# The secretory immunoglobulin A response to *Mycobacterium tuberculosis* in a childhood population

## Resposta da imunoglobulina A secretória ao *Mycobacterium tuberculosis* em população infantil

Carlos Fernández de Larrea[1], Jacobus Henry de Waard[2], Francesca Giampietro[3] and Zaida Araujo[3]

**ABSTRACT**

*We report on the measurement of saliva anti-Purified Protein Derivative sIgA and 38kDa antibodies from 127 children, of whom 31 were strong tuberculosis suspects and 96 were healthy contact children. The results concerning the percentage of children with antibody reactivity to PPD and 38kDa antigens showed that, of these 2 antigens, 38kDa induced higher reactivity in patients positive and negative for the Tuberculin Skin Test (28% and 16.6%, respectively) in comparison to controls positive and negative for the TST (11.7% and 7.1%, respectively). There was a statistically significant difference between patients positive and controls negative for the TST. In relation to the Purified Protein Derivative antigen, while 14.2% of patients positive for the TST showed antibody reactivity to the PPD antigen, no patients negative for the TST had reactivity to this antigen. The findings suggest that these two antigens seem be associated with a different development of the mucosal defence mechanisms mediated by sIgA against Mycobacterium tuberculosis.*

**Key-words:** *Tuberculosis. Warao. Secretory IgA. Tuberculin skin test.*

**RESUMO**

*Foram dosados anticorpos sIgA anti-Purified Protein Derivative e 38kDa da saliva de 127 crianças, das quais 31 eram de pacientes altamente suspeitos de tuberculose e 96 eram provenientes de crianças saudáveis, que tiveram contato com pacientes. Os resultados referentes à porcentagem de crianças, reativas ao PPD e ao antígeno 38kDa, mostraram que destes dois antígenos, o 38kDa induziu maior reatividade em pacientes positivos e negativos ao Tuberculin Skin Test (28% e 16,6%, respectivamente), em comparação aos controles positivos e negativos ao TST (11,7% e 7,1%, respectivamente). Houve diferença estatisticamente significativa entre pacientes positivos e controles negativos ao Tuberculin Skin Test. Em relação ao antígeno PPD, enquanto 14,2% de pacientes positivos ao TST mostraram anticorpos reativos ao antígeno Purified Protein Derivative, nenhum paciente negativo ao TST foi reativo ao antígeno. Os achados sugerem que, aparentemente, estes dois antígenos estão associados a desenvolvimento distinto dos mecanismos de defesa da mucosa mediados por sIgA contra Mycobacterium tuberculosis.*

**Palavras-chaves:** *Tuberculose. Warao. IgA secretória. Teste cutâneo da tuberculina.*

Mycobacteria, including those that cause tuberculosis (TB), cross mucosal barriers and enter mucosal lymphoepithelial sites, which include oropharyngeal and nasopharyngeal tonsils[22]. Dendritic cells and macrophages in these sites allow for mycobacterial replication, because of the permissive immunological enviroment in lymphoepithelial tissues, where bacteria appear to adapt their immediate environment to favor survival and may carry out essential immunoregulatory mechanisms designed to minimize immune pathology or the inappropriate activation of immune effectors. Thus *Mycobacterium tuberculosis* can establish life-long chronic infections in their hosts after an acute infection period involving the activation of both the innate and acquired immune systems[22].

Mucosal surfaces represent a very large area, and in the absence of specific immune defense mechanisms we would rapidly be overwhelmed by bacterial pathogens. Even in the presence of effective mucosal immune mechanisms, bacterial infections of the gastrointestinal, respiratory and urogenital mucosae are sufficiently common to represent a major global health problem[4 12 17]. Thus, enteric, chronic and acute respiratory tract infections are the three leading causes of illness and death globally. They affect mainly childhood populations in developing countries, such as Venezuela, where a chronic respiratory infection like TB in some indigenous communities towns, such as Murako and Koamuho, reach a prevalence of nearly 2% within a childhood population[20].

