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ORIGINAL ARTICLE

Patients Exposed to *Mycobacterium tuberculosis* Infection with a Prominent IgE Response

Zaida Araujo,[a] Francesca Giampietro,[a] Bruno Rivas-Santiago,[b] Julieta Luna-Herrera,[c] Albina Wide,[d] Wilman Clark,[e] and Jacobus Henry de Waard[f]

[a]*Laboratorio de Inmunología de Enfermedades Infecciosas, Instituto de Biomedicina, Universidad Central de Venezuela, Caracas, Venezuela*
[b]*Unidad de Investigación Médica Zacatecas, Instituto Mexicano del Seguro Social, Zacatecas, Mexico*
[c]*Escuela Nacional de Ciencias Biológicas del Instituto Politécnico Nacional, Ciudad de México, Mexico*
[d]*Cátedra de Parasitología, Instituto de Medicina Tropical, Facultad de Medicina, Universidad Central de Venezuela, Caracas, Venezuela*
[e]*Unidad de Informática, Instituto de Biomedicina, Caracas, Venezuela*
[f]*Laboratorio de Tuberculosis, Instituto de Biomedicina, Universidad Central de Venezuela, Caracas, Venezuela*

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Background and Aims. Even though it has been reported that chronic immune activation associated with intestinal helminthic infections results in a predominant IgE response, specific IgE antibodies that are also interleukin 4 (IL-4) dependent have been reported in tuberculosis patients; however, this fact has not been widely reported. This study was aimed at investigating the levels of circulating IgE in Warao (an indigenous population) of the Orinoco river delta, an area isolated from contact with the tubercle bacillus for millennia until the mid-1960s as compared to Creole (nonindigenous population).

Methods. A total of 294 individuals were studied, 161 Warao and 136 Creole. Patient group was comprised of 86 Warao patients (WP) and 60 Creole patients (CP). Control group was comprised of 75 Warao controls (WC) and 76 Creole controls (CC). Total serum IgE and IgE and IgG_4 reactivities to *M. tuberculosis* antigens were measured by an enzyme-linked immunosorbent assay (ELISA).

Results. Levels of total serum IgE were significantly elevated in WP (13002.0 ± 11200.0 IU/mL) and WC (2763.5 ± 2596.2 IU/mL) than in CP (385.9 ± 155.1 IU/mL) and CC (356.6 ± 157.5 IU/mL) ($p < 0.0001$). Anti-PPD and anti-H37Rv IgE were significantly higher in WP (0.240 ± 0.145 and 0.230 ± 0.155) than in CP (0.127 ± 0.152 and 0.97 ± 0.103, respectively) and also between WC (0.240 ± 0.273 and 0.147 ± 0.158) and CC (0.115 ± 0.136 and 0.43 ± 0.46, respectively) ($p < 0.0001$). Anti-PPD and anti-H37Rv IgG_4 did not show differences among groups; however, anti-H37Rv IgG_4 was affected by anti-TB treatment, which could be predictive of treatment outcome.

Conclusions. The findings suggest that for the Warao population there is an intrinsic propensity to produce a high IgE response, which could be incompatible with the protective response to *M. tuberculosis*. © 2012 IMSS. Published by Elsevier Inc.

Key Words: Tuberculosis, Immunoglobulin E, Immunoglobulin G_4, Derived purified protein derivate, *M. tuberculosis* H37Rv sonicate.

Introduction

It is widely accepted that protection against tuberculosis (TB) is provided by the formation of Th1 immune response, which is characterized mainly by the production of interferon-gamma (IFN-γ), interleukin 2 (IL-2) and tumor necrosis factor-alpha (TNF-α). However, Th2 immune response characterized by the production of interleukin 4 (IL-4) and interleukin 5 (IL-5) is also present; the role of IL-4 in the generation and regulation of antigen (Ag)-driven isotype responses has been determined. The findings support the concept that IgE and IgG4 productions are

Address reprint requests to: Zaida Araujo, Laboratorio de Inmunología de Enfermedades Infecciosas, Instituto de Biomedicina, Universidad Central de Venezuela, Caracas, Venezuela; Phone: +58-212-860-4636; FAX: +58-212-861-1258; E-mail: zaraujogarcia@yahoo.com

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linked and related to the quantities of IL-4 induced by Ag-specific T cells (1). Even though it has been reported that chronic immune activation associated with intestinal helminthic infections results in a predominant IgE response (2), specific IgE antibodies that are IL-4 dependent have also been reported in patients with TB (3–5). In this context, IgE antibodies were detected in the sera from 500 patients with TB by using the ELISA assay with PPD and ultrasonic disintegrated *M. tuberculosis* H37Rv (sonicate). These antigens recognized by IgE antibodies were found to be localized in the cell wall of mycobacteria (6). Taken together, a study documented high serum total IgE concentrations in adolescent patients in South Africa, a community with a high incidence of TB and a high rate of parasite infestation (7). Several authors found a significant association between intestinal helminthic infections and mycobacterial diseases (1,8,9).

