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HOST GENETICS OF SUSCEPTIBILITY

Cytokine genes are associated with tuberculin skin test response in a native Brazilian population

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SUMMARY

Tuberculosis was a major cause of population decline among Brazilian indigenous peoples and remains a leading cause of morbidity and mortality among them. Despite high BCG coverage, results of Tuberculin Skin Test (TST) reactivity have shown high rates of anergy in Amazonian Indians. Given the high prevalence of anergy in these populations and the fact that genetic host factors play an important role in susceptibility to *Mycobacterium tuberculosis* (MTB), the aim of this study was to evaluate the association of nineteen polymorphisms in fifteen genes related to immune response and anergy in the Xavante, an indigenous group from Brazil. A total of 481 individuals were investigated. TST anergy was observed in 69% of them. Polymorphisms in four genes showed absence or very low variability: *SP110*, *PTPN22*, *IL12RB1* and *IL6*. *IFNG* +874 A/T heterozygotes and *IL4*-590 C/C homozygotes were more frequent in those individuals who presented a positive TST (prevalence ratios of 1.9 and 2.0 respectively). The risk of anergy was 1.5 in *IL10*-1082 G/G homozygotes when compared to carriers for the A allele. In indigenous groups such as the Xavante exposure to a variety of infections, associated with specific genetic factors, may disturb the T-helper 1 and T-helper 2 balance leading to increased immunological susceptibility.

1. Introduction

Tuberculosis (TB) constitutes a major infectious disease, leading to 1.6 million deaths annually worldwide. The estimate is that about one third of the world's populations are currently infected with *Mycobacterium tuberculosis* (MTB), accounting for 8–10 million new cases each year.[1,2] The scenario of TB is also alarming in Brazil. The incidence rate was 50 per 100,000 in 2007, and the country ranks as the 14th most affected in the world.[1] TB incidence rates in Brazil can surpass 70 cases per 100,000 in some municipalities of the Amazon region.[3]

Although TB is a significant health problem for most of the world's populations, some have been specially affected. Incidence rates among indigenous groups tend to be higher than in the general population. In indigenous peoples from Brazil, TB has been historically a major cause of population decline and remains a leading cause of morbidity and mortality. Recent studies indicate that incidence rates among Amazonian indigenous peoples may be as much as ten times higher than those of the general Brazilian population.[4–6]

Environmental factors such as socioeconomic conditions and malnutrition play a role in TB susceptibility.[7] It has also been described that genetic factors related to both the bacterium and human host are also of relevance. The ability to develop adequate immunity to intracellular bacterial pathogens is unequally distributed in humans. Infection with MTB leads to disease in 5–10% of the exposed individuals, whereas the great majority successfully controls the infection process,[8] a situation known as latent infection. Most of the individuals infected with MTB develop a delayed-type hypersensitivity response in two to four weeks after infection. Immunologically speaking, this can be assessed by means of a positive response (skin induration) to intradermal injection with purified protein derivative (PPD) from MTB. PPD or tuberculin skin test (TST) is routinely used not only to identify subjects infected with MTB, but also to assess cell-mediated immune response to MTB in order to guide decisions about chemoprophylaxis and treatment.[9] However, its applicability is limited by lack of specificity, which might lead to false-positive results in individuals that were vaccinated with BCG or had been exposed to other mycobacteria.[10] The absence of skin induration following PPD intradermal injection,

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defined as anergy, is observed in many individuals. Immunologically, anergy involves an inability of T cells to produce interleukin-2 (IL-2),[11] which occurs together with a decreased production of interferon-γ (IFN-γ), particularly in severe disease.[9,12] Anergy has also been correlated with the expansion of IL-10-producing T cells.[12] The interpretation of TST results might also be affected by the immune status of the individual due to conditions that interfere with generalized cell-mediated delayed-type hypersensitivity responses, including HIV infection, chemotherapy, steroid use, and cancer.[9]