About 60% of the total immunoglobulin produced in humans is IgA. Although IgA makes up only 10-15% of serum immunoglobulin, it is the predominant immunoglobulin in mucosal secretions. There are two subclasses of IgA in humans: IgA1 and IgA2, serum contains 80-90% IgA1 whereas mucosal secretions contain up to 60% IgA2. The association of IgA dimers with a secretory component molecule facilitates transport of this isotype into the lumen, where they can interact with antigens. The oligomeric nature of IgA enhances its ability to interact with high avidity with viruses and bacteria that are present in secretions[12 37]. IgA antibodies also bind well to Fc receptors on neutrophils. In this regard, since the identification of receptors for IgA on the surface of blood leukocytes and alveolar macrophages was reported, the role of secretory IgA (sIgA) in the defence of mucosal surfaces at the level of the respiratory tract has now expanded from the limited role of exogenous material scavenger to a broader protective function with a potential protective role[26]. It has been reported that IgG is the predominant immunoglobulin in the lower respiratory tract, followed by sIgA[16]. Most IgA deficient individuals experience respiratory infections, malabsorption and autoimmune disorders[9 14].

Secretory IgA was found to be significantly higher in smear negative and culture positive cases of TB compared with culture negative cases[33]. In addition, intranasal inoculation of mice with IgA against *M. tuberculosis* antigen diminished the tuberculosis infection in the lungs[28]. Few studies have addressed the specific mucosal immune response in childhood TB or recognized the importance of sIgA in mucosal homeostasis in the respiratory tract. In this regard, the present research studied the levels of anti-PPD and 38kDa sIgA in a Warao child population with TB. The results might partially contribute to explaining the extraordinarily high prevalence of children with active TB present in this population.

## MATERIAL AND METHODS

In a remote area of an indigenous population from northeastern Venezuela, a study was conducted in 15 Warao indigenous communities from two Municipal Districts of the Delta Amacuro State (Tucupita and Antonio Díaz). A total of 127 saliva samples were collected from children of both genders, aged 1 month to 15 years. The mean age of cases was $8.09 \pm 3.80$ years old. Radiological studies suggested that 31 children were strong TB suspects. The children were grouped as follows: Patient Group, children with probable active TB before treatment (n=31, 25 positive and 6 negative for the TST); Control Group, healthy contact controls (n=96, 53 positive and 43 negative for the TST). The latter group was evaluated and no-one was found with characteristic signs suggesting TB. A second control group of 40 children from Caracas donated saliva samples for the determination of normal sIgA levels. Informed consent was obtained from all participants or their legal representatives (children or parents, respectively), who signed a *consent form* agreement before blood and saliva samples were taken. The approved consent of the Ethical Commission of the Biomedicine Institute was also obtained.

During the present study specific attention was given to Warao children less than 15 years old with respiratory symptoms. In this regard, a scheme previously reported by our research group was used[20], which takes into account the clinical and epidemiological criteria that children presented: 1) clinical and nutritional criteria and positive reactivity to tuberculin, and 2) clinical and nutritional criteria, with negative tuberculin and positive household contact. Children that were TB suspects were further evaluated with a chest X-ray.

**Clinical, epidemiological and bacteriological criteria for tuberculosis diagnosis.** Since 1996, the Regional Program of Tuberculosis of Delta Amacuro State and the Tuberculosis Laboratory of the Institute of Biomedicine have actively diagnosed tuberculosis cases among the Warao communities based on respiratory symptoms characteristic of TB, the TST and smears and/or cultures, and prescribed specific treatments.

**Clinical and nutritional criteria.** A complete basic clinical and nutritional evaluation was carried out. The latter was based on an anthropometric evaluation, contained in the Transversal Study of Caracas, Fundacredesa[3]. The clinical evaluation included: recent weight loss or inadequate progress of weight gain; prolonged febrile syndrome; night sweats; coughing or wheezing for more than two weeks; large painless adenopathy, with or without fistulas; angular deformity of the spine; increased articular or bone volume, or fistulas; inexplicable abdominal mass or ascitis; behavior or sensory system alterations; any other neurological manifestation suggestive of tuberculous meningitis; and registration of the presence or absence of BCG scars.

**Radiological criteria.** Thorax radiology was performed on all highly suspect cases according to standard techniques in post-anterior projection. This took place in the radiology service of the Hospital Luis Razzetti, Tucupita. Radiological evaluation was performed by two pneumonologists.

Epidemiological criteria. A complete basic epidemiological evaluation was carried out, which was based on the TST and household contacts, defined as continuous contact with an adult patient with active lung tuberculosis or who had recently received treatment.

