Surfactant Protein Genetic Marker Alleles Identify a Subgroup of Tuberculosis in a Mexican Population

Joanna Floros,[1,2] Hung-Mo Lin,[3] Andrea García,[4] Miguel Angel Salazar,[4] Xiaoxuan Guo,[1] Susan DiAngelo,[1] Martha Montaño,[4] Junming Luo,[1] Annie Pardo,[5] and Moises Selman[4]

Departments of [1]Cellular and Molecular Physiology, [2]Pediatrics, and [3]Health Evaluation Sciences, Pennsylvania State University College of Medicine, Hershey; [4]Instituto Nacional de Enfermedades Respiratorias and [5]Facultad de Ciencias Universidad Nacional Autonoma de Mexico, Mexico City, Mexico

Pulmonary surfactant and its components are essential for normal lung function and are involved in local host defense. Surfactant protein (SP)–A and SP-D bind to and modulate phagocytosis of *Mycobacterium tuberculosis* by macrophages. Frequency comparisons of SP marker alleles in tuberculosis patients and healthy control subjects (tuberculin–skin test positive or general population) were performed. Regression analyses of the tuberculosis and the tuberculin–skin test positive groups revealed, on the basis of odds ratios, tuberculosis susceptibility *(DA11_C* and *GATA_3)* and protective *(AAGG_2)* marker alleles. Similarly, between tuberculosis patients and general population control subjects, susceptibility *1A*^{3}, *6A*^{4}, and *B1013_A* and protective *AAGG_1,* and *AAGG_7* marker alleles were observed. Moreover, interactions were seen between alleles *6A*^{2} and *1A*^{3} ($P = .0064$) and between *1A*^{3} and *B1013_A* ($P = .036$). The findings indicate a possible involvement of SP alleles in tuberculosis pathogenesis.

There is a growing concern about the increasing incidence of tuberculosis worldwide [1]. *Mycobacterium tuberculosis,* an intracellular pathogen, employs several mechanisms [2] to enter human macrophages, where it survives well and from where it continues its pathogenetic life cycle [1]. Pulmonary surfactant protein (SP)–A and SP-D play important roles in the innate host defense and the regulation of inflammatory processes of the lung [3]. These proteins are members of the C-type lectins and are known as collectins, because they contain both collagen-like and carbohydrate-binding recognition domains (CRDs). SP-A, SP-D, and mannose-binding protein (another collectin) gene loci have been physically mapped on chromosome 10 [4, 5]. The human *SP-A* locus consists of 2 functional genes in opposite transcriptional orientation, *SP-A1* and *SP-A2,* and 1 pseudogene [4]. A number of alleles have been characterized for each human *SP-A* gene [5–7]. Polymorphic marker loci for *SP-D* also have been characterized [8], and methods that allow for the detection of specific *SP-A* or *SP-D* alleles have been developed [8].

SP-A and SP-D bind *M. tuberculosis* and modulate phagocytosis by alveolar macrophages. The binding of SP-A or SP-D to *M. tuberculosis* appears to be saturable, calcium dependent, and inhibited by carbohydrates [9–11]. Bronchoalveolar lavage (BAL) fluid from human immunodeficiency virus (HIV)–infected persons significantly promotes the attachment of *M. tuberculosis* to alveolar macrophages, and SP-A has been implicated as the molecule that promotes the enhanced attachment. SP-A levels are increased in BAL fluid of HIV-infected persons [12, 13]. The SP-D binding of *M. tuberculosis* results in agglutination of bacteria, but it is not known whether the agglutinated *M. tuberculosis* are cleared by yet unknown mechanisms or whether bacterial agglutination promotes their uptake into the host cell by novel mechanisms [9]. Therefore, it is not known whether SP-D should be considered a risk or a protective factor for *M. tuberculosis* infection.

