Lack of effects of the TNF-α and IL-10 gene polymorphisms in Mexican patients with lepromatous leprosy

JESÚS SALVADOR VELARDE FÉLIX*, SILVESTRE CÁZARES-SALAZAR*, JUAN JOSÉ RÍOS-TOSTADO**, AURELIO FLORES-GARCÍA***, HÉCTOR RANGEL-VILLALOBOS**** & JOEL MURILLO-LLANES*****

*Centro de Medicina Genómica del Hospital General de Culiacán “Dr. Bernardo J. Gastélum”, Servicios de Salud de Sinaloa. Culiacán, Sinaloa, México
**Unidad Académica de Biología, Universidad Autónoma de Sinaloa. Culiacán, Sinaloa, México
***Unidad Académica de Medicina, Universidad Autónoma de Nayarit, Tepic, Nayarit, México
****Instituto de Investigación en Genética Molecular, Centro Universitario de la Ciénega, Universidad de Guadalajara (CUCiéne-ga-UdeG), Ocotlán, Jalisco, México
*****Dpto. de Investigación del Hospital General de Culiacán “Dr. Bernardo J. Gastélum”, Servicios de Salud de Sinaloa. Culiacán, Sinaloa, México

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Summary Several human genetic variants have been associated with susceptibility or resistance to leprosy. The aim of this study was to assess whether gene polymorphisms of -308 G/A TNF-α and -819 T/C IL-10 are associated with lepromatous leprosy in Mexican mestizos patients from northwest Mexico. We genotyped these polymorphisms by means of polymerase chain reaction (PCR) and restriction fragment length polymorphisms (RFLPs) in 68 patients with lepromatous leprosy and 144 healthy Mexican Mestizos controls. We found that the -308G TNF-α allele was predominant in both cases (94.3%) and controls (92.3%) without statistical

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significance and the frequencies of -819C IL-10 allele were also similar for the cases (56.0%) and controls (59.0%). These negative findings suggest that other genes or polymorphisms may be important in the susceptibility to leprosy infection in the Mexican mestizos.

Introduction

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* that manifests as a clinical broad spectrum. The benign or tuberculoid (TT) form is characterised by strong cell-mediated immunity and T helper 1 (Th1) cytokine profile; the patients exhibit resistance to the microorganism and the lesions contain fewer bacilli. On the other hand, the lepromatous leprosy (LL) is the most severe clinical manifestation of leprosy characterised by immune responses with T helper 2 (Th2) cytokine, a strong humoral response and contains multiple bacilli in the skin and nerves.\(^1\) In such a spectrum of disease, several human genes have been implicated controlling susceptibility and severity of disease. Among the candidate genes involved in this host–pathogen interaction, cytokines evidently play a critical role.\(^2\)

TNF-α is a pleiotropic cytokine capable to perform pro-inflammatory activities including the macrophages activation,\(^3\) which participate in protective immunity during the granuloma formation\(^4\) and inhibiting mycobacterial growth *in vitro*.\(^5\) However, the effects of TNF-α may also result in immunopathology, such as nerve damage\(^6\) and tissue necrosis.\(^7\) Another important cytokine is the IL-10, which possesses immune regulatory property and is secreted by cells of the monocyte/macrophage lineage and T cell subsets such as Th1, Treg cells and Th17. This suppresses the production of inflammatory mediators as well as antigen presentation. High levels of IL-10 are observed in multibacillary leprosy compared with paucibacillary leprosy and a low TNF-α/IL-10 ratio is correlated to disease progression.\(^8\)

Polymorphisms in the regulatory regions of cytokine genes might affect the protein production level, controlling the susceptibility/predisposition to infectious diseases as well as different clinical outcomes. The G → A substitution at position -308 of the TNF-α gene has been associated to susceptibility to cerebral malaria,\(^9\) mucocutaneous leishmaniasis,\(^10\) brucellosis,\(^11\) fatal meningococcal disease.\(^12\) Similarly, IL-10 gene contains three distinct SNPs in its promoter region: A1082G, C819T and C592A; these have been analysed in hepatitis C virus infection,\(^13\) meningitis,\(^14\) cytomegalovirus infections.\(^15\) In leprosy, studies on the polymorphisms of the G308A TNF-α\(^16,17,18\) and C819T IL-10\(^18,19,20\) have shown different results between populations.

The aim of this study was to see whether the occurrence of G308A TNF-α and C819T IL-10 gene polymorphisms are increased in Mexican Mestizos patients with lepromatous leprosy from an endemic region in the northwest of Mexico.

Materials and Methods

A case-control study of outpatients with lepromatous leprosy from Dermatologic Center of Sinaloa was performed. The case group consisted of 68 patients (47 males and 21 females) that were diagnosed with lepromatous leprosy during 1994–2005 by dermatologists following clinical and histopathological criteria.\(^21\) At clinical examination of these leprosy patients, incapacity grade 1 or 2 in both extremities, was observed. Slit skin smear examination for mycobacteria from the lesions revealed that six patients (8.8%) were negative for bacterial index; the remaining were at least 2 + (1 to 10 acid fast bacilli per 10 fields on Ridley’s
logarithmic scale) and the lesions were levels 2 and 3. All patients were treated for multibacillary leprosy, as recommended by the World Health Organization (WHO).

