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TIRAP (MAL) S180L polymorphism is a common protective factor against developing tuberculosis and systemic lupus erythematosus

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ABSTRACT

Background and aim: The involvement of Toll-like receptor (TLR)-mediated pathways in infectious and autoimmunity has been suggested. The MyD88 adaptor-like (Mal) protein, also known as the TIR domain-containing adaptor protein (TIRAP), is implicated in the TLR2- and TLR4-mediated MyD88-dependent signaling pathway. The aim of this study was to investigate the influence of the functional *TIRAP* (MAL) S180L polymorphism on tuberculosis (TB) and four autoimmune diseases namely: rheumatoid arthritis (RA), primary Sjögren's syndrome (pSS), systemic lupus erythematosus (SLE) and type 1 diabetes mellitus (T1D).

Methods: This was a case–control and family based association study in which 1325 individuals from a well-defined Colombian population were involved. *TIRAP* (MAL) S180L genotyping was done by using a polymerase chain reaction-restriction fragment length polymorphism technique and by direct sequencing.

Results: Leu180 allele was found to be a protective factor against developing TB (odd ratio (OR): 0.53, 95% confidence interval (CI): 0.29–0.97) and SLE (OR: 0.29, 95% CI: 0.14–0.61) while no significant influence on RA, pSS and T1D was observed.

Conclusion: These results support the influence of *TIRAP* (MAL) S180L polymorphism on TB and indicate that TB and SLE might share a common immunogenetic pathway in the innate immune response.

1. Introduction

The involvement of Toll-like receptors (TLRs)-mediated pathways in infectious, autoimmune and inflammatory disease has been shown (Mansell et al., 2004; Takeda, 2005). TLRs are pattern recognition receptors of the innate immune system that recognize a wide variety of molecules which lead to the transcription of proinflammatory genes through a complex signaling cascade (Horng et al., 2001). Thus, activation of TLRs pathways may control not only host defense against pathogens but also autoimmune responses.

The MyD88 adaptor-like (Mal), also known as TIR domain-containing adaptor protein (TIRAP), is a cytoplasmic structure of 221 amino acids in length encoded by a gene holding the same name at chromosome 11q24.2. Functionally, TIRAP is implicated in the TLR2- and TLR4-mediated MyD88-dependent signaling pathway. The MyD88 was the first adapter in the family of the TIR domain-containing adaptor proteins to be described (Janssens and Beyaert, 2002). TIRAP resembles MyD88 in that it is involved in the early activation of NFκB and MAP kinases, but its use is essential and restricted to TLR2 and TLR4 (Mansell et al., 2004). Heterozygous carriage of the *TIRAP* single nucleotide polymorphism (SNP) rs8177374 (C/T), which encodes a leucine substitution at serine 180 of Mal (S180L) was showed to be associated with a decreased risk against pneumococcal disease, bacteremia, malaria and tuberculosis (TB) in English and African populations (Khor et al., 2007). Since S180L leads to an amino acid substitution in which Mal attenuates TLR2 and TLR4 signaling and thereby protects against excessive inflammation (Mansell et al., 2004), we hypothesized that variations at this *TIRAP* rs8177374 SNP might influence the susceptibility not only to common infectious diseases but also to autoimmune diseases. Therefore, we underwent the current study to examine the effect of *TIRAP* (MAL) polymorphism S180L in patients with TB and autoimmune diseases from a Colombian population (Latin American individuals).

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TB is a contagious and potentially fatal disease caused by *Mycobacterium tuberculosis* that affects almost any part of the body but manifests mainly as an infection of the lungs. Systemic lupus erythematosus (SLE) is characterized by the production of pathogenic autoantibodies, B-cell hyperactivation, and defective clearance of immune complexes, affecting several organs (Tan et al., 1982). Primary Sjögren's syndrome (pSS) is a late-onset autoimmune disease characterized by lymphocytic and plasma cell infiltration of the salivary and lachrymal glands, as well as by the production of autoantibodies leading to dryness of mucosa, mainly oral and lachrymal (Vitali et al., 2002). Rheumatoid arthritis (RA) is characterized by an autoimmune polyarthritis with progressive damage of diarthrodial joints leading to disability, increased comorbidity and premature mortality (Arnett et al., 1988). Type 1 diabetes mellitus (T1D) is an organ-specific autoimmune disease resulting from the damage of insulin-producing pancreatic β cells (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997).