Because of the importance of SP-A and SP-D in the local host defense of the lung, the potential role of these proteins in *M. tuberculosis* infection, and the seriousness of the *M. tuberculosis* health problem, we undertook the present study. Specifically, we determined the frequencies of *SP-A, SP-D,* or *SP-B* alleles in persons with active tuberculosis, in persons without tuberculosis who were tuberculin–skin test positive and who lived in an environment similar to the persons with tuberculosis, and a general control group composed of healthy persons with unknown living conditions and with no information regarding their ability to react to *M. tuberculosis.* We included *SP-B,* because SP-B is essential for normal lung function [14], and suboptimal levels of SP-B appear to compromise lung function [15]. Thus, we reasoned that this locus may play an indirect role by compromising

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Informed consent was obtained according to National Institute of Respiratory Diseases (INER) institutional review board guidelines, and the protocol was approved by the INER ethics committee.

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Reprints or correspondence: Dr. Joanna Floros, Dept. of Cellular and Molecular Physiology, H166 Pennsylvania State University College of Medicine, P.O. Box 850, 500 University Dr., Hershey, PA 17033 (jfloros@psu.edu).

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normal lung function, which may promote *M. tuberculosis* infection.

Patients and Methods

Study population. Study entry of patients and control subjects and sample procurement were conducted at the National Institute of Respiratory Diseases in Mexico City (INER). INER is one of the National Institutes of Health in Mexico and is a tertiary referral and research center. The study was composed of 3 groups of persons whose families had been born in Mexico for at least 3 generations.

Group 1 was composed of 107 patients with active pulmonary tuberculosis. These patients (mean age, 38.8 ± 16.3 years; 46 women and 61 men) were randomly recruited from all inpatients and outpatients attending the INER over 1.5 years on the basis of their willingness to participate. Diagnosis was confirmed by positive sputum specimens for acid-fast bacilli on microscopic examination and/or positive sputum culture for *M. tuberculosis.* Persons who were diabetic or HIV-infected were excluded from the study.

Group 2 was composed of 71 tuberculosis contacts recruited from the tuberculosis contact clinic (mean age, 32.3 ± 11.5 years; 41 women and 30 men). All had household contact with tuberculosis patients and had a positive tuberculin skin test, as defined by an induration >10 mm. Tuberculin test screening was done by the Mantoux method, using 2 tuberculin units of purified protein derivative (PPD) RT 23. Of these tuberculin–skin test positive subjects, 17 (24%) were relatives of the tuberculosis patients. All contacts were symptom-free and had normal chest radiographs.

The third group was composed of 101 sequential, unrelated, nonsmoking healthy adult INER blood donors (mean age, 32.3 ± 10.3 years; 20 women and 81 men) who served as general control subjects. The transfusion service at INER serves the geographic areas from which the patients were recruited. We estimate that this population is 10%–30% PPD positive [16, 17] (Olvera R, INER, unpublished data).

Genotype analysis. *SP-A, SP-B,* and *SP-D* genotype analyses were conducted, using a polymerase chain reaction (PCR)–based converted restriction fragment length polymorphism method, as described elsewhere in detail [8, 18]. Genomic DNA was used as template for PCR.

SP-A. The *SP-A* genotyping was done as described elsewhere [8]. In brief, a 3.3 kb *SP-A1*– or *SP-A2*–specific fragment was amplified and served as a template for subsequent reactions to score specific *SP-A1* or *SP-A2* alleles. The specificity of the 3.3-kb gene-specific fragments was further confirmed by converted PCR at amino acid 85, which is a cysteine (TGT) in all *SP-A1* alleles and an arginine (CGT) in all *SP-A2* alleles [6]. To distinguish individual *SP-A1* alleles, converted PCR was done at 5 amino acid positions: *AA19, AA50, AA62, AA133,* and *AA219.* This nucleotide change may or may not result in a different encoded amino acid. To distinguish *SP-A2* alleles, converted PCR was done, as described elsewhere [8], at 4 amino acid positions: *AA9, AA91, AA140,* and *AA223.*

SP-B. The *SP-B* genotyping was carried out as described elsewhere [18]. In brief, an 11-kb fragment that contains the entire *SP-B* gene was amplified from genomic DNA, and this 11-kb fragment served as a template in subsequent converted PCRs. To distinguish *SP-B* alleles, converted PCR was carried out at nucleotide positions *−18(A/C), 1013 (A/C), 1580 (C/T),* and *9306 (A/G)* [18]. The scoring of alleles was made according to the DNA patterns, as described elsewhere [18].

