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Analysis of DC-SIGN (*CD209*) Functional Variants in Patients with Tuberculosis

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ABSTRACT: Several lines of evidence suggest that host genetic factors controlling the immune response influence infection by *Mycobacterium tuberculosis*. Recently, DC-SIGN has been shown to be the major *M. tuberculosis* receptor on dendritic cells (DCs). The aim of this study was to investigate the influence of *DC-SIGN* functional polymorphisms -336G/A SNP in the promoter region and insertion/deletion in the "neck" region on the predisposition to tuberculosis. We performed an association study in 110 HIV-negative tuberculosis patients and 299 matched controls. In addition, a total of 155 healthy controls were screened for the tuberculin skin test (TST). *DC-SIGN* −336 SNP detection was performed by the real-time polymerase chain reaction technology, using the TaqMan 5′ allele. The insertion/deletion in the "neck" region was analyzed by polymerase chain reaction with specific primers. Although an increased frequency of the G allele in tuberculosis patients (23%), as compared with controls (19%), was observed, differences were not statistically significant (OR = 1.31, 95% CI = 0.89-1.94, $P = 0.14$). On the other hand, *DC-SIGN* repeat polymorphism in the "neck" region had a very low frequency in the analyzed population. We conclude that the studied polymorphisms are not relevant risk factors for developing tuberculosis in Northwestern Colombian individuals. *Human Immunology 67, 808–811 (2006).* © American Society for Histocompatibility and Immunogenetics, 2006. Published by Elsevier Inc.

KEYWORDS: DC-SIGN; *CD209*; tuberculosis; tuberculin skin test; Colombia

ABBREVIATIONS

DC-SIGN	DC-specific intercellular adhesion molecule–3 grabbing nonintegrin
DC	dendritic cell
PRR	pathogen-recognition receptor
TLR	Toll-like receptor
ICAM-3	intercellular adhesion molecule–3
HIV	human immunodeficiency virus
SNP	single nucleotide polymorphism
TST	tuberculin skin test
PPD	purified protein derivate

INTRODUCTION

Natural immune mechanisms such as macrophages, dendritic cells (DCs), natural killer cells, and neutrophils probably have an important role in the primary response to *Mycobacterium tuberculosis*, one of the most common causes of morbidity and mortality in humans [1]. Although the function of DC as an antigen-presenting cell is pivotal for the initiation of the immune response against pathogens, certain pathogens have evolved to subvert DC functions so that they can survive and infect the host. One of these pathogens is *M. tuberculosis.* DCs express a repertoire of pathogen-recognition receptors (PRRs), including Toll-like receptors (TLRs) and C-type lectins that can recognize molecular patterns expressed by pathogens. Specifically, myeloid DCs express the C-type lectin DC-specific intercellular adhesion molecule-3 grabbing nonintegrin (*DC-SIGN*), encoded by the *CD209* gene [2]. *DC-SIGN* is organized into three domains: an N-terminal cytoplasmic region, a neck region containing 8 repeats of a 23-aa sequence, and a C-terminal C-type lectin domain [3]. In addition, *DC-SIGN* has been found to recognize a large range of pathogens, including *Ebola virus*, [4] *Leishmania amastigotes* [5], *Aspergillus fumigatus*, [6] and *M. tuberculosis* [7].

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The *CD209* gene is located on chromosome 19p13.2-3 and is highly polymorphic. Numerous single nucleotide polymorphisms (SNPs) have been reported [8, 9]. One of these SNPs represents a Guanine (G) to Adenine (A) transition at position −336 within the *CD209* gene promoter. This site affects predicted multiple transcription factor binding sites for Sp1/GATA1/CACCC and CAC-binding transcription factors. The *DC-SIGN* -336 SNP has been associated with increased risk for parenteral acquisition of HIV-1 infection [10] and severity of dengue disease [9]. Other genetic polymorphisms also exit affecting the number of repeats within the neck region of *DC-SIGN* [10]. Given the functional relevance of *DC-SIGN* in *M. tuberculosis* binding by DCs, we hypothesized that polymorphisms within the gene promoter region and in the "neck" domain could be a risk factor for developing tuberculosis.

SUBJECTS AND METHODS

Subjects

Ninety-four women and 16 men with tuberculosis and a mean age of 40 ± 15 years were included in the study at the time of treatment for their disease. Tuberculosis was diagnosed by the presence of alcohol acid-resistant bacilli in sputum or by isolation of *M. tuberculosis* in culture. In all cases, patients with tuberculosis were negative for HIV 1/2 infection (Axsym assays, Abbott Laboratories, Chicago, USA). The patients formed part of a project on immunogenetics of tuberculosis in the Corporacion para Investigaciones Biologicas, Medellin, Colombia [11]. Controls included 299 individuals without inflammatory autoimmune disease or history of chronic infectious disease, including tuberculosis and HIV infection. They were matched to patients by sex, ethnicity, and socioeconomic status and were unrelated to the patients. The local ethics committee approved the study.