On the other hand, TB has emerged as a prime example of the role of genetic factors (10). It has been reported that the annual death rate from TB reached 10% when the disease first became prevalent in the Qu'appelle Valley Indian reservation in Canada, eliminating half the Indian families in the first three generations, yet 40 years later the annual death rate dwindled to 0.2%, suggesting selection for host resistance (11). Different genetic backgrounds between Venezuelan Warao and Creole populations exist. Immunological studies performed in Warao population confirmed inheritance and segregation of DW, a class II HLA antigen (human leukocyte antigen), DW 8.3, DW 16 and DW 22, defined only with homozygous typing cells of Warao origin (12). In regard to the HLA class I and class II allele and haplotype, distributions among Venezuelan Creoles showed that genes of Mongoloid, Negroid, and Caucasoid origin have created a distinctive HLA genetic profile in this hybrid Creole population (13). Because the role of genetic and nongenetic factors in influencing the incidence of pulmonary tuberculosis in a population is accepted, we wanted to study the levels of circulating IgE in the Warao people from the indigenous population of the Orinoco river delta and the forest of the Orinoco delta, an area isolated from contact with the tubercle bacillus for millennia until the mid-1960s as compared to a nonindigenous population.

Subjects and Methods

Study Participants

A prospective trial was performed and included the Warao people living in a rural area. Within the Warao indigenous population, a high prevalence of active TB is observed, indicating a high susceptibility to disease even among bacille Calmette-Guerin-vaccinated individuals (14). The humoral immune response of the Warao people was compared with individual residents of the capital of Venezuela (urban area), nonindigenous persons from the Creole population. A total of 297 adults aged 15 to 60 years were studied: 161 Warao and 136 Creole. Individuals were grouped according to patient group: Warao patients (WP, $n = 86$) and Creole patients (CP, $n = 60$). These individuals had TB disease activity according to the clinical evaluation. The control group consisted of Warao healthy controls (WC, $n = 75$) and Creole healthy controls (CC, $n = 76$). These individuals were without symptoms or without TB disease activity.

Clinical and Inclusion and Exclusion Criteria

All patients had clinical features consistent with TB supported by typical chest x-ray findings where the clinical evaluation included recent weight loss or inadequate progress of weight gain, prolonged febrile syndrome, night sweats, coughing or wheezing for more than 2 weeks and positive cultures for *M. tuberculosis*. For confirmatory diagnosis of TB, sputum smears were stained by the Ziehl-Neelsen direct method. For each specimen (sputum), two tubes of modified Ogawa egg medium and Lowenstein-Jensen were inoculated, using a method reported by Kudoh and Kudoh (15). Exclusion criteria included patients and healthy controls who were HIV positive, patients taking immunosuppressive drugs (e.g., corticosteroids, azathioprine and cyclophosphamide) and participants who did not sign an informed consent. Specific treatments were initiated in all newly identified cases of tuberculosis prior to studies and according to the norms of the National Tuberculosis Program (16).

This study was approved by the Ethics Committee of the Institute of Biomedicine (protocol No. 09–006256–2006/18/09/06), and written informed consent was voluntarily signed by all patients and control subjects.

Skin Test and BCG Vaccination Status

Tuberculin skin test (TST) was performed on all study individuals using a test previously described: tuberculin-purified protein derivative (0–1 mL; 2 tuberculin units; Statens Seruminstitut, Copenhagen) was injected intradermally on the right forearm. The criterion for positive test reactivity was based on measurements of induration values >10 mm and, after 72 h, the diameter of induration was measured (17). Vaccination status was inferred from the presence of BCG scars according to the National Tuberculosis Program, which recommends international BCG immunization policies (18).

