Lepr Rev (2012) **83,** 34–39

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

JESÚS SALVADOR VELARDE FÉLIX*,**,
SILVESTRE CÁZAREZ-SALAZAR*,
JUAN JOSÉ RÍOS-TOSTADO*,**,
AURELIO FLORES-GARCIA***,
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 (CUCiénega-UdeG), Ocotlán, Jalisco, México*

******Dpto. de Investigación del Hospital General de Culiacán "Dr. Bernardo J. Gastélum", Servicios de Salud de Sinaloa. Culiacán, Sinaloa, México*

Accepted for publication 15 December 2011

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

Correspondence to: Jesús Salvador Velarde Félix, Centro de Medicina Genómica, Hospital General de Culiacán Bernardo J. Gastélum. Juan Aldama y Nayarit s/n. Col. Rosales. Culiacán, Sinaloa, México. CP. 80230 (Tel: +52667 716 8560, ext. 196; Fax: +52667 716 9825; e-mail: jsvelfe@hotmail.com)

 0305-7518/12/064053+06 $1.00

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 Tcell 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[18–20] 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 histopatological 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, bloods 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,[22] 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′.[10] For the SNP C819T of IL-10, the primers were: forward 5′- TCATTCTATGTGCTGGAGATGG 3′ and reverse 5′- TGGGGGAAGTGGGTAAGAGT-3′.[23] 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.[10] 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.[23] 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 ($P > 0{\cdot}05$), 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

Table 1. Comparison of Allele and Genotype Distribution between Lepromatous Leprosy (LL) patients and healthy controls

	TNF alpha, *n* (%)				IL-10, *n* (%)			
	Allele†	Genotypes			Allele†	Genotypes		
	G	G/G	G/A	A/A	C	C/C	C/T	T/T
LL Cases	128 (94·3)	60 (88·2)	8 (11·7)	0 (0)	72 (56)	18 (28·1)	36 (56·2)	10 (15·6)
Controls	266 (92·3)	123 (85·4)	20 (13·9)	1 (0·7)	164 (59)	46 (33·3)	72 (52·1)	20 (14·5)
HWE tests		$P = 0{\cdot}58$; controls $P = 1{\cdot}0$; cases				$P = 0{\cdot}38$; controls $P = 0{\cdot}31$; cases		
Association test		OR = 0·78; $P = 0{\cdot}50$				OR = 1·1 $P = 0{\cdot}52$		

HWE = Hardy-Weinberg equilibrium, exact test (P – value).
† Wild-type allele frequency is presented.

Indian population in Calcutta.[16] In southern Brazil, the GG genotyped was found to be associated with both tuberculoid and borderline leprosy (BB).[20] 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 in its production.[24] 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;[18] in a Brazilian population, however, the -819T variant was found to be associated with leprosy susceptibility.[25] 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.[26] For Latin American Mestizo populations, as in our study, this is critical because the great inter-populational genetic variability demonstrated by genome-wide analysis.[27,28] 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.[28,29] This could explain the heterogeneous distribution of the genotype G308A TNF-α polymorphism in healthy individuals from states of Mexico: Jalisco (West Mexico);[30] Mexico City (Centre);[31] 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.[32,33] 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.

Acknowledgements

This study was supported by the Consejo Nacional de Ciencia y Tecnología to J.S.V-F (Grant SIN-2005-CO1-02; FOMIX-CONACYT). We thank to the CECyT-Sinaloa and Universidad Autónoma de Sinaloa (UAS). In addition, we are grateful to Dr. Rafael Castro Velázquez's team from the Dermatologic Center Jesús Rodolfo Acedo Cárdenas at Culiacán, Sinaloa, for patient recruitment.