Studies which have investigated the characteristics of TST reactivity in Amazonian Indians have reported high rates of anergy, usually over 60%, even when relatively high BCG coverage is observed.[13–17] It has been suggested that the recurrent low reactivity to TST observed in Amazonian Indians, despite high BCG coverage and high incidence rates of the disease, may be due to unclear immunological mechanisms that may cause diminished cell-mediated immune response against MTB.[13,18,19]

Cytokines are important modulatory molecules in immune responses against microorganisms, and they also have an important role in the development of immunological disorders, mainly those related to Th1 (T-helper 1) or Th2 cell populations. The balance between these two types of cells affects cytokine expression and physiological and pathological processes as well.[20] Th1 cells preferentially produce cytokines as IFN-γ, TNF-α (tumor necrosis factor-α), IL-12 and IL-2. Th2 cells drive humoral immunity, up-regulating antibody production and producing IL-4 and IL-10.[21] Response against MTB is preferentially Th1-like, reflected by the production of high titers of IFN-γ and TNF-α with low or undetectable levels of IL-4.[22] IL-12 also leads the development of Th1 responses by enhancing IFN-γ and antagonizing IL-4 and IL-10, thereby down-regulating Th2 responses.[23] IL-4 and IL-10 are known to significantly inhibit the development of delayed-type hypersensitivity responses in mice[24]; IL-10 also inhibits Th1 activity through macrophage deactivation and the blocking of IFN-γ release by Th1 lymphocytes.[25]

Once infected with MTB, the risk of an individual to develop the disease is influenced by many factors, including nutritional status, presence of co-infections, exposure to other environmental microbes, and previous vaccination. However, it is clear that genetic host factors also play an important role in controlling disease susceptibility to intracellular pathogens.[8] Human immune response genes are more diverse than any other gene group, suggesting that infectious diseases have been important driving forces in human evolution.[26] Candidate genes studies have unveiled several immunogenetic polymorphisms associated with infectious diseases in humans. Candidate genes have been identified in several ways, most through studies of immune mechanisms of infectious diseases influenced by Major Histocompatibility Complex (MHC) and of cytokine genes.[27,28]

Several studies have been carried out in order to evaluate the association between immune response genes with susceptibility to infection by MTB or TB disease.[26,29–31] However, few investigations have explored the role of these genes on delayed-type hypersensitivity, and more specifically in PPD response or anergy. Given the high prevalence of PPD anergy in Amazonian Indians,[13,14,17] these populations offer a unique opportunity to investigate genes potentially involved with lack of response to TST. In this study we evaluate the association of nineteen single nucleotide polymorphisms (SNPs) in fifteen genes related to the immune response to PPD and anergy among the Xavante Indians of Central Brazil.

2. Materials and methods

2.1. Study population

The Xavante population comprised approximately 13,000 individuals at the time of fieldwork (July 2006), living in over 100 villages in seven reserves in the state of Mato Grosso, Central Brazil. Data collection for this study was conducted at the Pimentel Barbosa village (approximately 13°19′09″S; 51°40′36″W), which was the largest village in the reserve of the same name.

Permanent contact of the Xavante of Pimentel Barbosa with the national Brazilian society took place in the late 1940s. During the next two decades nearly half of the population died due to epidemics of infectious diseases.[32]

Current health conditions among the Xavante are characterized by high infant mortality (over 80 per 1000), mostly due to diarrhea and pneumonia, which account for 23% and 35% of all hospitalizations, respectively, and high prevalence rates of undernutrition (18.9% in children < 5 years of age).[32,33] Sanitary conditions are markedly deficient, intestinal parasitism is moderately prevalent, and pemphigus foliaceus is endemic, but with a few number of cases.[34,35] To the best of our knowledge HIV or HTLV infections were not reported in the study population.