Isotype Reactivities

Multiple antigen blot assay (MABA) test was used to confirm mycobacteria-purified protein derivative (PPD) and H37Rv (sonicate) as antigens for the purposes of studying IgE and IgG4 isotype reactivities (19). Briefly, MABA permits the simultaneous screening of different antigens, sera based on a dot-blot ELISA test and using

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an acrylic device (Minibloter 28 S-L, Immunetics Inc., Cambridge, MA) containing at least six parallel troughs. Antigens were distributed and immobilized onto a nitrocellulose membrane. Strips were then cut perpendicularly and exposed to immune samples (sera diluted 1:50) for 1 h at 37°C. Anti-human IgE biotin-labeled (Vector Laboratories, Burlingame, CA) and peroxidase-conjugated monoclonal antibody anti-IgG4 (AP009, The Binding Site, UK) were used as secondary antibodies.

Total IgE Levels

Concentrations were determined in serum by an enzyme-linked immunosorbent assay (ELISA) as previously reported (20). Briefly, polystyrene 96-well microtiter plates (Immunolon, Birmingham, UK) were coated at 4°C overnight with anti-human IgE, Epsilon Chain Specific (Vector Laboratories) in carbonate−bicarbonate buffer (pH 9.6). Plates were blocked with 1% BSA in PBS at 37°C for 1 h and washed with PBS. Serum was diluted 1:10 in PBS containing 1% BSA and added to coated wells in duplicate and incubated for 1 h at 37°C. Positive and negative control sera were included in duplicate. Goat anti-human immunoglobulin E conjugated with biotin (IgE biotin-labeled, Vector Laboratories) was used as secondary antibody. Enzyme activity was amplified by incubation for 1 h at room temperature with streptavidin-HRP. Ten mg o-phenylenediamine (OPD) dihydrochloride (Sigma-Aldrich, St. Louis, MO) was used as substrate. Optical density (OD) at 492 nm was measured with an automatic microplate reader. Standard curves were generated for calculating concentration of total IgE. Levels of total serum IgE are shown as international units (IU), and normal values are between 50 and 200 IU/mL.

Antibody Responses

ELISA was also performed for the measurement of IgE reactivity against PPD and H37Rv as previously mentioned. Briefly, 96-well microtiter plates (Immunolon) were coated with PPD (Statens Seruminstitut) or *Mycobacterium tuberculosis* H37Rv strain sonicate (Instituto Politécnico Nacional de México) antigen, (1 μg/well) overnight at 4°C. Samples diluted 1:10 were added and plates were incubated for 1 h at 37°C. Biotin-labeled anti-human IgE (Vector Laboratories) was used as secondary antibody, and enzyme activity was amplified by incubation streptavidin-HRP.

IgG_4 reactivity against PPD and H37Rv antigens was determined by a similar ELISA as previously mentioned for IgE. Sera were added at 1:50 and peroxidase-conjugated monoclonal antibody anti-IgG_4 (AP009, The Binding Site) was used as the secondary antibody.

Other Serological Markers

HIV diagnostic testing was done with the Passive Particle Agglutination Test for the detection of antibodies to HIV-1 and/or HIV-2 of FUJIREBIO Diagnostics (Abbott Laboratorie−Dainabot Co. Ltd., Tokyo, Japan).

Statistical Analysis

The differences of the isotype reactivities and the levels of total serum IgE were compared among groups using non-parametric tests: Kruskal−Wallis test and Mann−Whitney U test, respectively. In this regard, reactivities before and after treatment were compared using Wilcoxon signed rank test. Comparisons of the average age among groups were done by Student t test, and Fisher's exact test was used to compare the significance of the differences between the percentage values of individuals positive and negative for the TST; p values <0.05 were considered significant.

Results

Age and Microbiological Methods

The age results are shown as mean ± SD. Average age was 32.86 ± 12.45 years, 42.25 ± 23.13 years, 36.25 ± 11.9 years and 33.11 ± 6.02 years for WP, WC, CP and CC, respectively. There was a statistically significant difference between WC and WP ($p < 0.0009$) and CC ($p < 0.007$). Sensitivities of the smear tests were 70.0% and 64.0% for the WP and CP, respectively, whereas smear plus culture tests showed a sensitivity of 100.0% (Table 1).