**The tuberculin skin test.** The TSTs were performed on all the individuals of this study using two tuberculin units of purified protein derivative (PPD) of *Mycobacterium tuberculosis*, strain RT-23, from the Statens Seruminstitut in Copenhagen, Denmark. Testing and reading were done according to international guidelines[2]; induration of [3] 10mm was used as the criterion for infection with *M. tuberculosis*.

**Bacteriological criteria.** Since invasive procedures cannot be used to take samples in these communities, a study of secretions of the pharynx and attempts to obtain samples of sputum by expectoration in older children was carried out in all highly suspect cases. Smears from sputum were stained by the Ziehl-Neelsen direct method. For each specimen two tubes of modified Ogawa egg medium and Lowënstein-Jensen were inoculated using the swab method of Kudoh and Kudoh, for both sputum and oozing secretions[19].

**Treatment of tuberculosis.** Specific treatments were initiated following the norms of the Venezuelan National Program of Tuberculosis Control[6] in all newly identified cases of tuberculosis, where radiological evidence suggestive of tuberculosis or bacteriological confirmation by bacilloscopy or culture was found. Clinical and nutritional monitoring in all highly suspect patients was carried out, to evaluate the improvement of these aspects as therapeutic evidence, which allowed for the corroboration of the diagnosis.

**Determination of the antibodies of *Mycobacterium tuberculosis* antigens.** *Anti-PPD sIgA*: the detection of anti-PPD sIgA levels was performed by capture immunoenzymatic assays (ELISA). The assay was developed and standardized in our laboratory for the detection of sIgA against PPD antigen. Each individual assay included positive and negative sera and also blanks to control non-specific binding. Microtiter plates (ThermoLabsystems), Dynatech Laboratories, Inc.) were coated with PPD (*Statens Seruminstitut, Copenhagen*) (1µg/well in carbonate-bicarbonate buffer pH 9.6) overnight at 4°C. Excess protein binding sites were blocked by incubation with horse serum in PBS (1:30) at 37°C for 2h, then the plates were washed four times with PBS containing 0.1% Tween 20. Optimal dilutions of saliva samples (1:50) were added and plates were incubated for 2h at 37°C and washed four times; then incubated for 1 hour at 37°C with the secondary antibody for sIgA (peroxidase-conjugated monoclonal antibody anti-alpha chain IgA, *Sigma-Aldrich, USA,* diluted). After washing, substrate solution (30µl of 30% $H_2O_2$ and 10mg o-phenylenediamine (OPD) dihydrochloride, (*Sigma-Aldrich, USA*) in 25ml citrate buffer, pH 5) was added and incubated

for 16 minutes at room temperature. Color development was measured in an ELISA reader at 492nm.

*Anti-38kDa sIgA*: the levels of anti-38kDa sIgA in saliva were determined by a similar ELISA to that described for anti-PPD sIgA. Briefly, microtiter plates (ThermoLabsystems) were coated overnight at 4°C with 38kDa antigen (1µg/well of each antigen in carbonate-bicarbonate buffer pH 9.6). The saliva samples were diluted 1:50 and peroxidase-conjugated monoclonal antibody anti-alpha chain IgA *(Sigma-Aldrich, USA)* was used as the secondary antibody diluted.

**Statistical analysis.** The statistical significance of the differences between the mean ± SD of the optical density (OD) values of the patients and controls was estimated by the Students "t" test. The evaluation of positive saliva was based on a positive score represented by levels greater than OD mean plus two standard deviations of saliva from a healthy control group negative for the TST from Caracas in north-central Venezuela. The proportions of patients with positive results for the different evaluations were compared by Fisher's exact test.

## RESULTS

**Secretory IgA specific levels according to the tuberculin skin test.** The results in relation to the TST showed that in the TB patient group, 25 were positive for the tuberculin skin test (TST) and 6 negative for the TST; and in the healthy contact children, 53 were positive for the TST and 43 negative for the TST (Table 1).

The mean ± SD of the optical density (OD) values of anti-PPD and anti-38kDa sIgA levels of patient and control children are shown in Table 1. In relation to the mean anti-PPD sIgA levels, in the patient group, there was no statistically significant difference between patients positive and negative for the TST (0.422±0.197 and 0.338±0.158, respectively). In the control group, the mean anti-PPD sIgA levels were significantly increased in the control group positive for the TST (0.320±0.195), in comparison to the control group negative for the TST (0.237±0.166), p<0.02, (Table 1).