SP-B–*linked microsatellite markers.* Genotyping was carried out for 4 *SP-B* flanking microsatellite markers, as described elsewhere [19]. Three of these markers *(D2S2232, D2S388,* and $[AAGG]_n$*)* are located at the 5′ end of the *SP-B* gene at ~130, 64, and 27 kb, respectively [19, 20]. The fourth marker *(GATA41E01)* is located at the 3′ end of the *SP-B* gene at ~1064 kb [19, 20]. For each marker, a specific end-labeled ^{32}P-γATP primer was used for PCR, the PCR products were run on a 6% PAGE sequencing gel (Bio-Rad Laboratories, Hercules, CA), and the gel was dried and exposed to X-AR film (Kodak). The scoring of alleles was made according to control allele DNA patterns, as described elsewhere [19].

SP-D. For *SP-D* genotyping, 2 1-kb fragments were amplified from genomic DNA. One fragment contained amino acid 11 *(DA11),* and the other contained amino acid 160 *(DA160).* Each 1-kb fragment served as template for converted PCR at *DA11* or *DA160* sites [8].

Statistical analyses. The statistical analyses included univariate and multiple logistic regression analyses. The 3 subject groups were (1) tuberculosis patients, (2) healthy tuberculin–skin test positive subjects, and (3) general control subjects (healthy nonsmokers). The variables (marker alleles) considered are shown in table 1.

Univariate analyses. For each of the *SP-A, SP-B,* and *SP-D* biallelic markers, analyses were also performed in 2 ways by logistic regression. First, we considered that the alleles have a dose effect, that is, being homozygous for a given allele has a different effect than being heterozygous. In logistic regression, an indicator allele for heterozygosity and an indicator for allele homozygosity were created. We also repeated the same analysis while switching the original baseline with its counterpart. Because very few tests for dose effect were significant regardless of the allele chosen for baseline, we considered it reasonable to consider that 1 allele has the same effect as 2 identical alleles. In the second analysis, an indicator was created for each marker for the presence of ⩾1 copy of the particular allele that resulted in the less significant *P* value for testing the dose effect. Test for significance was performed using logistic regression, except we used the Fisher's exact test when the expected frequency of the markers was too small. Because the markers shown to be significant in the univariate analysis would be considered, at least initially, in the multivariate analysis, a less strict *P* value ($P = .1$) was used.

Analyses were performed in a similar way for the *SP-A2* and *SP-*

Table 1. *SP-A–, SP-B–, SP-D–,* and *SP-B*–linked microsatellite markers used in statistical analyses.

Marker(s)	Marker alleles
SP-A2	*AA9, AA91, AA140, AA223, 1A, 1A^1, 1A^0, 1A^2, 1A^3, 1A^4, 1A^5, 1A^7, 1A^8*
SP-A1	*AA19, AA50, AA62, AA133, AA219; 6A, 6A^2, 6A^3, 6A^4, 6A^6, 6A^9, 6A^{11}, 6A^{14}, 6A^{18}*
SP-B	*AC18, AC1013, CT1580, AG9306*
SP-D	*A11D, A160D*
Microsatellites	*D2S388, D2S2232, AAGG, GATA*

NOTE. SP, surfactant protein.

Table 2. Tuberculosis (TB) vs. skin test positive for TB (univariate analysis).

Allele[a]	Percentage of TB *(n)*	Percentage of skin test positive for TB *(n)*	OR (90% CI)	*P*
AA19_C	42.1 (107)	29.6 (71)	1.72 (1.01–2.95)	.092
AA50_G	62.6 (107)	74.7 (71)	0.56 (0.32–0.99)	.095
AA62_A	84.1 (107)	93.0 (71)	0.40 (0.16–0.96)	.087
AA133_G	42.1 (107)	29.6 (71)	1.72 (0.01–2.95)	.092
1A	45.7 (107)	30.4 (69)	1.92 (1.12–3.29)	.045
DA11_C	69.8 (106)	46.5 (71)	2.66 (1.57–4.49)	.002
D2S388_7	0.9 (107)	5.7 (70)	0.15 (0.02–0.99)	.08[b]
AAGG_2	2.8 (106)	10.1 (69)	0.25 (0.08–0.82)	.051[b]
GATA_3	51.4 (107)	33.3 (69)	2.11 (1.24–3.58)	.019

NOTE. CI, confidence interval; *n,* no. of samples; OR, odds ratio.
[a] ⩾1 Copy.
[b] Fisher's exact test.