Tuberculin Skin Response

When skin test reactivity was carried out in order to study the delayed-type hypersensitivity (DTH), >10-mm reactions were found in 80/86 (93.0%) and 47/60 (78.3%) of

Table 1. Age and bacteriological and immunological markers between Warao and Creole groups

	Patients		Controls	
Marker	Warao	Creole	Warao	Creole
Age (years)	32.8 ± 12.4[a]	36.2 ± 11.9	42.2 ± 23.1[b]	33.1 ± 6.0[c]
$Smear^+$ (%)	70.0	64.0	0	0
$Smear^+$ + culture (%)	100.0	100.0	0	0
TST+ (%)	93.0[d]	78.3[e]	70.6	44.7
Anti-PPD IgE (%)	62.0	16.0	64.0	80.0
Anti-H37Rv IgE (%)	72.0	28.0	61.4	93.0
Anti-H37Rv IgG_4 (%)	40.6	16.6	74.7	81.6

Age results are shown as mean ± SD. There was a statistically significant difference for the average age between a,b and b,c. TB cases were confirmed through Ziehl-Neelsen-stained smears and microbiological cultures. Tuberculin skin tests (TSTs) were performed using 2 tuberculin units of PPD of *M. tuberculosis*, strain RT-23 from the Statens Serum Institut in Copenhagen. There was a statistically significant difference for the positive group for the TST between d,e.

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the untreated WP (TST+) and CP (TST+), respectively. There was a statistically significant difference between the patient groups ($p < 0.01$). Within the control groups, positive reactions were found in 53/75 (70.6%) and 34/76 (44.7%) of the healthy controls; WC (TST+) and CC (TST+), respectively. There was no statistically significant difference between the WC and CC groups (Table 1). Patients and controls <20 years of age were highly likely to have received one BCG vaccination as neonates and/or during childhood as part of the increasingly effective Venezuelan national BCG vaccination program (data not shown).

Total Serum IgE

Figure 1 shows the unequal distribution of levels of total serum IgE between Warao and Creole groups. Results are shown as mean of levels of total serum IgE (international unit/mL, IU/mL) ± standard deviation (mean ± SD). The production of total serum IgE was significantly elevated in WP (13,548.5 ± 11,200.2 IU/mL) than in WC (2763.5 ± 2596.2 IU/mL), CP (385.91 ± 155.1 IU/mL) and CC (356.6 ± 157.5 IU/mL), $p < 0.0001$. Levels of total serum IgE were also considerably higher in WC than in Creole groups; CP and CC, $p < 0.0001$ (Figure 1).

In regard to the levels of total serum IgE and individuals positive and negative for the TST, the findings showed that there were statistically significant differences between Warao and Creole groups, independent of the TST status (data not shown). When levels of total serum IgE were measured after treatment in a subset of Warao patients ($n = 40$), it was found that these levels did not differ significantly from pretreatment levels (data not shown).

IgE and IgG$_4$ Antibodies

Figure 2 shows IgE reactivity against PPD and H37Rv antigens according to the TST status. The results are shown as mean optical density (OD) ± standard deviation. Evaluation of IgE reactivity against PPD showed significantly

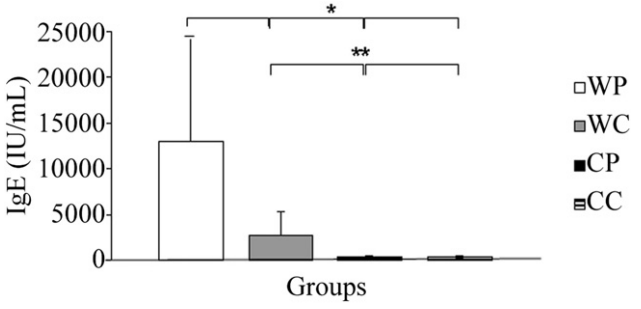

Figure 1. Total IgE levels. Data representing mean ± SD of levels of total serum IgE (IU/mL) in each group; (*) represents the significance between Warao patients (WP) $n = 86$ (□) and the rest of the groups. Warao controls WC, $n = 75$ (■) Creole patients CP, $n = 60$ (■) and Creole controls CC, $n = 76$ (▤). (**) represent the significance between WC and both Creole groups.