In relation to the mean anti-38kDa sIgA levels, in the patient group, although patients positive for the TST presented a high mean of these levels, there was no statistically significant difference between patients positive and negative for the TST (0.645±0.340 and 0.363±0.229, respectively). In the control group, the mean anti-38kDa sIgA levels were significantly increased in the control group positive for the

*Table 1 - Specific sIgA levels in patients and controls according to the response to the tuberculin skin test.*

| Groups | Reactivity to PPD | | Reactivity to 38kDa | | |
|---|---|---|---|---|---|
| | TST + | TST - | TST + | TST - | |
| Patients | 0.422±0.197 | 0. 338±0.158 | 0.645±0.340 | 0.363±0.229 | |
| | (25) | (6) | (25) | (6) | |
| Controls | 0.320±0.195[(a)] | 0.237±0.166[(b)] | 0.577±0.225[(c)] | 0.238±0.166[(d)] | a b and c d |
| | (53) | (43) | (34) | (42) | 0.02>p>0.0001 |

TST (0.577±0.225), in comparison to the control group negative for the TST (0.238±0.166), p<0.0001 (Table 1).

When the results are shown as percentage of children with antibodies to specific antigens according to the tuberculin skin test, it was found that regarding the sIgA specific response against PPD according to the TST, salivas from patients positive for the TST presented a significant percentage of patients with specific sIgA (14.2%) in comparison to patients negative for the TST (0%). The Figure 1 shows that in the control group, there was no significant difference between controls positive and negative for the TST (3.7% and 4.6%, respectively).

The percentages of patients and controls with positive responses to 38kDa antigen according to the TST are shown in Figure 2. Regarding the sIgA specific response according to the TST, in patient group, there was statistically significant differences in patients positive for the TST that presented anti-38kDa levels (28%) in comparison to patients and controls negative for the TST (16.6% and 7.1%, respectively), p<0.04 (Figure 2). Those patients positive for the TST presented twice the anti-38kDa sIgA levels in comparison to those that produced anti-PPD sIgA levels. There was no statistically significant differences in the percentage of controls with specific anti-38kDa sIgA response between children positive and negative for the TST.
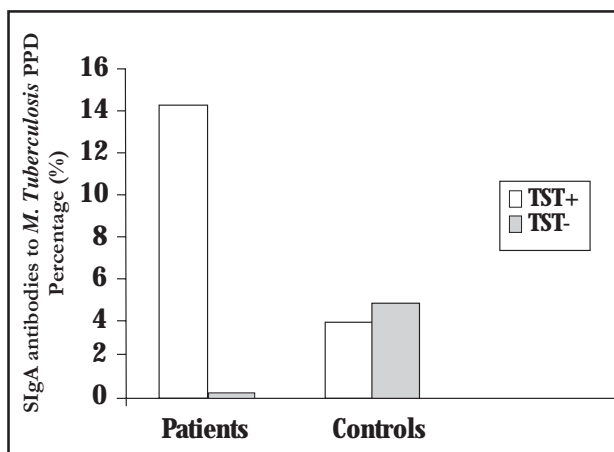


Data representing the percentages of patients (n=31) and controls (n=96).

*Figure 1 - Anti-PPD sIgA according to the tuberculin skin test.*
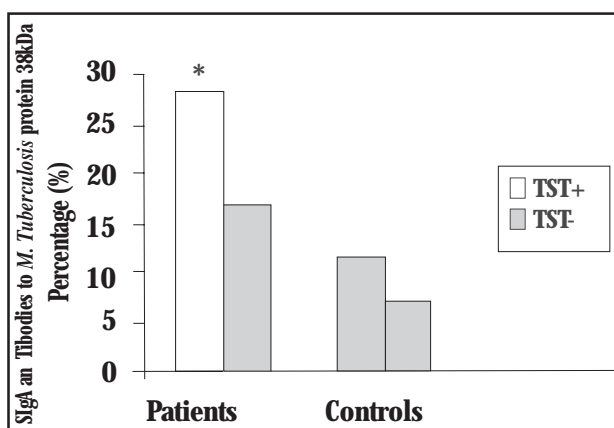