TB has been an important cause of morbidity and mortality in the Xavante.[32,36] Analysis of TB morbidity from the Brazilian National Diseases Surveillance System (SINAN) databank and from the National Health Foundation (FUNASA) revealed that, during the period 1999–2004, 37 subjects from Pimentel Barbosa village were treated for TB. The mean rate of incidence for this period was 1289.6 per 100,000 inhabitants, with a balanced distribution by sex (51.4% female) and a clear majority of children $\leq$ 15 years of age (67.6%). Results from a recent TB survey carried out in 2006 among the Pimentel Barbosa Xavante indicated a moderately high annual risk of infection for TB (0.94%) and moderate prevalence of infection (16.6%), defined as TST $\geq$ 10 mm. It was also observed that, after controlling for a number of variables, age was the only predictor of TB infection, with individuals older than 15 years of age showing an adjusted odds-ratio of 20.0 (P.C. Basta, C.E.A. Coimbra, J.R. Welch, L.C. Alves, R.V. Santos and L.A. Camacho. Tuberculosis among the Xavante Indians of the Brazilian Amazon: An epidemiological and ethnographic assessment. Submitted).

2.2. Field research

The field research for the present study was carried out as part of a clinical and epidemiological investigation aiming to detect TB cases, following guidelines put forward by the American Thoracic Society.[10] All TST results used in this study were obtained during this opportunity. PPD reactivity was defined as induration at 72 h after inoculation. Each participant was injected intradermally on the volar side of the left forearm with 0.1 mL (2 TU) of purified protein derivative (PPD-RT23) (Statens Serum Institute, Copenhagen, Denmark). Tuberculin testing was done by a team of three nurses, each of whom read each test independently. The transverse diameter of indurations was measured at 72 h after inoculation. Agreement among measurements was high (intraclass correlation coefficient > 0.98). A total of 69% of the population tested were anergic (TST $= 0$ mm) (P.C. Basta, C.E.A. Coimbra, J.R. Welch, L.C. Alves, R.V. Santos and L.A. Camacho. Tuberculosis among the Xavante Indians of the Brazilian Amazon: An epidemiological and ethnographic assessment. Submitted).

The village population was 560 individuals, of whom 50.7% were female and 59.8% were children and adolescents $\leq$ 15 years of age. The population was distributed in 34 houses with an average of 20 persons per house (median $=$ 16; with a minimum of 4 and maximum of 42).

The study sample for the genetic analyses comprised 481 subjects (86% of the village population), all of whom had been clinically examined and tested with PPD. Among the studied sample 46.4% were males and age varied from 0.3 to 91.7 years, with an average of 18.8 $\pm$ 18.9 years. Exclusion criteria were pregnant women and children under 3 months.

Table 1
Polymorphisms investigated in this study.

Gene	Chromosome Location	Polymorphism	dbSNP ID
SLC11A1 (NRAMP1)	2q35	INT4 (496 + 14 G > C)	rs3731865
VDR	12q13.11	*Fok* I A > G	rs10735810
SP110	2q37.1	exon 11 C > T	rs3948464
		intron 6 C > T	rs2114592
P2X7	12q24	1513 A > C	rs3751143
PTPN22	1p13.3-p13.1	1858 C > T	rs2476601
IL1B	2q14	intron 2 C > T	rs1143629
IL12RB1	19p13.1	641 A > G	rs11575934
		1094 T > C	rs375947
IFNGR1	6q23.3	−611G > A	rs1327474
		−56 T > C	rs2234711
TNFR1	12p13.2	intron 1A > G	rs4149622
IFNG	12q14	+874 A > T	rs2430561
IL2	4q26-q27	−330 T > G	rs2069762
IL10	1q31-q32	−592 A > C	rs1800872
		−1082 A > G	rs1800896
IL6	7p21	−174 G > C	rs1800795
IL4	5q31.1	−590 T > C	rs2243250
IL4R	16p12.1-p11.2	1902 A > G	rs1801275

Ethical approval was obtained from the Brazilian National Committee on Research Ethics (CONEP). A signed informed consent was obtained from the village leaders since most subjects were not literate in Portuguese, but all subjects provided verbal assent to participate. A Xavante health agent, who helped explain the aims and scope of the study to individuals, accompanied all activities.

2.3. Genotyping

Genomic DNA was extracted from buccal swabs (BuccalAmp™ DNA Extraction Kit - Epicentre, Madison, WI, USA), and genotyping (except to *SLC11A1*) was carried out by TaqMan® SNP Genotyping Assay methods (Applied Biosystems, Foster City, USA). The *SLC11A1* polymorphism was genotyped by PCR-RFLP as described by Zhang and colleagues.[37] Polymorphisms investigated in this study are described in Table 1.