References

[1] Yamamura M, Uyemura K, Deans RJ *et al.* Defining protective responses to pathogens: cytokine profiles in leprosy lesions. *Science*, 1991; **254**: 277–279.
[2] Moraes MO, Pacheco AG, Schonkeren JJ *et al.* Interleukin-10 promoter single-nucleotide polymorphisms as markers for disease susceptibility and disease severity in leprosy. *Genes Immun*, 2004; **5**: 592–595.
[3] Huang Y, Krein PM, Muruve DA *et al.* Complement factor B gene regulation synergistic effects of TNF-alpha and IFN-gamma in macrophages. *J Immunol*, 2002; **169**: 2627–2635.
[4] Kindler V, Sappino AP, Grau GE *et al.* The inducing role of tumor necrosis factor in the development of bactericidal granulomas during BCG infection. *Cell*, 1989; **56**: 731–740.
[5] Bermudez LE, Young LS. Tumor necrosis factor, alone or in combination with IL-2, but not IFN-gamma, is associated with macrophage killing of *Mycobacterium avium* complex. *J Immunol*, 1988; **140**: 3006–3013.
[6] Selmaj KW, Raine CS. Tumor necrosis factor mediates myelin and oligodendrocyte damage *in vitro*. *Ann Neurol*, 1988; **23**: 339–346.
[7] Rook GA, Attiyab RA, Foley N. The role of cytokines in the immunopathology of tuberculosis, and the regulation of agalactosyl IgG. *Lymphokine Res*, 1989; **8**: 323–328.
[8] Cardoso CC, Pereira AC, de Sales Marques C *et al.* Leprosy susceptibility: genetic variations regulate innate and adaptive immunity, and disease outcome. *Future Microbiol*, 2011; **6**: 533–549.
[9] McGuire W, Hill AV, Allsopp CE. Variation in the TNF-alpha promoter region associated with susceptibility to cerebral malaria. *Nature*, 1994; **371**: 508–510.
[10] Cabrera M, Shaw MA, Sharples C *et al.* Polymorphism in tumor necrosis factor genes associated with mucocutaneous leishmaniasis. *J Exp Med*, 1995; **182**: 1259–1264.
[11] Caballero A, Bravo MJ, Nieto A *et al.* TNFA promoter polymorphism and susceptibility to brucellosis. *Clin Exp Immunol*, 2000; **121**: 480–483.
[12] Nadel S, Newport MJ, Booy R *et al.* Variation in the tumor necrosis factor-alpha gene promoter region may be associated with death from meningococcal disease. *J Infect Dis*, 1996; **174**: 878–880.
[13] Lio D, Caruso C, Di Stefano R *et al.* IL-10 and TNF-alpha polymorphisms and the recovery from HCV infection. *Hum Immunol*, 2003; **64**: 674–680.
[14] Oztuzcu S, Cakmak EA, Sivasli E *et al.* Gene Expression and Promoter Region Polymorphisms of Interleukin-10 in Meningitis Patients. *Genet Test Mol Biomarkers*, 2011; **15**: 327–331.
[15] Mitsani D, Nguyen MH, Girnita DM *et al.* A polymorphism linked to elevated levels of interferon-γ is associated with an increased risk of cytomegalovirus disease among Caucasian lung transplant recipients at a single center. *J Heart Lung Transplant*, 2011; **30**: 523–529.
[16] Roy S, McGuire W, Mascie-Taylor CG *et al.* Tumor necrosis factor promoter polymorphism and susceptibility to lepromatous leprosy. *J Infect Dis*, 1997; **176**: 530–532.
[17] Santos AR, Almeida AS, Suffys PN *et al.* Tumor necrosis factor promoter polymorphism (TNF2) seemed to protect against development of severe forms of leprosy in a pilot study in Brazilian patients. *Int J Lepr Other Mycobact Dis*, 2000; **68**: 325–327.
[18] Fitness J, Floyd S, Warndorff DK *et al.* Large-scale candidate gene study of leprosy susceptibility in the Karonga district of northern Malawi. *Am J Trop Med Hyg*, 2004; **71**: 330–340.
[19] Pereira AC, Brito-de-Souza VN, Cardoso CC *et al.* Genetic, epidemiological and biological analysis of interleukin-10 promoter single-nucleotide polymorphisms suggests a definitive role for -819C/T in leprosy susceptibility. *Genes Immun*, 2009; **10**: 174–180.
[20] Franceschi DS, Mazini PS, Rudnick CC *et al.* Influence of TNF and IL10 gene polymorphisms in the immunopathogenesis of leprosy in the south of Brazil. *Int J Infect Dis*, 2009; **13**: 493–498.
[21] Ridley DS, Jopling W. Classification of leprosy according to immunity. A five-group system. *Int J Lepr Other Mycobact Dis*, 1966; **34**: 255–273.
[22] Gustincich S, Manfioletti G, Del Sal G *et al.* A fast method for high-quality genomic DNA extraction from whole human blood. *Biotechniques*, 1991; **11**: 298–302.

