 0.002). The DQB1*02 allele occurred less frequently in patients with TB than in healthy controls (26% *v* 47%; $\chi^2 = 9.51$; p = 0.002; pc = 0.01). There was no difference between the two groups in the frequency of the remaining alleles (DQB1*03, DQB1*04, and DQB1*06).

DISCUSSION

There have been few reports of the correlation between the frequency of DQB1 alleles and the susceptibility to pulmonary TB.[2–7] A significant association was found between the occurrence of DQB1*0501, *0502 and *0601 alleles and TB in subjects from North India.[2] In South India the results of studies on the association of DQB1 alleles with the development of clinical TB have been inconsistent[3 4]; Ravikumar *et al*[3] found a higher frequency of DQB1*0601 in those with TB while Sanjeevi *et al*[4] found no correlation between TB and DQB1 alleles. In Mexican patients with TB a significant positive association with DQB1*0501 was reported by Teran-Escandon *et al*,[5] whereas in a Thai population DQB1* 0502 was found more frequently in subjects with TB.[6] In Cambodia an association was found between TB and DQB1*0503 but not with DQB1*0501 or DQB1*0601.[7] A negative association between the presence of *M tuberculosis* and DQB1*0301 in Thai subjects and DQB1*0402 in Mexicans was noted.[5 6]

The finding in our study of a high frequency of DQB1*05 in patients with TB is in agreement with that of previous studies in many populations.[2 5–7] However, we were not able to confirm a positive association with DQB1*06 or a negative association with DQB1*03 or DQB1*04 alleles as has been reported in other populations.[2 3 5 6] There have been no reports on the frequency of the DQB1*02 allele in TB.

The discrepancies in the results may be caused by different methods and/or the high degree of polymorphism of the DQB1 allele in different ethnic groups, in which significant geographical variations have been observed. Stern *et al*[9] showed that the DQB1*0503 allele encodes a change in the amino acid position 57 of the β chain which influences the charge in the putative peptide binding pocket (P9) of the DQ molecule. The negatively charged P9 binding pocket may bind TB antigens less effectively or elicit a diminished immunogenic response.[9] The findings of Goldfeld *et al*[7] support the evidence for an association between a specific DQB1*0503 allele and progressive clinical TB. Geluk *et al*[10] recently identified three new DQA1*0301/DQB1*0302 restricted T cell epitopes of mycobacterial heat shock protein 65 which mounted an efficient response to *M tuberculosis*. Epidemiological and experimental studies suggest that the high degree of molecular diversity in HLA molecules influences the variability in the human response to *M tuberculosis*.

ACKNOWLEDGEMENTS

This study was funded by the Polish State Committee for Scientific Researches grant no. 3PO5B 15522.

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