2.4. Statistical analysis

Allele and genotype frequencies of individual polymorphisms were determined by direct counting, and compared between groups (anergics vs. non-anergics) using exact χ^2 tests. Association between genotypes and response to PPD was also evaluated using prevalence ratios (PR) estimated by Poisson regression with robust variance, controlling for age as a continuous variable. Sex was not included in the regression model because it was not significant. The calculations were performed using the SPSS 16.0 program.

3. Results

Allele frequencies for the polymorphisms investigated, together with the frequencies reported for Europeans, Africans and Asians are shown in Table 2. Differences in the total number of Xavante individuals tested for each variant were due to genotyping problems. Among the 19 polymorphisms screened, six showed absence or very low variability (minor allele frequency $\leq$ 0.01) in the Xavante (*SP110* rs3948464, rs2114592; *PTPN22* rs2476601; *IL12RB1* rs11575934, rs375947; and *IL6* rs1800795) despite being polymorphic in other major ethnic groups.

Association tests of genotype frequencies between anergic vs. non-anergic subjects are shown in Table 3. For all markers with a χ^2 *P* value equal or lower than 0.2, prevalence ratios (PR) were estimated after adjustment for age. Reactivity to PPD was associated with *IFNG* +874A > T (rs2430561), and *IL4*-590 C > T (rs2243250) polymorphisms. The adjusted PRs for *IFNG* + 874 T/A and *IL4*-590 C/C genotypes were 1.9 (CI: 1.1–3.1) and 2.0 (CI: 1.2–3.3), respectively (Table 4). On the other hand, *IL10*-1082A > G (rs1800896) SNP was associated with a higher prevalence of anergy. The risk of anergy

Table 2
Allele frequencies for the polymorphisms in Xavante and their frequency range in world populations.

Gene	dbSNP ID	N*	Allele	MAF			
				Xavante	Africans (n†)	Europeans (n†)	Asians (n†)
SLC11A1	rs3731865	150	C	40	2–13 (2)	12–27 (2)	8–14 (4)
VDR	rs2228570	474	A	32	17–24 (4)	34–44 (5)	26–51 (4)
SP110	rs3948464	474	T	<1	11–27 (8)	8–15 (3)	0–1 (3)
	rs2114592	399	T	0	14–28 (5)	11 (1)	15–26 (3)
P2X7	rs3751143	473	C	15	0–11 (3)	14–29 (6)	21–24 (2)
PTPN22	rs2476601	466	T	0	3 (1)	1–21 (7)	2–6 (2)
IL1B	rs1143629	467	T	30	52–59 (5)	63–83 (4)	46–60 (8)
IL12RB1	rs11575934	473	G	1	10–24 (5)	30–38 (3)	34–49 (6)
	rs375947	465	C	1	23–75 (5)	30–72 (4)	27–52 (6)
IFNGR1	rs1327474	466	G	6	3–98 (4)	34–44 (4)	3–6 (3)
	rs2234711	468	C	22	40–47 (3)	35–55 (3)	44–50 (3)
TNFR1	rs4149622	475	G	5	43–65 (3)	0 (2)	10–13 (2)
IFNG	rs2430561	426	A	2	68–76 (1)	49–73 (3)	64–68 (2)
IL2	rs2069762	410	G	17	0–8 (4)	23–31 (4)	24–56 (7)
IL10	rs1800872	466	C	32	50–77 (4)	45–80 (5)	27–77 (7)
	rs1800896	465	G	13	31–33 (2)	40–62 (5)	4–39 (10)
IL6	rs1800795	459	C	0	0–4 (4)	20–57 (6)	0–40 (6)
IL4	rs2243250	417	C	16	17–29 (3)	62–90 (4)	22–89 (5)
IL4R	rs1801275	458	G	33	57–90 (4)	23–34 (5)	7–23 (3)

dbSNP is a public database of single nucleotide polymorphisms (SNPs) found at <http://www.ncbi.nlm.nih.gov/projects/SNP/>
rs: Reference SNP cluster 'rs' ID's are created by NCBI during periodic 'builds' of the database.
MAF: minor allele frequencies (%).