[23] Edwards-Smith CJ, Jonsson JR, Purdie DM *et al.* Interleukin-10 Promoter Polymorphism Predicts Initial Response of Chronic Hepatitis C to Interferon Alfa. *Hepatology*, 1999; **30**: 526–530.
[24] Gibson AW, Edberg JC, Wu J *et al.* Novel single nucleotide polymorphisms in the distal IL-10 promoter affect IL-10 production and enhance the risk of systemic lupus erythematosus. *J Immunol*, 2001; **166**: 3915–3922.
[25] Santos AR, Suffys PN, Vanderborght PR *et al.* Role of tumor necrosis factor-alpha and interleukin-10 promoter gene polymorphisms in leprosy. *J Infect Dis*, 2002; **186**: 1687–1691.
[26] Price AL, Zaitlen NA, Reich D *et al.* New approaches to population stratification in genome-wide association studies. *Nat Rev Genet*, 2010; **11**: 459–463.
[27] Bryc K, Velez C, Karafet T *et al.* Genome-wide patterns of population structure and admixture among Hispanic/Latino populations. *Proc Natl Acad Sci*, 2010; **107**: 8954–8961.
[28] Silva-Zolezzi I, Hidalgo-Miranda A, Estrada-Gil J *et al.* Analysis of genomic diversity in Mexican Mestizo populations to develop genomic medicine in Mexico. *Proc Natl Acad Sci*, 2009; **106**: 8611–8616.
[29] Rubi-Castellanos R, Martínez-Cortés G, Muñoz-Valle JF *et al.* Pre-Hispanic Mesoamerican demography approximates the present-day ancestry of Mestizos throughout the territory of Mexico. *Am J Phys Anthropol*, 2009; **139**: 284–294.
[30] González-Enríquez GV, Rubio-Benítez MI, García-Gallegos V *et al.* Contribution of TNF-308A and CCL2-2518A to carotid intima-media thickness in obese mexican children and adolescents. *Arch Med Res*, 2008; **39**: 753–759.
[31] Rodríguez-Carreón AA, Zúñiga J, Hernández-Pacheco G *et al.* Tumor necrosis factor-alpha -308 promoter polymorphism contributes independently to HLA alleles in the severity of rheumatoid arthritis in Mexicans. *J Autoimmun*, 2005; **24**: 63–68.
[32] Alvarado-Navarro A, Montoya-Buelna M, Muñoz-Valle JF *et al.* The 3′UTR 1188 A/C polymorphism in the interleukin-12p40 gene (IL-12B) is associated with lepromatous leprosy in the west of Mexico. *Immunol Lett*, 2008; **118**: 148–151.
[33] Velarde-Felix JS, Rendón-Maldonado JG, Ochoa Ramírez LA *et al.* Lack of association between 3′ UTR 1188 A/C polymorphism in the IL- 12p40 gene and lepromatous leprosy in Sinaloa, México. *Int J Dermatol*, 2011; Apr 6. doi: 10.1111/j.1365-4632.2010.04597.