A1 multiallelic markers and for the microsatellites. The allele *1A* of *SP-A2* and allele *6A* of *SP-A1* served as the baseline for the creation of indicators. These alleles were not significantly different in either comparison: tuberculosis patients versus tuberculin–skin test positive subjects or tuberculosis patients versus healthy control subjects. In addition, dummy variables were created for each microsatellite that had a count >10, indicating for which subjects that this was true. In these analyses, only the *D2S388* and *D2S2232* variables had any subjects with counts >10.

Multivariate analysis. When we assumed no dose effect for the alleles, we constructed multivariate logistic regression models, using backward selection methods with staying significance levels equal to .05. The alleles that entered the model were preselected, that is, only the alleles shown to be significant by univariate analysis ($P < .1$) were considered in the model.

Allele interactions. The *P* values shown for the allele interactions were determined by Breslow-Day test [21] instead of by logistic regression in the cases in which the odds ratio (OR) of 1 subgroup was either infinity or zero.

Results

Tuberculosis group versus tuberculin–skin test positive group. Significant differences ($P < .1$) in the univariate analysis (table 2) were observed for *SP-A1* marker alleles *(AA19_C, AA50_G, AA62_A,* and *AA133_G)* for *SP-A2 (1A),* for SP-D *(DA11_C),* and for microsatellite marker alleles *(D2S388_7, AAGG_2,* and *GATA_3;* table 2). Of these, on the basis of an OR < 1, the *AA50_G, AA62_A, D2S388_7,* and *AAGG_2* marker alleles appeared to be associated with a decreased risk for tuberculosis, whereas the other marker alleles (OR, >1) appeared to be associated with an increased risk for tuberculosis.

When these marker alleles were considered in the multivariate analysis (table 3), 3 alleles were significant: the SP-D *(DA11_C)* and the microsatellite marker alleles *AAGG_2* and *GATA_3.* The *DA11_C* and the *GATA_3* appeared, on the basis of ORs of 2.81 and 2.13, respectively, to be associated with an increased risk for tuberculosis, whereas the *AAGG_2* marker allele (OR, 0.201) was associated with a decreased risk for tuberculosis. The *DA11_C* marker allele corresponds to a threonine (ACG) at amino acid 11 of SP-D, whereas the *DA11_T* allele corresponds to a methionine (ATG). This regression model was good, with a *c* statistic of 0.695 [22]. The *c* statistic should be $0.5 < c < 1.0$; the larger the *c* statistic, the better the predictive ability of the model.

Tuberculosis group versus general control group. The univariate analysis (table 4) showed that the frequency of several marker alleles differed ($P < .1$) between these 2 groups. These included *SP-A1* marker alleles *(AA50_G, AA219_T, 6A*^{2}, and *6A*^{4}*), SP-A2 (1A*^{3}*), SP-B (B1013_A, B1580_C,* and *B9306_G),* and microsatellite marker alleles *(D2S2232_7, AAGG_1, AAGG_7,* and *GATA_1).* Of these, on the basis of the ORs shown in table 4, half were associated with a decreased risk (OR, <1) and half with an increased risk (OR, >1) for tuberculosis.

When the marker alleles shown in table 4 were considered in multiple logistic regression analysis, 5 marker alleles were significant (table 5). The *SP-A1 (6A*^{4}*), SP-A2 (1A*^{3}*),* and *SP-B (B1013_A)* marker alleles, on the basis of their ORs (4.51, 9.57, and 2.36, respectively), appear to be associated with an increased risk for tuberculosis, whereas the microsatellite alleles *AAGG_1* (OR, 0.12) and *AAGG_7* (OR, 0.26) appear to be associated with a decreased risk for tuberculosis. The *6A*^{4} allele differs at amino acid 219 from all other commonly found *SP-A* alleles (Trp for *6A*^{4} and Arg for others); the *1A*^{3} differs at amino acid 223 (Lys for *1A*^{3} and Gln for others, except for *1A*^{1}) from other alleles. These differences are located within the CRD of SP-A, a domain that is implicated in the binding of *M. tuberculosis* [10, 11]. Whether these amino acid differences within the CRD of SP-A have an impact on the binding of *M. tuberculosis* remains to be determined.