2. Methods

The whole sample was constituted by 1325 individuals, of whom 976 were included in a case–control study. The rest of individuals were T1D affected probands ($n = 68$) and their relatives ($n = 281$) who were included in a family based study ($n = 65$). They belonged to a well defined and homogeneous northwestern Colombian population with a low rate of admixture (Bravo et al., 1996), with a calculated ancestral ethnic component of 85% Caucasian and 15% Amerindian (Correa et al., 2002; Bedoya et al., 2006). All the individuals were from Medellin, and were enrolled at the Cellular Biology and Immunogenetics Unit of the "Corporación para Investigaciones Biológicas" (CIB), in Medellin, Colombia. This research was conducted in compliance with resolution 008430 of 1993 of the ministry of health of Colombia, and was classified as research with minimal risk. The institutional review board of the CIB approved the study design.

2.1. Patients

The study included 150 women and 13 men with SLE, with a mean age ± S.D. of 36 ± 13 years; the mean duration of disease was 8 ± 6.3 years, 60% of them tested positive for anti-DNA antibodies. The main clinical manifestations were musculoskeletal (85%), dermatological (59%), and renal (51%). There were 168 women and 18 men with RA, their age was 49 ± 15 years, the mean duration of disease was 12 ± 9 years, and 45% had at least one extra-articular manifestation. Rheumatoid factor and anti-cyclic citrullinated peptide antibodies were registered in 80% and 86% of cases, respectively. There were 89 patients with primary SS, their mean age was 55 ± 13.5 years, the mean duration of pSS was 7.7 ± 6.7 years, and anti-Ro and anti-La antibodies were present in 91% and 64% of cases, respectively. Moreover, T1D affected probands ($n = 68$, 37 girls and 31 boys with a mean age of 8.7 ± 5.8 years) and their relatives ($n = 281$) were included to carry on a transmission disequilibrium test (TDT). All patients with autoimmune diseases met the international classification criteria for their respective disease (Tan et al., 1982; Vitali et al., 2002; Arnett et al., 1988; Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997). Clinical and immunological characteristics of patients with autoimmune diseases were similar to those previously reported (Correa et al., 2005; Tobon et al., 2006). None of these patients had previous or current evidence of TB from clinical history and chest radiograph.

Patients with pulmonary TB ($n = 147$) were enrolled for the study at the time of treatment of their disease. TB was diagnosed by the presence of alcohol acid-resistant bacilli in sputum smears or by isolation of *M. tuberculosis* in culture. All patients with TB were negative for human immunodeficiency virus (HIV) 1/2 infection (by Axsym assays, Abbott Laboratories, Chicago, USA).

2.2. Control subjects

Three hundred and ninety-one individuals without inflammatory disease, autoimmune disease or history of chronic infectious disease such as TB and HIV infection represented control individuals and were gender-, age-, socio-economic- and ethnically matched to patients. Socio-economic status ranked from 1 to 6 according to the Colombian public services classification, taking 1–3 as low income and 4–6 as moderate to high income, as reported (Cadena et al., 2003).

2.3. *TIRAP* (MAL) S180L typing

Genomic DNA was extracted from EDTA-anticoagulated peripheral blood from each individual using a salting out procedure (Miller et al., 1988). The typing was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique by means of an iCycler BioRad Thermal Cycler (BioRad, Hertfordshire, UK). *TIRAP* (MAL) S108L polymorphism was screened using primers 5′-CTC CAG GGG CCG AGG GCT GCA CCA TCC CCA TGC TG-3′ and 5′-TAC TGT AGC TGA CCG TTC C-3′ (Invitrogen Life Technologies, Frederick, MD, USA), as previously reported (Khor et al., 2007). PCR products were analyzed by 3% agarose gel electrophoresis (Seakem LE Agarose, BioWhittaker, Rockland, ME, USA) and were visualized by ethidium bromide staining (Fig. 1). Ten percent of individuals from each study group were randomly chosen, directly sequenced and used as genotyping positive controls.