Tuberculin Skin Test

All control individuals were tested to assess the delayed-type hypersensitivity (DTH) skin test response to 0.1 ml (5TU) of the purified protein derivate (PPD) antigen (Tuberculin PPD powder master lot #154616, Public Health Service, National Center for Disease Control, USA) injected intradermally in the forearm following the Mantoux method [12]. The skin test response was measured as the diameter of induration 48 to 72 hours after injection. We classified these subjects into those who were naturally infected with *M. tuberculosis* (*i.e.*, diameter of induration ≥10 mm) and those who were uninfected at the time of DTH testing (diameter of induration >10 mm); 74 subjects (sim;48%) were considered to be naturally infected while 81 (∼52%) were considered non-infected, tuberculin skin test (TST) negative. The mean age of these individuals was 45 ± 16 years for TST– healthy controls and 47 ± 15 years for TST^+ healthy controls. All were negative for tuberculosis disease.

DC-SIGN -336 Genotyping

Genomic DNA was isolated from 10 ml of ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood sample using the standard salting-out technique. Genotyping for *DC-SIGN* -336 G/A was carried out using a Custom TaqMan SNP Genotyping Assays method (Applied Biosystems, Foster City, CA, USA). The primer sequences were 5′-GGACAGTGCTTCCAGGAACT-3′ (sense) and 5′-TGTGTTACACCCCCTCCACTAG-3′ (antisense). The Taqman minor groove binder probe sequences were 5′-TACCTGCCTACCCTTG-3′, and 5′-CTGCCCACCCTTG-3′. The probes were labeled with the fluorescent dyes VIC and fluorescein-Aminohexyl Amidite (FAM), respectively. The polymerase chain reaction (PCR) was carried out in total volume of 12.5 μl using the following amplification protocol: denaturation at 95°C for 10 minutes, followed by 40 cycles of denaturation at 92°C for 15 seconds, followed by annealing and extension at 60°C for 1 minute. After the PCR, the genotype of each sample was attributed by measuring the allele-specific fluorescence in the ABI Prism 7000 Sequence Detection System, using SDS 1.1 software for allele discrimination (Applied Biosystems).

Genotyping of Insertion/Deletion

The *DC-SIGN* repeat region in exon 4 was amplified from genomic DNA with the following pairs of primers 5′-GGGATTAACCAAGACCTTGGCTC-3′ (forward) and 5′-CCCAACTTCTCCTAGTCTGGAGG-3′ (reverse) designed from GeneBank sequence AF209479. PCR amplification was performed in a volume of 50 μl using standard conditions as previously described [13]. The cycle conditions were 5 minutes at 95°C, followed by 35 cycles of 45 seconds at 95°C, 30 seconds at 64°C, and 72°C and then 1 cycle of a 10 minutes at 72°C. Alleles were distinguished on 3% agarose gel electrophoresis with ethidium bromide staining.

STATISTICAL METHODS

Data were managed and stored using the SPSS program (V9.05 for Windows, Chicago, IL, USA). Allele and genotype frequencies of *DC-SIGN* -336 were obtained by direct counting. Differences between allele and genotype frequencies were determined using two-sided X^2 and Fisher's exact test as appropriate. The influence of *DC-SIGN* -336 on the patients was analyzed by logistic regression. Crude odds ratio (OR) was calculated with 95% confidence intervals (CI). A p value <0.05 was considered statistically significant.

TABLE 1 Frequency of *DC-SIGN* −336G→A genotypes and alleles in tuberculosis patients and healthy controls

	No. (%) in tuberculosis patients	No. (%) in healthy controls
DC-SIGN−336G→A genotypes		
No. of genotypes	110	299
GG	6 (5.45)	10 (3.34)
GA	40 (36.36)	94 (31.44)
AA	64 (58.18)	195 (65.22)
DC-SIGN −336G→A alleles		
No. of alleles	220	598
G	52 (23.66)	114 (19.1)
A	168 (76.4)	484 (80.9)

Comparison of genotype frequencies between tuberculosis patients and controls, using a 2 × 3 contingency table, χ^2 (2df) = 2.14p = 0.34. Comparison of G/G + G/T versus AA genotype frequencies in tuberculosis patients and controls χ^2 (1df) = 1.71, p = 0.2, OR 1.35, and 95% CI 0.84–2.16. Comparison of allele G versus allele A in tuberculosis patients and controls, χ^2 (1df) = 2.08, p = 0.14, OR 1.31, and 95% CI 0.89–1.94.