A nucleotide substitution (A/C) at the splice junction of intron 2–exon 3 of SP-B [18] is noted by the *B1013_A* marker allele. Although a transversion from C→A occurs frequently (74%–87%) at the specific position of the consensus splice sequence, its impact (if any) on splicing is unknown. The *AAGG_1* and *AAGG_7* marker alleles flank *SP-B* and may represent unknown *SP-B*–linked genes that are involved in the pathogenesis of tuberculosis.

Assessment of marker alleles of tuberculosis versus tuberculin–skin test positive (homogeneous) or general (heterogeneous) control groups: allele/allele interactions. To study whether the presence of marker allele X would affect the susceptibility to (or protection from) tuberculosis for persons with marker allele Y, we studied allele/allele interactions for alleles from the 2

Table 3. Tuberculosis ($n = 98$) vs. skin test–positive ($n = 63$) results for tuberculosis (multiple logistic regression analysis).

Allele	OR (95% CI)	*P*
DA11_C	2.81 (1.46–5.51)	.002
AAGG_2	0.201 (0.04–0.78)	.026
GATA_3	2.13 (1.09–4.23)	.027

NOTE. Max-rescaled $R^2 = 0.154$; $c = 0.695$. CI, confidence interval; OR, odds ratio.

Table 4. Tuberculosis (TB) group vs. healthy control group (univariate analysis).

Allele[a]	Percentage of TB *(n)*	Percentage of controls *(n)*	OR (90% CI)	*P*
AA50_G	62.6 (107)	75.3 (101)	0.55 (0.33–0.91)	.050
AA219_T	16.8 (107)	7.9 (101)	2.35 (1.12–4.92)	.057
$6A^2$	62.6 (107)	74.3 (101)	0.58 (0.35–0.95)	.072
$6A^4$	16.8 (107)	6.9 (101)	2.71 (1.25–5.87)	.033
$1A^3$	8.6 (105)	1.0 (100)	9.28 (1.61–53.39)	.018[b]
B1013_A	77.1 (105)	66.3 (101)	1.71 (1.02–2.86)	.086
B1580_C	75.7 (107)	86 (100)	0.50 (0.27–0.92)	.063
B9306_G	44.9 (107)	33.7 (101)	1.60 (1.00–2.56)	.099
D2S2232_7	1.9 (103)	7.1 (99)	0.26 (0.06–0.99)	.096[b]
AAGG_1	2.8 (106)	15.0 (100)	0.16 (0.05–0.48)	.002[b]
AAGG_7	35.9 (106)	51.0 (100)	0.53 (0.33–0.85)	.028
GATA_1	42.1 (107)	26.7 (101)	1.98 (1.21–3.24)	.021

NOTE. CI, confidence interval; *n,* no. of samples; OR, odds ratio.
[a] ⩾1 Copy.
[b] Fisher's exact test.

comparisons (tuberculosis vs. tuberculin–skin test positive and tuberculosis vs. general control) summarized in figure 1. We also assessed the *SP-A1* marker allele, *AA50_G,* which was significant in both sets of univariate comparisons. A similar OR was observed in both comparisons (tables 2 and 4).