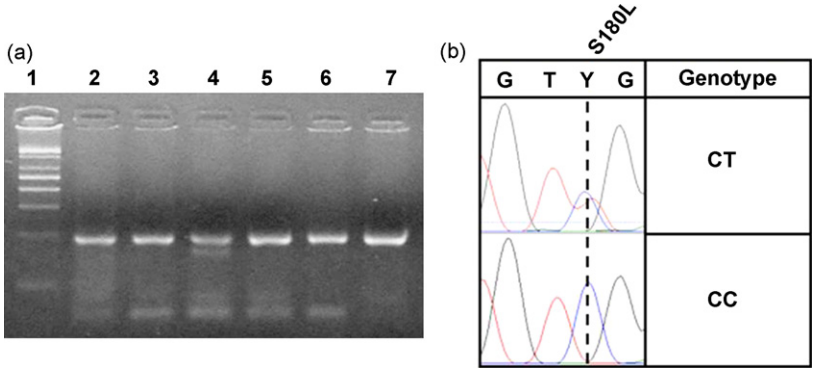

Fig. 1. PCR-RFLP and direct sequencing performed to genotype TIRAP (MAL) S180L polymorphism. (a) 3% agarose gel with sequenced positive control individuals. Individuals in wells 2, 3 and 5–7 are homozygous (CC) for Ser180. Individual in well 4 is heterozygous (CT) for S180L. (b) Obtained phenograms from the randomly chosen individuals by direct sequencing. Y = C or T.

2.4. Statistical analysis

Data was analyzed using the Statistical Package for the Social Sciences (SPSS) (v15 for Windows, Chicago, IL). Crude odd ratios (OR) as well as 95% confidence interval (CI) were estimated. Allelic relative frequencies were calculated by the direct counting method. Wright's *F* statistics (Fst) were evaluated using Arlequin Version 3.01 (Excoffier et al., 2005). Exact tests were performed to identify departure from Hardy–Weinberg (HW) proportions. The false-positive report probability was calculated according to Wacholder et al. (2004). TDT and case–control analyses were accomplished by means of the TDTPHASE and COCAPHASE programs from the UNPHASED software, respectively (Dudbridge, 2003).

3. Results

All patient and control groups fulfilled the Hardy–Weinberg proportions for the polymorphism S180L. As expected, the population involved in this study was not stratified (Fst = 0.01). Differences in the allelic frequencies according to gender were not observed. The Leu180 allele was associated with a decrease of 73% in the risk of SLE and a decrease of 50% in the risk of TB (Table 1). The calculated false-positive report probability for the Leu180 allele was 0.02–0.013 in SLE, while for TB was 0.14–0.17 for prior probabilities of the hypothesis ranging from 0.1 (high) to moderate (0.01), below the 0.2 level proposed by Wacholder et al. (2004). This analysis suggests that the S180L associations are noteworthy, with a low risk of being a false-positive finding. No other autoimmune disease presented an association with this polymorphism.

The TDT was performed to assess the allele transmission rates in families of T1D to test for the deviation from the expected 50% transmission. The effect of the Leu180 allele (OR = 0.78, 95% CI: 0.92–2.50, $p = 0.35$) and the heterozygous genotype (OR = 1.06, 95% CI: 0.85–2.97, $p = 0.25$) showed a non-significant decrease on the risk to develop T1D.

Association between *TIRAP* (MAL) S108L and clinical or immunological (i.e. autoantibodies) characteristics was not observed in any group of patients.

4. Discussion

Replication of promising initial results is a necessary step to assess the genetic contribution to human disease. With this goal, the present study was conducted to confirm earlier findings in TB, and to explore a possible influence of *TIRAP* (MAL) polymorphism on autoimmune diseases. When data are interpreted in the context of earlier results (Khor et al., 2007), association between *TIRAP* (MAL) S180L and TB was reproduced and confirmed to be true (Manly, 2005). Nevertheless, as for many polymorphisms in complex diseases the influence of this SNP on TB varies according to ethnicity (Nejentsev et al., 2008). In addition, we support a recent report showing a lack of association between this polymorphism and RA (Sheedy et al., 2008). On the other hand, our data provide new information about the protective effect of *TIRAP* (MAL) S180L on the risk of developing SLE. Noteworthy, *TIRAP* locus is close to previous loci associated or linked to SLE (Moser et al., 1998; Lindqvist et al., 2000). Results with respect to RA, pSS and T1D showed no association with *TIRAP* (MAL) S180L polymorphism but represented a novel research in this topic. Familial data in T1D revealed no statistically significant differences in the transmission pattern of S180L, plus a non-biased transmission to the affected offspring from parents.