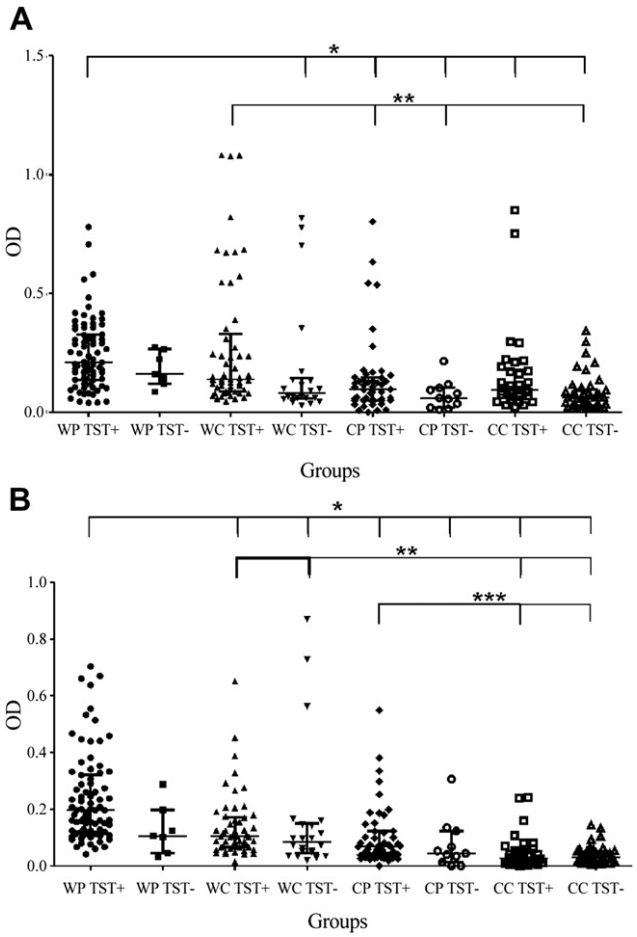

Figure 2. Reactivity detections of anti-PPD and anti-H37Rv IgE concerning tuberculin skin test status. Data representing mean OD ± SD of IgE reactivity. Warao groups: patients (WP) and controls (WC). Creole groups: patients (CP) and controls (CC). Tuberculin skin test (TST). WP (TST+), $n = 79$ (●), WP (TST−), $n = 7$ (■), WC (TST+), $n = 53$ (▲), WC (TST−), $n = 22$ (▼), CP (TST+), $n = 49$ (◆), CP (TST−), $n = 11$ (○), CC (TST+), $n = 34$ (□) and CC (TST−), $n = 42$ (△). (A) For anti-PPD IgE, (*) represents the significance between WP (TST+) and WC (TST−), CP (TST+), CP (TST−), CC (TST+) and CC (TST−), $p < 0.0001$. (**) represents the significance between WC (TST+) and CP (TST+), CP (TST−) and CC (TST−), $p < 0.0001$. (B) For anti-H37Rv IgE, (*) represents the significance between WP (TST+) and WC (TST+), WC (TST−), CP (TST+), CP (TST−), CC (TST+) and CC (TST−), $0.0001 < p < 0.0002$. (**) represents the significance between WC (TST+) and WC (TST−) and CC (TST+) and CC (TST−), $p < 0.0001$. (***) represents the significance between CP (TST+) and CC (TST+) and CC (TST), $p < 0.04$.

higher anti-PPD IgE in WP (TST+) (0.245 ± 0.148) than in WC (TST) (0.185 ± 0.246), CP (TST+) (0.139 ± 0.164), CP (TST−) (0.073 ± 0.059), CC (TST+) (0.155 ± 0.179) and CC (TST−) (0.082 ± 0.076), $p < 0.0001$. Significantly higher anti-PPD IgE was also observed in WC (TST+) (0.273 ± 0.281) than in CP (TST+) (0.139 ± 0.164), CP (TST−) (0.0734 ± 0.059) and CC (TST−) (0.082 ± 0.076), $p < 0.0001$ (Figure 2A).

With regard to IgE reactivity against H37Rv, it was significantly higher in WP (TST+) (0.239 ± 0.156) than

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in WC (TST+) (0.138 ± 0.115), WC (TST−) (0.168 ± 0.233), CP (TST+) (0.102 ± 0.106) CP (TST−) (0.074 ± 0.088), CC (TST+) (0.046 ± 0.059) and CC (TST−) (0.039 ± 0.031), $0.0001 < p < 0.0002$. Higher anti-H37Rv IgE was also observed in WC (TST+) (0.138 ± 0.115) and WC (TST) (0.168 ± 0.233) than in CC (TST+) (0.046 ± 0.059) and CC (TST−) (0.039 ± 0.031), $p < 0.001$ and also between CP (TST+) (0.102 ± 0.106) and CC (TST+) (0.046 ± 0.059) and CC (TST-) (0.039 ± 0.031), $p < 0.04$ (Figure 2B).