Significant interactions for the tuberculosis versus tuberculin–skin test positive comparison were observed between alleles $1A^3$ and *B1013_A* ($P = .036$; figure 2). For the tuberculosis versus general control comparison, interactions were observed between alleles *AA50_G* and $1A^3$ ($P = .005$; figure 3). Because the *SP-A1* allele $6A^2$ is distinguished from all other *SP-A1* alleles by *AA50_G,* we wished to assess whether the 2 *(*$6A^2$ and *AA50_G)* marker alleles can be interchanged and, by extension, whether these alleles identify the same subgroup. Thus, we studied allele interactions ($1A^3$, X) in which we replaced *AA50_G* with allele $6A^2$. Figure 3 shows that similar results were observed in the respective allele interactions with either *AA50_G* or $6A^2$. For example, persons who have both alleles *AA50_G* and $1A^3$ or $6A^2$ and $1A^3$ always develop tuberculosis (100% of the time; figure 3), presumably under certain environmental conditions (i.e., after exposure to *M. tuberculosis*). These observations suggest that *AA50_G* and $6A^2$ identify the same subgroup. However, although the *P* value for the interactions shown in figure 3 is very low, caution should be exercised in the interpretation of this interaction, because the $1A^3$ allele is not one of the most common alleles [8] and also because the *P* value in the univariate analysis of tuberculosis versus tuberculin–skin test positive groups is not very low ($P = .095$). Therefore, a much larger sample size is needed to firmly establish this putative interaction.

For the observed interactions (figures 2 and 3), the effect on disease outcome (i.e., tuberculosis) is additive. For example, of the subjects with active tuberculosis, 73 (57.9%) of 126 subjects and 1 (33.3%) of 3 subjects had marker alleles $1A^3$ and *B1013_A*, respectively (figure 2). However, if both marker alleles are present in the same person, 8 (88.8%) of 9 subjects of the $1A^3(+)$/*B1013_A(+)* are identified with tuberculosis.

Discussion

M. tuberculosis as an intracellular pathogen employs several mechanisms to gain entry into macrophages to secure its survival [2]. Therefore, molecules that may promote its uptake by alveolar macrophages may be viewed as being advantageous for its survival but detrimental to the host's well being, whereas molecules that promote its clearance may be considered as detrimental for *M. tuberculosis* and advantageous to the host. Because SP-A and SP-D bind to *M. tuberculosis* [9–11] and to alveolar macrophages [10, 11], these molecules may play a role in *M. tuberculosis* infection. Moreover, because SP-B is essential for normal lung function [15], SP-B may play an indirect role in *M. tuberculosis* infection via its ability to further compromise lung function in the presence of *M. tuberculosis* in infection. Since several alleles have been described for *SP-A, SP-D,* and *SP-B,* in this study we investigated the hypothesis that certain *SP* alleles are found with higher frequency in persons with active tuberculosis. Such alleles may be viewed as contributors to tuberculosis and may explain, in part, the individual variability in the susceptibility to tuberculosis. The results revealed that certain *SP-A, SP-B, SP-D,* and microsatellite *SP-B*–linked marker alleles associate with increased or decreased risk for tuberculosis. These studies also revealed allele interactions with additive effects on disease outcome, indicating that the use of multiple markers may better predict risk for disease.

The marker alleles that appear to play a role in the pathogenesis of tuberculosis, as assessed by their association with increased or decreased risk for tuberculosis, are shown in figure 1. The *SP-D (DA11_C), SP-A2 (*$1A^3$*),* and *SP-A1 (*$6A^4$*)* alleles are distinguished from other alleles by amino acid differences. The *DA11_C* marker allele of SP-D has a threonine (ACG) at amino acid 11, whereas the *DA11_T* allele corresponds to a methionine. Although the significance of this change is unknown, this change may identify an allele that may aggregate *M. tuberculosis* more or less efficiently and thus have an impact on its clearance or on its uptake by alveolar macrophages [9]. SP-D agglutinates *M. tuberculosis* through its ability to bind, via its CRD, to terminal oligosaccharide of the lipoglycan, lipoarabinomannan, on the surface of the *M. tuberculosis.* On the basis of available information, it is unknown whether the agglutinated bacteria are cleared by the mucociliary system or taken up by the alveolar macrophages via unknown mechanisms and, by extension, whether SP-D is beneficial to *M. tu-*

Table 5. Tuberculosis group ($n = 98$) vs. control group ($n = 97$; multiple logistic regression analysis).