* Number of individuals tested.

† Number of studies published.Sources of allelic frequencies in world populations (Supplementary material): http://www.ncbi.nlm.nih.gov; Liu et al. (1995); Bellamy et al. (1998); Yang et al. (2000); Delgado et al. (2002); Li et al. (2002); Abe et al. (2003); López-Maderuelo et al. (2003); Rossouw et al. (2003); Scola et al. (2003); Bornman et al. (2004); Remus et al. (2004); Sellick et al. (2004); Uitterlinden et al. (2004); Gomez et al. (2005); Orozco et al. (2005); Shin et al. (2005); van Oene et al. (2005); Zhang et al. (2005); Amirzargar et al. (2006); Bulat-Kardum et al. (2006); Etokebe et al. (2006); Henao et al. (2006); Pierer et al. (2006); Shemon et al. (2006); Thye et al. (2006); Tosh et al. (2006); Vidyarani et al. (2006); Zammiti et al. (2006); Babb et al. (2007); Fernando et al. (2007); Kusuhara et al. (2007); Moreno et al. (2007); Prabhu Anand et al. (2007); Sahiratmadja et al. (2007); Stein et al. (2007); Selvaraj et al. (2008); Smith et al. (2008); Ates et al. (2009).

Table 3
Association of genotype frequencies with response to PPD.

Gene	dbSNP ID	Genotypes	PPD = 0	PPD > 0	*P* value[§]
SLC11A1	rs3731865	G/G	33	22	0.927
		G/C	40	30	
		C/C	14	11	
VDR	rs2228570	G/G	162	79	0.752
		G/A	117	50	
		A/A	47	19	
SP110	rs3948464	C/C	324	148	0.570
		C/T	2	0	
	rs2114592	C/C	266	133	–
P2X7	rs3751143	C/C	11	5	0.784
		C/A	76	31	
		A/A	237	113	
PTPN22	rs2476601	C/C	319	147	–
IL1B	rs1143629	C/C	160	66	0.458
		C/T	135	71	
		T/T	25	10	
IL12RB1	rs11575934	A/A	320	147	0.705
		A/G	3	1	
		G/G	2	0	
	rs375947	C/C	1	1	0.265
		C/T	6	0	
		T/T	311	146	
IFNGR1	rs1327474	A/A	284	128	0.756
		A/G	36	18	
	rs2234711	C/C	12	4	0.739
		C/T	119	53	
		T/T	188	92	
TNFR1	rs4149622	A/A	301	132	**0.200**
		A/G	23	17	
		G/G	2	0	
IFNG	rs2430561	T/T	277	133	**0.059**
		T/A	7	9	
IL2	rs2069762	T/T	189	91	0.532
		T/G	76	41	
		G/G	7	6	
IL10	rs1800872	C/C	15	4	0.596
		C/A	177	83	
		A/A	126	61	
	rs1800896	G/G	13	0	**0.042**
		G/A	64	33	
		A/A	241	114	
IL6	rs1800795	G/G	312	147	–
IL4	rs2243250	T/T	193	105	**0.045**
		T/C	73	29	
		C/C	7	10	
IL4R	rs1801275	G/G	33	19	0.586
		G/A	137	57	
		A/A	143	69	

In bold are the *P*-values equal or lower than 0.2.
[§] χ^2 test.

was 1.5 (CI: 1.2–1.7) in G/G homozygous individuals when compared with carriers of the A allele (Table 5).