The control group consisted of 144 (69 males and 75 females) unrelated healthy blood donors from the General Hospital of Culiacan Blood Bank, without family history of mycobacterial disease, nor HIV, hepatitis C or B viruses. After written informed consent was obtained, blood samples were collected from all participants.

Patients and controls were recruited considering their origin from the northwest state of Sinaloa (parents and grandparents) and of Mexican Mestizos ethnicity. The range age of patients was 24 to 88 years and of controls was 18 to 62 years. The study was approved by the Ethics and Research committee of the General Hospital of Culiacan.

GENETIC ANALYSIS

The genomic DNA was obtained by CTAB-DTAB method, and subjected to PCR amplification. The primers used for SNP G308A of TNF-α were forward- 5'-GAGCAA-TAGGTTTTGAGCGCCAT-3', and reverse 5' GGGACACACAAGCATCAAG-3'. For the SNP C819T of IL-10, the primers were: forward 5' - TCATTCTATGTGCTGGAGATGG 3' and reverse 5' - TGGGGGAAGTGGGTAAGAGT-3'. Polymorphisms at TNF-α and IL-10 gene promoter regions at positions -308 and -819 were identified by restriction fragment length polymorphisms (RFLPs) by digestion with Nco I and Mae III, (Fermentas-Molecular Biology Tools, Canada), respectively. For the G308A TNF-α promoter region, a 108 pb PCR was amplified; fragments of 87 bp and 20 bp for were generated for allele G, whereas allele A remains undigested. For the C819T polymorphism in the IL-10 promoter, we amplified a PCR product of 209 bp that was digested with the endonuclease Mae III. The allele C generates 125 and 84 bp fragments, whereas T is the uncut allele. Restriction patterns were observed by 6% (29:1) polyacrylamide gel electrophoresis with silver staining.

The allele and genotype frequencies were established by direct counting. Genotype distribution deviations from Hardy-Weinberg expectations, and comparison between groups were evaluated by Fisher’s exact tests. De Finetti program was employed for these analyses (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl).

Results

The allele and genotype frequencies in case and control groups are shown in Table 1.

For the TNF-α polymorphism, the allele -308G was predominant in both cases (94.3%) and controls (92.3%). For the IL-10 polymorphisms, a more even allele distribution was observed, with a slight prevalence of the allele 819C in controls (59%) compared to cases (56%). Observed genotype distributions at these two polymorphic loci were in general agreement with Hardy-Weinberg equilibrium expectations, in both groups. The case-control comparison was not significant (P > 0.05), including an additional assessment classifying cases by gender (data not shown).

Discussion

Two studies have shown a relationship between TNF 308 G/A polymorphism with different types of leprosy. For lepromatous leprosy, an association was observed with AA genotype an
Indian population in Calcutta. In southern Brazil, the GG genotyped was found to be associated with both tuberculoid and borderline leprosy (BB). However, a study from northern Malawi did not find any association with the TNF-308 G/A polymorphisms. The result from our study is also negative, similar to the Malawi study.

The cytokine IL10 has multiple effects in regulation of the immune system, including the inhibition of Th1 cytokine secretion and T cell proliferation. It has been noted that several polymorphisms in the promoter region of IL-10 gene influence its production. A few genetic variants of the IL-10 promoter gene, such as A1082G, C819T and C592A, alone or in haplotype were studied in patients with leprosy, but discordant results were noted. In Malawi, the frequencies of these SNP’s were similar between controls and patients; in a Brazilian population, however, the -819T variant was found to be associated with leprosy susceptibility. In our study, the lack of an association with the C819T IL-10 polymorphism was again similar to the Malawi study.

A limitation concerning case-control association studies is that samples should have similar ancestral components, in order for the importance of the polymorphisms as a disease-predisposing factor to be apparent. For Latin American Mestizo populations, as in our study, this is critical because the great inter-populational genetic variability demonstrated by genome-wide analysis. In Mexico, the population structures have been largely attributed to differences in Amerindian and European ancestral components, with a specific geographic distribution throughout the country. This could explain the heterogeneous distribution of the genotype G308A TNF-α polymorphism in healthy individuals from states of Mexico: Jalisco (West Mexico); Mexico City (Centre); and Sinaloa (North West) (present study), whose frequencies varied as follows, 27.8, 8.64, and 11.7%, respectively. A similar variability was observed for the 3’ UTR 1188 A/C polymorphism in lepromatous leprosy patients from two nearby states from the West region of Mexico. In our study, the ancestral component variation was considered during the sampling process, recruiting only local individuals from the state of Sinaloa.

In conclusion, the present study does not find any association of G308A TNF-α or C819T IL-10 gene polymorphisms with lepromatous leprosy in Mexican Mestizos from the Northwest region. The observed inter-population heterogeneity in leprosy susceptibility might involve other immune regulatory genes and environmental factors.
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References

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