Data representing the percentages of patients (n=31) and controls (n=96). *There was statistically significant differences in patients TST + that presented anti-38kDa levels in comparison to patients and controls TST-, p<0.04

*Figure 2 - Anti-38kDa sIgA according to the tuberculin skin test.*

## DISCUSSION

Since in indigenous Warao communities in a remote indigenous population from north-eastern Venezuela, invasive procedures cannot be used to take samples due to ethical considerations, the assessment of TB among a population of children with an high prevalence of active TB in adults offered an opportunity to study the development of the mucosal defence mechanisms mediated by sIgA against *M. tuberculosis* antigens and to attempt to improve diagnostic methods, such as the specific sIgA test.

The role of type-2 responses and humoral immunity in TB infection is generally considered to be marginal[15 34]. However, bearing in mind that the identification of receptors for IgA on the surface of blood leukocytes and alveolar macrophages that perform a protective role in chronic respiratory infections, such as TB, has been reported and that this provides a measure of the integrity of the specific mucosal response to *M. Tuberculosi*[16 22 25 32], the measurement of specific sIgA was carried out. Concerning the anti-PPD sIgA antibody response and the response to the TST, while patients positive for the TST presented antibodies reactive to PPD antigen, no tuberculin negative patients presented antibodies reactive to this antigen, so the latter correlates with a lack of the cellular specific response in these children. The results show that child patients produced *M. tuberculosis*-reactive sIgA antibodies during active infection; moreover, children in this patient group failed to react to PPD, as has been suggested by other studies which showed that during active TB, signs of immune depression were related to the presence of a significant immune depression in response to the TST and with antibody test unresponsiveness or anergy, when PPD was used[7]. In addition, it has been reported that in another approach, when a correlation was found between the presence of TB disease and anti-PPD sIgA, 7% of patients displayed a selective sIgA deficiency[35]. the present findings suggest that an absence of this specific sIgA immune defence in a percentage of these children does occur, which might be associated with higher susceptibility to TB and probably to other infections, particularly acute respiratory tract infections that are frequently observed in these childhood communities[10]. On the other hand, other factors could be involved, such as nutritional status, type of feeding[7] and genetic aspects[6 21 27], alternatively, proinflammatory factors, such as cytokines (for example, IL-6), traditionally involved in the polyclonal activation seen in TB, may play a role in sIgA elevation[13 18]. In this regard, several studies have reported that the human airway epithelium constitutively produces IL-2, TGF-beta, IL-6 and IL-10, factors which are essential for B-cell clonal proliferation, IgA isotype switch and differentiation into IgA-producing plasma cells[5 11]. Additionally, it has been reported that a transient absence of salivary IgA in the first years of life was associated with an increased risk of developing atopy, asthma or bronchial hyperreactivity later in life[23 24]; whereas low levels of salivary IgA, particularly the IgA1 subclass, have been associated with an increased risk of respiratory illness[8].

In relation to the anti-38kDa mucosal response, a significant percentage of both tuberculin positive patients and controls,

and even a percentage of tuberculin negative patients, produced anti-38kDa sIgA antibodies, which could be due to the fact that the mucosal immune system in these children is associated with an appropriate mucosal immune response that it is capable of mounting a better response to 38kDa antigen than the PPD antigen. The similar lower percentage of tuberculin negative controls with reactivity to 38kDa and PPD could be conditioned by a high prevalence of atypical mycobacteria, which can induce a cross-reacting antibody immune response. Although in the presence of active infection a certain amount of immune depression related to PPD antibody test unresponsiveness or anergy occurred, the sIgA antibodies to 38kDa antigen were produced both in patients positive and negative for the TST. Bearing in mind that the use of ELISA for immunodiagnosis of TB has shown that the sensitivity of the tests remained limited in the diagnosis of childhood TB[29 30 31], and since the immunological activity of 38kDa antigen of *M. tuberculosis* has been reported and used for the serodiagnosis of TB[36], it seems important that a combination including anti-38kDa sIgA provided improvement in the diagnosis of this population, as previously reported by our group[1].