RESULTS

DC-SIGN -336 Polymorphism

Allelic and genotype frequencies corresponding to *DC-SIGN* -336 SNP in 110 tuberculosis patients and 299 controls are shown in Table 1. Both patients and controls were in Hardy-Weinberg equilibrium. GG genotype homozygosis was observed in 5.5% of the tuberculosis patients and 3.3% of healthy controls, although this difference did not reach statistical significance (Table 1). We did not observe statistically significant differences in the distribution of *DC-SIGN* -336 alleles in tuberculosis patients as compared with controls.

In the control group, 155 individuals were screened for DTH using the TST. Seventy-four were TST^+ and 81 were TST^-. The presence of G allele was slightly increased in TST^+ individuals compared with TST^- (Table 2). The frequency of G allele was similar in TST^+ controls to that in tuberculosis patients (23.66% vs 23.7).

Next, we examined the influence of *DC-SIGN* -336 with regard to clinical forms of the disease. No association between *DC-SIGN* -336 SNP and pulmonary or extrapulmonary forms of tuberculosis (data not shown). Similarly, there were no significant differences between *DC-SIGN* -336 alleles and genotypes when men and women were compared (data not shown).

DC-SIGN Insertion/Deletion in Neck Region

The neck region of DC-SIGN between the C-terminal domain and the transmembrane domain is formed by repeats of 69 bp and encode repeating units of 23 aa. Analysis of this region in our cohort of 409 individuals revealed, on the basis of the number of repeats (range 7-8 repeats), the presence of two alleles, 7 and 8 repeats, and two genotypes of 7/7 and 7/8. The allele frequencies found for allele 8 were 100% for patients and 99.4% for controls. In addition the frequencies for allele 7 were 0% and 0.6% in tuberculosis patients and controls, respectively (data not shown). This data shows that the *DC-SIGN* repeat polymorphism has a very low frequency in our population, sustaining previous findings [10].

DISCUSSION

Although there are varying associations of certain gene polymorphisms, such as interferon γ receptor, natural resistance-associated macrophage 1 (NRAMP1) [14, 15] and tumor necrosis factor [11]. Nevertheless, the genetic factors that determine host response to the disease remain poorly identified; studies on additional candidate genes are therefore required.

DC-SIGN -336 SNP plays an important role in the susceptibility in other infectious diseases. The -336G allele was originally shown to be associated with parental HIV-1 but not mucosal, acquisition of HIV-1 infection in the European-American population [8]. Interestingly, in the Asiatic population an association was found with -336G allele and risk of Dengue fever but not Dengue hemorrhagic fever [9]. A recent report from an African

TABLE 2 Frequency of *DC-SIGN* −336G→A allele according to TST status

DC-SIGN −336G→A alleles	Tuberculosis patients	TST+ controls	TST− controls
No. of alleles	220	148	162
G	52 (23.66)	35 (23.7)	30 (18.5)
A	168 (76.4)	113 (76.3)	132 (81.5)

Comparison between tuberculosis patients and TST− healthy controls, χ^2 (1df) = 1.45, p = 0.2, OR 1.36, and 95% CI 0.80–2.33. Comparison between tuberculosis patients and TST+ healthy controls, χ^2 (1df) = 0.01, p = 0.99, OR 1.0, and 95% CI 0.6–1.68. Comparison between TST+ healthy controls and TST− healthy controls χ^2 (1df) = 1.23, p = 0.26, OR 1.36, and 95% CI 0.76–2.45.

population indicated that the -336A allele was associated with a protective role against tuberculosis [16].

In addition, although a putative functional role for the *DC-SIGN* -336 SNP has been shown, caution should be taken in extrapolating the results from the *in vitro* experiments to the individual patient, since other factors within the disease environment may affect the *DC-SIGN* production and its biologic activity. Since other polymorphisms in linkage disequilibrium might also influence the promoter activity, further detailed molecular promoter studies using cell lines of different origins are needed to define the overall functional importance of *DC-SIGN* -336 SNP.

In spite of a lack of association herein reported, our results offer the SNP frequencies in a Northwestern Colombian population, which might be useful for future studies. On the other hand, there may be differential interaction between *M. tuberculosis* and *DC-SIGN*, depending on the variability of *Mycobacterium tuberculosis* strain affecting the level of infectivity. Further studies examining the relation between *M. tuberculosis* strain and functional variants of *DC-SIGN* are warranted to assess both *in vitro* and *in vivo* the functional consequences of *CD209* variants on the quality of the host immune response against pathogens, including *M. tuberculosis.*

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