Figure 3 shows IgG_4 reactivity against H37Rv according to the TST status. The means of OD ± SD obtained for the anti-H37Rv IgG_4 did not show statistically significant differences between Warao and Creole groups; WP (TST+) (0.292 ± 0.302), WP (TST−) (0.288 ± 0.293), WC (TST+) (0.242 ± 0.231), WC (TST−) (0.181 ± 0.226), CP (TST+) (0.189 ± 0.233), CP (TST−) (0.135 ± 0.177), CC (TST+) (0.224 ± 0.211) and CC (TST−) (0.155 ± 0.182) (Figure 3).

Reactivity and Treatment

Reactivity before and after treatments is shown in Figure 4. Measurements of IgE reactivities against PPD and H37Rv antigens and IgG_4 reactivity against H37Rv were compared before and after treatments in a subset of Warao patients. Posttreatment results showed that anti-PPD IgE was higher (0.645 ± 0.411) than before (0.245 ± 0.148), $p < 0.001$ (Figure 4A). Pretreatment results of anti-H37Rv IgE did not differ significantly (0.239 ± 0.156) from posttreatment (0.232 ± 0.180) (Figure 4B), whereas after treatment, a lower anti-H37Rv IgG4 (0.077 ± 0.048) was observed than before (0.292 ± 0.302), $p < 0.004$ (Figure 4C). Anti-PPD IgG4 was not detected.

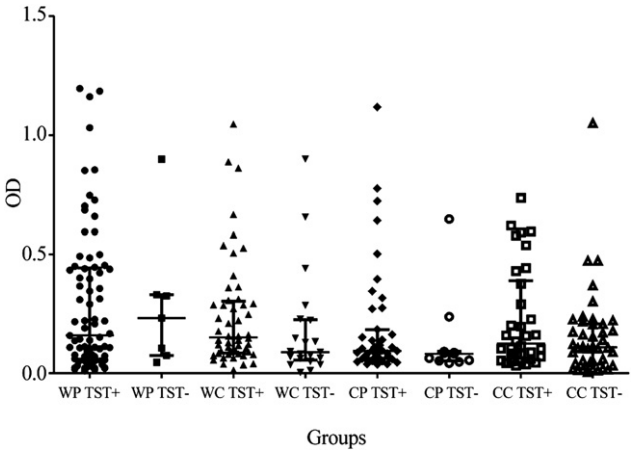

Figure 3. IgG4 reactivity against H37Rv according to the tuberculin skin test. Data representing mean OD ± SD in regard to IgG4 reactivity against H37Rv. Warao groups: Patients (WP) and controls (WC). Creole groups: Patients (CP) and controls (CC). Tuberculin skin test (TST). WP (TST+), $n = 79$ (●), WP (TST), $n = 7$ (■), WC (TST+), $n = 53$ (▲), WC (TST−), $n = 22$ (▼), CP (TST+), $n = 49$ (◆), CP (TST−), $n = 11$ (○), CC (TST+), $n = 34$ (□) and CC (TST−), $n = 42$ (△). IgG_4 reactivity against H37Rv antigen did not show differences among groups.

Discussion

Venezuelan Health Services reported among nonindigenous populations a rate of between 26.1 and 24.8 and among indigenous populations a rate of between 155.6 and 129.4/100,000 inhabitants between 1997 and 2001 (16). Since 1999, the state of Delta Amacuro has presented the highest rates, between 93.2 and 81/100,000 inhabitants. Of these, 90% of the cases were present in the Warao population with a very high prevalence of adult TB (16,18). In the present study, observational studies on humoral immune response suggest differences in immunological responsiveness to *M. tuberculosis* infection in the Warao population as compared to Creole residents of the capital of Venezuela. Warao individuals had higher levels of total serum IgE and IgE reactivities against PPD and H37Rv antigens than in Creole, so that the Warao/Creole population heterogeneity of antigen recognition was significantly associated with high IgE profile in the Warao people. The latter probably may be in relation to the findings about human leukocyte antigen (HLA), confirming the existence of the DR/DW dissociation previously observed in North American Indian, Japanese and Caucasian populations and showing inheritance and segregation of DW specificities (DW 8.3, DW 16, DW 22) defined only with homozygous typing cells of Warao origin. These data illustrated HLA haplotypes, linkage-disequilibrium, and DR/DQ associations not seen previously in other human populations (12). It has been reported that patients with active TB clearly had high *M. tuberculosis* specific IgE antibodies that are IL-4 dependent (3,4,6). In this context, we investigated the ability of PBMCs from Warao and Creole individuals with TB to secrete cytokines; the findings revealed that PPD-induced responses observed in patients from both populations could be divided into two groups, one group that activates Creole PBMCs to preferentially secrete TNF-α, IL-12 and IFN-γ and another group that activates preferential secretion of IL-4 and IL-5 in Warao PBMCs (21).