4. Discussion

In the 1960s, human geneticist James Neel and colleagues studied the same Indian group investigated in this work, including some of those who still live at the Pimentel Barbosa village. At that time, the Xavante had not yet been exposed to BCG and just one person tested positive for PPD.[38] At present, although there is almost 90% BCG coverage in the population, a high percentage of anergy to PPD (69%) is observed (P.C. Basta, C.E.A. Coimbra, J.R. Welch, L.C. Alves, R.V. Santos and L.A. Camacho. Tuberculosis among the Xavante Indians of the Brazilian Amazon: An epidemiological and ethnographic assessment. Submitted). Despite this high prevalence of anergy to PPD, 16.6% of the individuals had indurations greater than 10 mm, an indication that they had probably been previously infected with MTB.

Allele frequencies observed in the Xavante population were different from those reported in other major ethnic groups (Table 2), and as was already mentioned *SP110*, *PTPN22*, *IL12RB1* and *IL6* SNPs were not polymorphic in this community. When these allele frequencies were compared with those described for Asians, with whom they share more recent common ancestors, we observed that for three SNPs, the minor allele presented higher frequencies and for eight SNPs lower prevalences of the minor allele was observed in the Xavante whereas the remaining eight polymorphisms presented allele frequencies in the same range of variation reported for Asian populations. It is clear, therefore, that the Xavante present a very distinctive pattern of frequencies for most of these systems. Since no information for other Amerindian samples has been reported for these polymorphisms, it is difficult to infer whether the variants lost and the other differences were due to microevolutionary processes or if these polymorphisms were not present in the founder group. The genetic structure of present-day Native American populations results from an intricate set of interacting factors which include different demographic changes, group fissions and fusions, intergroup contacts, and diverse mating systems. Therefore, to understand specific differences genetic results should be examined in conjunction with ethnohistoric data. The low genetic variability in these genes could be explained through a founder effect or by high inbreeding associated with isolation. The possibility also exists that these differences might be due to a restricted immune system shaped by natural selection. More native Brazilian populations should be investigated to disclose the full spectrum of variation of these immune response genes in Amerindians.

Three genes (*IFNG*, *IL10* and *IL4)* showed association with response to PPD. IFN-γ is a key Th1 cytokine in the immune response against MTB, because it activates macrophages that attack intracellular bacilli. IL-10 is an immunoregulatory Th2 cytokine produced by T cells, macrophages, and dendritic cells. It can deactivate macrophages and dampen the immune response to either prevent or limit the pathology from an over-exuberant inflammatory response to a given pathogen. It has been suggested that high IL-10 levels could be detrimental to bacterial control.[39] IL-4 is also a Th2 type cytokine which downregulates protective Th1 response.[40] Balikó and colleagues[41] found a significant higher ratio of IL-4 and IL-10 positive lymphocytes and a significant lower ratio

Table 4
Prevalence ratios for reaction to PPD.

Gene	dbSNP ID	Genotypes	PPD = 0 *N*(%)	PPD > 0 *N*(%)	Adjusted PR[**] (95% CI)	*P* value
TNFR1	rs4149622	A/A	301(92.3)	132(88.6)	0.8 (0.6–1.2)	0.314
		A/G	23(7.1)	17(11.4)		
		G/G	2(0.6)	0(0)		
IFNG	rs2430561	T/A	7(2.5)	9(6.3)	1.9 (1.1–3.1)	0.020
		T/T	277(97.5)	133(93.7)		
IL4	rs2243250	C/C	7(2.6)	10(6.9)	2.0 (1.2–3.3)	0.007
		C/T	73(26.7)	29(20.1)		
		T/T	193(70.7)	105(72.9)		

PR: prevalence ratio. Assuming a dominance model: *TNFR1* A/A vs. A/G and A/A genotypes; *IFNG* T/A vs. T/T genotype; *IL4* C/C vs. T/C and T/T genotypes.
[**] Adjusted for age.

Table 5
Prevalence ratios for anergy to PPD.