Table 1
Genotypic and allelic relative frequencies of TIRAP (MAL) S180L in patients with autoimmune diseases and pulmonary tuberculosis

Genotypes[a]	CC, *n* (%)	CT, *n* (%)	OR	95%CI	*p*-value
Controls (391)	328 (0.84)	63 (0.16)	1.00	–	–
SLE (163)	155 (0.95)	8 (0.05)	0.27	0.13–0.57	0.0002
RA (186)	161 (0.87)	25 (0.13)	0.81	0.49–1.33	0.46
pSS (89)	80 (0.90)	9 (0.10)	0.56	0.28–1.23	0.19
TB (147)	134 (0.91)	13 (0.09)	0.5	0.27–0.94	0.03
Alleles[b]	C, 2*n* (%)	T, 2*n* (%)	OR	95%CI	*p*-value
Controls (782)	719 (0.92)	63 (0.08)	1.00	–	–
SLE (326)	318 (0.97)	8 (0.02)	0.29	0.14–0.61	0.0002
RA (372)	347 (0.93)	25 (0.07)	0.82	0.51–1.33	0.48
pSS (178)	169 (0.95)	9 (0.05)	0.61	0.30–1.25	0.21
TB (294)	281 (0.96)	13 (0.04)	0.53	0.29–0.97	0.04

OR: odds ratio, CI: confidence interval, SLE: systemic lupus erythematosus, RA: rheumatoid arthritis, pSS: primary Sjögren's syndrome, TB: tuberculosis.

[a] Number of individuals in parenthesis.

[b] Number of chromosomes in parenthesis.

Since the S180L attenuates TLR2 signal transduction (Mansell et al., 2004; Khor et al., 2007), protein models generated have located the S180L residue on the edge of a surface-exposed loop implicated on downstream signaling molecules in which the serine substitution affects recruiting of TLR2 (Khor et al., 2007). This positional effect would relate the protective genetic effect obtained with a reduced or attenuated NFκB activation. Hence, the heterozygous protective outcome seen in this report for SLE and TB could be explained by an intermediate signaling through the genotypic state of *TIRAP*. If an individual were homozygous for the Ser180 his response potentially would be higher presenting an over-reactive and severe outcome (Khor et al., 2007).

Taken together, these results suggest that a specialized adaptive immune response is defined by innate immunity mechanisms tightly linked to acquire self or non-self response. Then, the genetic variation at *TIRAP* might underlie susceptibility to common infectious diseases as indicated by Khor et al. (2007) and also have a common evolutive genetic background within systemic autoimmune diseases such as SLE, given by selective pressures which limit the development of the disease, making the initial innate immune response the foremost barrier for any immunological exposure (self or non-self). As observed, this argument is applicable to SLE, a systemic autoimmune disease, and not to rather organ-specific autoimmune diseases such as RA, T1D and pSS, as shown in this report.

The genetic factors for autoimmune diseases consist of two forms: those common to many autoimmune diseases and those specific to a given disorder (Anaya et al., 2006). Accordingly, *TIRAP* (MAL) S180L polymorphism is a specific factor for SLE. The protective effect of *TIRAP* polymorphism on SLE might be related to the role of its protein in control TLR2 and TLR4 interactions with specific infectious pathogens. Infectious agents are important in the pathogenesis of autoimmune diseases since they are a major part of the environmental trigger of autoimmunity (Barzilai et al., 2007). Yet, the effect of infectious agents on autoimmune diseases may differ depending on the agent involved (i.e. cytomegalovirus, hepatitis viruses, others) and the latitudinal gradient (Pordeus et al., 2008).

In summary, our results confirm the influence of *TIRAP* (MAL) S180L polymorphism on TB and indicate a common genetic susceptibility pathway for TB and a systemic autoimmune disease (i.e. SLE).

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