Few studies have addressed the depression of both delayed-type hypersensitivity, manifest as depressed tuberculin skin test reaction, and the sIgA response to *M. tuberculosis* antigens, the present study permitted the suggestion that in the Warao childhood population where *M. tuberculosis* infection is prevalent, there was a clear separation of the two sIgA specific responses. These antigens seem to be associated with a distinct development of the mucosal defence mechanisms mediated by sIgA against *M. tuberculosis*. The identification of these mucosal mechanisms could be more clearly defined in future studies, leading to improved diagnosis of childhood TB and the design of targeted mucosal vaccines.

## ACKNOWLEDGEMENTS

## REFERENCES

1. Araujo Z, Waard JH, Larrea CF, López D, Fandiño C, Maldonado A, Hernández E, Ocaña Y, Ortega R, Singh M, Ottenhoff Tom HM, Arend SM, Convit J. Study of the Response against *Mycobacterium tuberculosis* Antigens in Warao amerindian Children in Venezuela. Memórias do Instituto Oswaldo Cruz 99: 517-524, 2004.

2. Arnadottir T, Rieder HL, Trébuq A, Waaler H. Guidelines for conducting tuberculin skin test surveys in high prevalence countries. Tubercle and Lung Disease 77: 1-20, 1996.

3. Blanco ML, Landaeta M. Manual de Crecimiento y Desarrollo. Fundacredesa. Caracas, 1991.

4. Boyaka PN, Lillard Jr JW, McGhee J. Interleukin 12 and innate molecules for enhanced mucosal immunity. Immunologic Research 20: 207-217, 1999.

5. Braciak TA, Gallichan WS, Graham FL, Richards CD, Ramsay AJ, Rosenthal KL, Gauldie J. Recombinant adenovirus vectors expressing interleukin-5 and-6 specifically enhance mucosal immunoglobulin A responses in the lung. Immunology 101: 388-396, 2000.

6. Davies P, Grange J. The genetics of host resistance and susceptibility tuberculosis. Annals of New York Academy of Sciences 953: 151-156, 2001.

7. Gatner EM, Anderson R. An *in vitro* assessment of cellular and humoral immune function in pulmonary tuberculosis: correction of defective neutrophil motility by ascorbate, levamisole, metoprolol and propanolol. Clinical and Experimental Immunology 40: 327-336, 1980.

8. Gleeson M. Mucosal immunity and respiratory illness in elite athletes. International Journal of Sports Medicine 21 33-43, 2000.

9. Gleeson M, Cripps A, Claney R. Modifiers of the human mucosal immune system. Immunology and Cell Biology 73: 397-404, 1995.

10. González N, De Cubeddu L, Waard JH, Fandiño C, Larrea CF, López D, Maldonado A, Ocaña Y, Hernández E, Ortega R, Convit J, Pujol FH, Castés M, Araujo Z. Study of the immune response in Warao children from an area with high prevalence of tuberculosis. Investigación Clínica 44: 303-318, 2003.

11. Goodrich ME, McGee DW. Effect of intestinal epithelial cell cytokines on mucosal B-cell IgA secretion: enhancing effect of epithelial-derived IL-6 but not TGF-beta on IgA+ B cells. Immunology Letters 67: 11-14, 1999.

12. Holmgren J, Rudin A. Mucosal Immunity and Bacterial. *In:* Mucosal Immunology. Second edition, Chapter 41. Academic Press USA, p. 685-690, 1999.

13. Hucklebridge F, Clow A, Evans P. The relationship between salivary secretory immunoglobulin A and cortisol: neuroendocrine response to awakening and the diurnal cycle. International Journal of Psychophysiology 31: 69-76, 1998.

14. Jemmott 3rd JB, Borysenko JZ, Borysenko M. Academic stress, power motivation, and decrease in secretion rate of salivary secretory immunoglobulin A. Lancet 1: 1400-1402, 1983.

15. Kamat HA, Williamson M, Koppikar GV. Humoral and cell mediated immune responses in patient with tuberculosis meningitis. Indian Journal of Medical Sciences 53: 343-348, 1999.

16. Kitz R, Ahrens P, Zielen S. Immunoglobulin levels in bronchoalveolar lavage fluid of children with chronic chest disease. Pediatric Pulmonology 29: 443-451, 2000.