Allele	OR (95% CI)	*P*
$6A^4$	4.51 (1.56–15.47)	.008
$1A^3$	9.57 (1.62–183.20)	.038
B1013_A	2.36 (1.10–5.21)	.029
AAGG_ 1	0.12 (0.03–0.47)	.004
AAGG_7	0.26 (0.13–0.5)	.0001

NOTE. Variables were significant ($P < .10$) in univariate tests; $R^2 = 0.114$; $c = 0.695$. CI, confidence interval; OR, odds ratio.

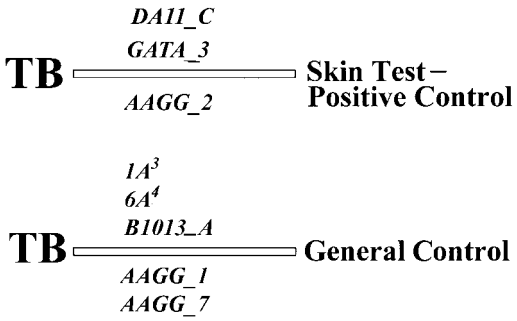

Figure 1. Summary of marker alleles of tuberculosis (TB) vs. various control groups (tables 3 and 5). Markers above bar are based on odds ratios (ORs) and may increase susceptibility to TB (OR, >1); markers below bar may decrease susceptibility to TB (OR, <1) in persons with certain genetic or environmental backgrounds.

berculosis or to the host [9]. However, the results from the present study indicate, on the basis of OR results, that the *SP-D* allele *(DA11_C)* may be a susceptibility factor for tuberculosis.

The *SP-A1 (6A*^{4}*)* allele and the *SP-A2 (1A*^{3}*)* alleles differ from most or all other alleles by amino acid 219 and amino acid 223 [5], respectively. Both of these amino acid differences are located within the CRD, which is implicated in the binding of *M. tuberculosis* [10, 11]. Should the amino acid differences (Trp for *6A*^{4} and Arg for other; Lys for *1A*^{3} and Gln for other, except *1A*^{1}) of *6A*^{4} and *1A*^{3} alleles within CRD have an impact on the efficiency of binding to *M. tuberculosis,* such differences may be magnified or minimized under certain circumstances. For example, a tryptophan *(6A*^{4}*)* is more susceptible to oxidation than an arginine [23]. Therefore, it is possible that amino acid 219 (Trp) of the *6A*^{4} allele in the presence of oxidative stress becomes oxidized, and this modification may have a further effect on functional differences among *SP-A* alleles. In addition, these 2 alleles *(SP-A1* [*6A*^{4}] and *SP-A2* [*1A*^{3}]) differ in amino acids that are located within the core sequence, which distinguishes *SP-A1* from *SP-A2* alleles [6], and these amino acids hold the potential for differential sensitivity to oxidation. These may have a further effect on the functional and/or structural capabilities of these alleles. In fact, oxidized SP-A, following in vitro or in vivo exposure to ozone, alters its structural and functional capabilities as assessed by its ability to bind carbohydrates and self-aggregate [24], its decreased ability to inhibit phosphatidylcholine secretion by type II cells [25], and its impaired interaction with alveolar macrophages [25].

Heterogeneity due to differences either in genetic background or environmental conditions is a major challenge in the study of human disease and is probably one source of apparent discrepancies in case-control study results in the literature. Each of the control groups in our study (i.e., tuberculin–skin test positive and general control) represents a slightly different group of subjects and may address different points. The tuberculin–skin test positive control group may be viewed as the resistant group because, despite exposure to mycobacterium, these persons did not develop disease. The general control, on the other hand, represents the general population, whose exposure and response to mycobacterium is unknown. Therefore, the findings from this comparison reflect findings that may be applicable to the general Mexican population rather than to a selective subgroup (i.e., more resistant to infection). In the present study, we observed differences in marker alleles associated with increased or decreased susceptibility to tuberculosis (figure 1), when the tuberculosis group was compared with a homogeneous control group (tuberculin–skin test positive) or to a heterogeneous general control, in which the positivity of the tuberculin skin test and living conditions were unknown. These differences indicate that caution should be exercised in the definition of experimental and control groups and that findings should be interpreted within the context of subject ascertainment and experimental design.