On the other hand, data from a study demonstrated that chronic immune activation associated with intestinal helminthic infections results in a predominant total IgE response (2). It is then possible to assume that PPD or H37Rv-specific IgE is produced preemptively in Warao individuals by chronic exposure TB, whereas high levels of total serum IgE may be the result of co-infection with intestinal parasites. In a preliminary study we found that, in the Warao population, both children and adults were polyparasitized with protozoa and helminthic parasites as, for example, *Entamoeba coli*, and *Ascaris lumbricoides* and/or Ancylostomidae, respectively (22). Taken together, chronic exposure to TB and co-infection with intestinal parasites contribute to the poorer outcome of infection by polarizing the immune response towards IgE response, which is IL-4 dependent as suggested by several authors (3,4,6). The latter is in relation with our results for the Warao population where Warao PBMCs stimulated secrete preferentially IL-4

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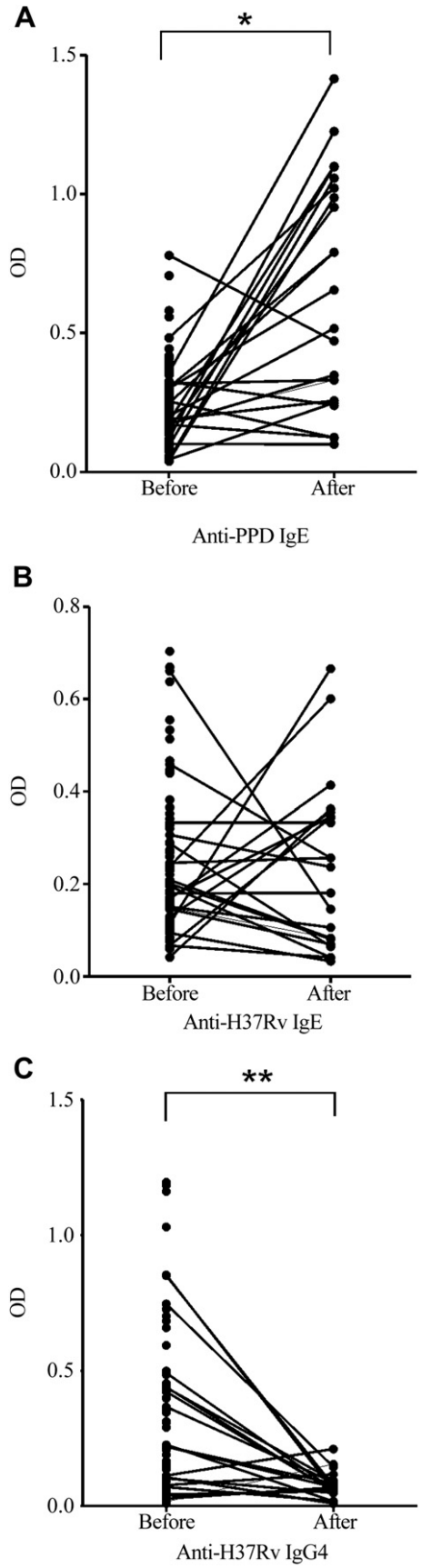

and IL-5 (21). Preferential secretion of IL-4 and IL-5 in Warao PBMCs may be due to greater parasitic burdens. This, in turn, may lead to greater disease susceptibility. A profile of IgE then seems to be significantly predominant among individuals from the Warao population as compared to the Creole population. Further studies are needed to understand the association between intestinal helminthic infections and the IgE response among the Creole population.