17. Kiyono H, Kweon MN, Hiroi T, Takahashi I. The mucosal immune system: from specialized immune defense to inflammation and allergy. Acta Odontologica Scandinavica 59: 145-153, 2001.

18. Kramer DR, Sutherland RM, Bao S, Husband AJ. Cytokine mediated effects in mucosal immunity. Immunology and Cell Biology 73: 389-396, 1995.

19. Kudoh S, Kudoh T. A simple technique for culturing tubercle bacilli. Bulletin of the World Health Organization 51: 71-82, 1974.

20. Larrea CF, Fandiño C, López D, del Nogal B, Rodríguez N, Convit J, Araujo Z, Waard JH. Childhood tuberculosis in the Warao population in Venezuela. Investigación Clínica 43: 35-48, 2002.

21. Layrisse Z, Heinen HD, Balbas O, García E, Stoikow Z. Unique HLA-DR/DQ associations revealed by family studies in Warao amerindians. Haplotype and homozygosity frequencies. Human Immunology 23: 45-57, 1988.

22. Lugton I. Mucosal-associated lymphoid tissues as sites for uptake, carriage and excretion of tubercle bacilli and other pathogenic mycobacteria. Immunology and Cell Biology 77: 364-372, 1999.

23. Maree G, Cripps AW, Clancy RL. Modifiers of the human mucosal immune system. Immunology and Cell Biology 73: 397-404, 1995.

24. Miletic ID, Schiffman SS, Miletic VD, Sattely-Miller EA. Salivary IgA secretion rate in young and eldery persons. Physiology & Behavior 60: 243-248, 1996.

25. Nagao AT, Pilagallo MI, Pereira AB. Quantitation of salivary, urinary and faecal sIgA in children living in different conditions of antigenic exposure. Journal of Tropical Pediatrics 39: 278-283, 1993.

26. Pilette C, Ouadrhiri Y, Godding V, Vaerman JP, Sibille Y. Lung mucosal immunity: immunoglobulin-A revisited. The European Respiratory Journal 18: 571-588, 2001.

27. Ramos M, Postigo JM, Vilches C, Layrisse Z, Castro JAL. Primary structure of a novel HLA-B39 allele (B*3909) from the Warao Indians of Venezuela. Further evidence for local HLA-B diversification in South America. Tissue Antigens 46: 401-404, 1995.

28. Reljic R, Clark SO, Williams A, Falero-Díaz G, Singh M, Challacombe S, Marsh PD, Ivanvi J. Intranasal IFNgamma extends passive IgA antibody protection of mice against _Mycobacterium tuberculosis_ lung infection. Clinical Experimental Immunology 143: 467-473, 2006.

29. Sant'Anna CC, Ferreira MAS, Fonseca LS. Evaluation of a serological method (ELISA) for the diagnosis of pulmonary tuberculosis in children. The International Journal of Tuberculosis and Lung Disease 3: 744-748, 1999.

30. Starke JR. Childhood tuberculosis: a diagnostic dilemma. Chest 104: 329-330, 1993.

31. Swaminathan S, Umadevi P, Shantha S, Radhakrishnan A, Datta 0. Sero diagnosis of tuberculosis in children using two ELISA kits. Indian Journal of Pediatrics 66: 837-884, 1999.

32. Tamada T, Sasaki T. The role of airway submucosal glands in the airway mucosal defense system. Nihon Kokyuki Gakkai Zasshi 39: 157-165, 2001.

33. Tomoda T, Takai A. Tubercle bacilli and the defence factors for infection in sputum and bronchoalveolar lavage fluid. Kekkaku 69: 743-749, 1994.

34. Van Crevel R, Ottenhoff THM, van der Meer JWM. Innate immunity to _Mycobacterium tuberculosis_. Clinical Microbiology Reviews. 15: 294-309, 2002.

35. Watson RR, McMurray DN. The effects of malnutrition on secretory and cellular immune processes. CRC Critical Reviews in Food Science and Nutrition 12: 113-159, 1979

36. Young D, Kent L, Rees A, Lamb J, Ivanyi, J. Immunological activity of a 38-kilodalton purified from _Mycobacterium tuberculosis_. Infection Immunity 54: 177-183, 1986.

37. Zakrzewski J. The significance of IgA1 proteases for infections of the upper respiratory tract. Reviews of Infectious Diseases 2: 211-212, 2000.