For the markers tested, there is a possibility that some results were significant by chance alone. For example, of the 35 markers considered in table 1, if each marker is tested individually at the α level of .05, the probability of observing $\geq$1 significant markers by chance is .83; for $\geq$3 markers, it is .25; and for $\geq$6 markers, .01. Three and 6 markers with $P < .05$ were identified in tables 2 and 3, respectively. Thus, some of the markers shown in tables 2 and 3 may be expected to be due to chance alone, and some may not. However, the probabilities (.25 and .01) are low.

In summary, we observed that certain *SP-A, SP-B, SP-D,* and *SP-B* flanking marker alleles associate with an increased or decreased risk for tuberculosis. We also observed interactions among some of these alleles with additive effects on the disease outcome. Furthermore, results obtained from the univariate, multivariate, and allele interaction analyses indicate that dif-

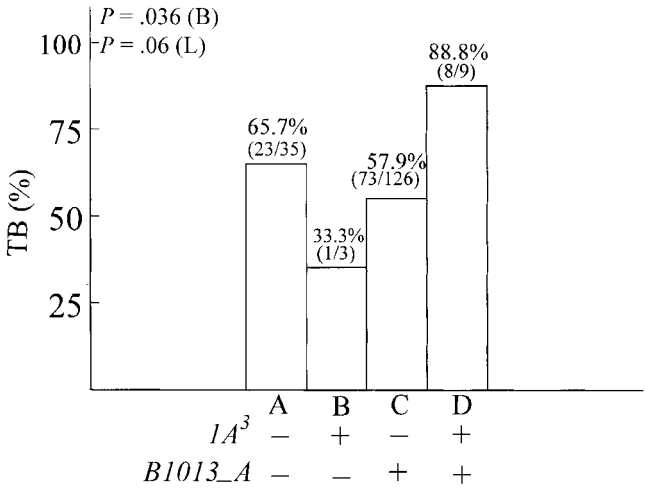

Figure 2. Interaction between marker alleles *1A*^{3} and *B1013_A* in tuberculosis (TB) vs. tuberculin–skin test positive controls. *P* values are from Breslow-Day test (B) or logistic regression (L). +, Presence of allele; −, absence of allele.

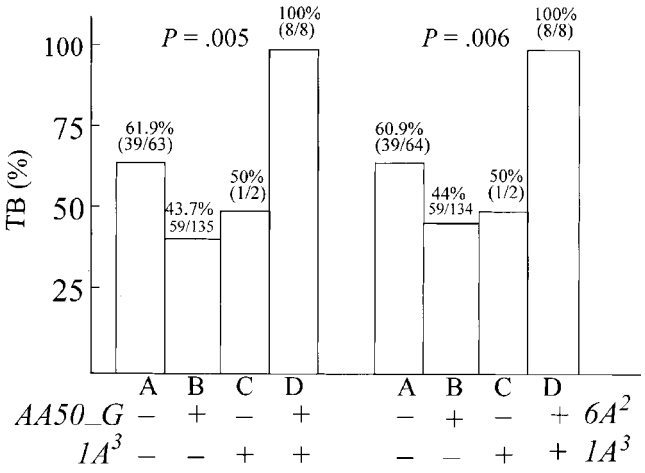

Figure 3. Interaction between marker alleles $1A^3$ and *AA50_G* or $6A^2$ in tuberculosis (TB) vs. general controls. *P* values are from Breslow-Day test. Figure depicts interchange of information between alleles *AA50_G* or $6A^2$ in their interaction with $1A^3$. Information obtained from allele *AA50_G* with its interaction with $1A^3$ is virtually identical to that obtained with allele $6A^2$ and its interaction with $1A^3$. Because $6A^2$ is distinguished from all other *SP-A1* alleles at amino acid 50 (AA50), it is likely that AA50 is entirely responsible for contribution of $6A^2$ to TB when $6A^2$ interacts with $1A^3$. +, Presence of allele; −, absence of allele.

ferences in genetic background or environment may have an effect on the disease outcome. Thus, clinical and/or genetic markers should be used to identify appropriate homogeneous groups for the study of human disease.

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