It has also been reported that intestinal parasites may cause false-negative TST and are risk factors for developing active TB (23). A significant association between intestinal helminthic infections and mycobacterial diseases such as pulmonary tuberculosis and multibacillary leprosy has been demonstrated by several authors (1,8,9,24). Authors investigated cytokine profiles in patients with TB and patients with concomitant TB and intestinal helminthic infection. The findings indicated that concomitant intestinal helminthic infection in patients with diagnosed TB skews their cytokine profile toward a T helper 2 response, which could favor persistent *M. tuberculosis* infection (25,26). In addition, differences between Warao and Creole groups such as socioeconomic status and access to health care may affect immunity to TB as well as genetic differences. In this context, socioeconomic status of the Warao population is lower than the Creole population, which may contribute to increase the risk for TB as well as parasitic worm infections prevalent in the Warao communities as compared to Creole communities (21). Thus, Warao communities require better control measures for these parasitic infections for effective shifting the immune response towards type 1, which is crucial for prognosis in TB patients. It is also important to focus and demonstrate that intestinal parasites are endemic in this indigenous population because these infections with *M. tuberculosis* and parasites not only stimulate the production of anti-*Mycobacterium* or anti-parasite IgE antibody but can nonspecifically induce polyclonal IgE synthesis that could result in highly elevated levels of total serum IgE. The latter may explain the considerably higher levels of total serum IgE found among the Warao population. In addition, it has been accepted that also malnutrition and the severity of the family TB exposure, cannot be discarded to contribute for developing TB within a population. As regards these points, it has been reported that the different nutritional profile in a population results from the specific patterns of social, cultural and economic conditions of each population. We have found that within the Warao people malnutrition at early ages is common (27).

Figure 4. Reactivity detections before and after treatment. IgE and IgG_4 against PPD and H37Rv antigens were measured after treatment in a subset of Warao patients ($n = 40$). Data representing mean OD ± SD of IgE reactivities before and after treatment. (A) Concerning anti-PPD IgE, (*) represents the significance between pre- and post-treatment, $p < 0.001$. (B) Post-treatment anti-H37Rv IgE did not differ from prior reactivity. (C) For anti-H37Rv IgG4, (**) represents the significance between pre- and post-treatment, $p < 0.004$.

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IgG_4 reactivity against H37Rv did not differ significantly between the Creole and Warao populations. In this context, in a previous study authors found that among the indigenous Yanomami, a very high prevalence of TB exists and also had higher titers of IgG_4 antibodies against *M. tuberculosis* antigens than the control subjects comprised of Brazilians of European descent (28). As mentioned above, anti-H37Rv IgG_4 responses were serologically indistinguishable between Warao and Creole groups; however, the *p* value obtained with the Kruskal−Wallis test was close to being significant, so further study in regard to specific-IgG_4 must be done between groups.

Measurements of IgE reactivity against H37Rv antigen showed that post-treatment did not differ from reactivity before, whereas IgE reactivity against PPD as well as levels of total serum IgE were high in patients who finished treatment. The latter is not in concordance with several studies reporting total and specific IgE decline after TB treatment (5,9). These findings could be explained because the study was performed among individuals from a nonindigenous population. Therefore, measurements of total serum IgE and IgE reactivity before and after treatment must be done among the Creole population, which may permit us to compare the results obtained in the Warao indigenous people from a population with a different genetic background and highly susceptibility to TB even among bacille Calmette-Guerin-vaccinated individuals (14).

In regard to IgG_4, reactivity against H37Rv significantly declined after treatment so IgG_4 was affected by anti-TB treatment, which could suggest that *M. tuberculosis* downregulates IgG_4 reactivity in Warao individuals. The findings permit us to suggest that this isotype is a marker of active infection and may be predictive of treatment outcome in this population such as has been reported by others authors who have suggested that the levels of antibodies to *M. tuberculosis* represent serological correlates of active disease because these were affected in an antigen-specific fashion by anti-tuberculosis treatment (29). In this context, among the Creole population further studies about anti-H37Rv IgG_4 must be done for determining if this reactivity is affected by anti-TB treatment.

Finally, this exploratory study on humoral immune response showed that a profile of one isotype, IgE seems to be significantly predominant among the Warao population as compared to the Creole population. This knowledge principally shows the importance of this response with regard to probable development of an intrinsic propensity to produce IgE, probably due to greater parasitic burdens as well as to genetic differences, which may favor persistent *M. tuberculosis* infection in this population.

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