Gene	dbSNP ID	Genotypes	PPD = 0 *N*(%)	PPD > 0 *N*(%)	Adjusted PR[††] (95% CI)	*P* value
IL10	rs1800896	G/G	13(4.1)	0(0)	1.5 (1.2–1.7)	<0.001
		G/A	64(20.1)	33(22.4)		
		A/A	241(75.8)	114(77.6)		

PR: prevalence ratio. Assuming a dominance model: *IL10* G/G vs. G/A and A/A genotypes.
[††] Adjusted for age.

of IL-12 in the peripheral blood of PPD anergic patients with active TB when compared with TB patients that were PPD positive or healthy donors. These findings suggest a Th2 biased immune response during the early course of the disease because there was no significant correlation between the severity of pulmonary TB and the in vitro parameters examined. Unless tuberculin reactivity was also considered. Montiel and colleagues,[42] based on their observation of an increase in IL-4 concentrations together with low concentrations of IFN-γ in anergic patients to PPD, suggested that a Th2 pattern of immune response is associated with anergy.

Two previous investigations reported that the G allele of *IL10*-1082 was associated with higher production of IL-10 in TB patients with anergy to PPD.[43,44] Therefore the higher prevalence of *IL10*-1082 G/G genotype in PPD anergic Xavante individuals is in line with these observations. The higher prevalence of *IL4*-590 C homozygous subjects among those who developed skin reactivity to PPD observed in our study (prevalence ratio of 2.0; CI: 1.2–3.3) points in the same direction since its alternative allele (T) is associated with increased IL-4 levels.[45,46] *IL4*-590 C homozygotes would have lower IL-4 levels and a Th1 pattern of response that would explain the positive PPD reaction more frequently observed in them.

Some studies have shown that the *IFNG* +874A > T polymorphism influences IFN-γ production by providing a binding site for NFκB. The A allele was found to predispose to TB in several populations.[47–50] Electrophoretic mobility shift assays showed specific binding of NFκB to the allelic sequence containing the +874T allele.[48] Since this transcription factor induces IFN-γ expression, the +874T and +874A alleles are probably correlated with high and low IFN-γ expression, respectively.[50] One study provided evidence that PPD-induced IFN-γ was significantly lower in homozygotes for the A allele when compared to those carrying one or two copies of T allele.[49] In another study, PPD reactivity was found to be significantly higher in T/T than in the A/A genotypes, with T/A patients showing an intermediate response.[51] Although we did not observe A/A homozygotes, we found an association between T/A genotype and response to PPD. Etokebe and colleagues[52] suggested that the *IFNG* +874A > T polymorphism might play a role in disease severity rather than in disease susceptibility. Clearly more studies are warranted to disclose the functional effect of these SNPs on cytokine levels and their effect on anergy and/or TB susceptibility.

If at the community level, socioeconomic and epidemiologic (including access to adequate health services) factors play the leading roles in TB morbidity and mortality, at the individual level the containment and cure of TB requires an effective cell-mediated immune response. Moreover, the absence of delayed-type hypersensitivity responses to mycobacterial antigens during active TB infection can be associated with poor clinical outcome.[11] If delayed-type hypersensitivity is indeed an important Th1 marker,[53] anergy could be due to a diminished production of Th1 cytokines and an associated increase in Th2 cytokines. Our results indicate that polymorphisms in Th2 immune profile genes (*IL4* and *IL10)* might be involved with positive and negative responses to PPD, respectively. In this context, knowledge about immunological mechanisms involved in maintaining a latent infection is crucial to the understanding of the clinical outcome of MTB infection. In Amazonian indigenous groups with a high prevalence of a variety of infections, the Th1/Th2 balance may be disturbed leading to eventual increased susceptibility to a given pathogen.[54] In addition, their sometimes unique gene pool may contribute to susceptibility or resistance to agents of disease in a way that differs from those of large, urban populations. Thus, it is important to investigate other immune response polymorphisms in indigenous communities. Although we sampled almost all individuals in the population the number of individuals with susceptibility alleles are low. Notice however, that this is the first study to address this question in Native Brazilians, therefore new studies in other Native Brazilian populations are warranted. In relation to human genetics studies of TB in Amazonian native groups, special attention should be given to individuals who have been exposed to MTB but who are anergic to PPD and had not developed the disease. They may help us to understand the immunological changes occurring in latent infection and that are associated with cell-mediated immune responses to MTB.

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Supplementary material

Supplemental data associated with this article can be found in online version at doi:10.1016/j.tube.2009.11.002.

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