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Infection by *Ascaris lumbricoides* and bronchial hyper reactivity: An outstanding association in Venezuelan school children from endemic areas

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Abstract

Asthma and other respiratory diseases have increased in the last years among Venezuelan children from helminthic endemic areas where the infection by *Ascaris lumbricoides* has been associated to bronchial airway inflammation in parasitized individuals. The aim of this work was to investigate the possible associations between the development of bronchial hyper reactivity and the immune response against *A. lumbricoides* in urban and rural children. We evaluated 470 school children from rural and urban communities. Pulmonary function tests were performed and $\geq$20% PC_{20} changes were considered as a positive diagnostic of bronchial hyper reactivity. The prevalence and intensity of *A. lumbricoides* infection was determined by faecal examination. Specific serum IgE levels using a modified ELISA and skin prick tests against *A. lumbricoides* and the common allergen *Dermatophagoides pteronyssinus* were done. The number of circulating lymphocyte sub populations was determined by flow cytometry analysis. In rural children, bronchial hyper reactivity was associated with increased specific levels of anti-*A. lumbricoides* IgE ($p < 0.0001$) and skin test positivity for *A. lumbricoides* ($p < 0.0001$). The percentage of FEV1 predictive values correlated inversely ($p < 0.0001$) with anti-*A. lumbricoides* IgE levels. Elevated numbers of circulating CD3+CD4+ and CD20+CD23+ cells were found in rural children with bronchial hyper reactivity compared to their asymptomatic counterparts. They correlated positively with anti-*A. lumbricoides* IgE levels ($p < 0.005$ and < 0.0001, respectively). In contrast, in urban children, bronchial hyper reactivity was associated with elevated anti-*D. pteronyssinus* IgE levels ($p = 0.0089$), skin hyper reactivity towards this aero allergen ($p = 0.003$) and to an increase in the number of CD3+CD8+ ($p < 0.0001$). Our results suggest that the IgE response against *A. lumbricoides* infection may be involved in the development of bronchial hyper reactivity among rural children from endemic areas and also that improved hygienic conditions in the urban environment is associated with increased responses to airborne allergens.

Keywords: *Ascaris* infection; Bronchial hyper reactivity; IgE antibodies; CD23 receptor

1. Introduction

There are close parallels between the immune response associated with allergic diseases and helminthic infection. Both are characterized by

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the secretion of Th2 type cytokines such as IL-4, IL-5 and IL13, high levels of serum IgE, tissue eosinophilia and mastocytosis (Cooper, 2002). In tropical countries, particularly in endemic rural areas, gastrointestinal helminths may influence the expression of allergic diseases. Intense helminthic infection can inhibit allergic reactivity through the induction of high levels of polyclonal IgE, suppressing the development of specific IgE responses against environmental allergens (Hagel et al., 1993a). Also these parasites stimulate the production of regulatory cytokines such as IL-10 and TGF-β that down regulate IgE dependent inflammatory responses (van den Biggelaar et al., 2000). In contrast, mild infections may induce non-specific stimulation of IgE and allergic reactivity towards environmental antigens (Hagel et al., 1993b).

Clinical and epidemiological studies suggest a possible participation of this parasite in bronchial asthma (Palmer et al., 2002; Aderele and Oduwole, 1981; Faniran et al., 1999). Early reports in Venezuela described significant changes in the pulmonary function of tropical asthmatics after bronchial provocation with *Ascaris lumbricoides* extract (Lynch et al., 1992a). Studies performed in tropical slum children from endemic areas have shown that non-asthmatic children can significant respond to bronchodilator inhalation, and that this response can be reversed by anti-helminthic treatment (Lynch et al., 1992b). However, the possible mechanisms by which these parasites stimulate the development of respiratory symptoms like wheezing, dyspnoea or persistent cough, are still not well elucidated.

Experimental models have demonstrated a role for specific anti-parasite IgE antibodies in bronchial hyper responsiveness. The migration of *Strongyloides venezuelenzis* larvae through the lungs of infected rats induces a local eosinophilic inflammation process characterized by mucus hyper secretion and thickening of bronchial epithelial muscle layers, accompanied by a strong local increase in IgE concentrations (Negrao-Correa et al., 2003).

Evidence obtained from experimental and *in vitro* models has shown the role of the low affinity receptor for IgE (CD23) in the stimulation of the IgE response (Bonnefoy et al., 1990, Yabuuchi et al., 2002). Epidemiological studies have reported an increase in the proportion of CD23+ positive B cells in asthmatic children allergic to *Dermatophagoides pteronyssinus*, with high levels of specific IgE against this allergen (Aberle et al., 1997). Also, specific allergic vaccination with birch pollen extracts has been shown to inhibit CD23-mediated allergen presentation in atopic subjects (van Neerven et al., 2004). Thereby, the expression of the low affinity IgE receptor (CD23) may be an important factor in IgE production, probably involved in the development of bronchial hyper reactivity induced by environmental allergens or helminth derived antigens.

Therefore, the aim of this study was to investigate the possible association between the presence of bronchial hyper reactivity and the IgE response against *A. lumbricoides*, in Venezuelan school children from urban and rural communities.

2. Methods

2.1. Study population

We performed a cross-sectional study among the school children population of two rural communities and from a slum area of Caracas, in which local collaboration of health authorities and other logistic facilities made our study suitable. Estimation of the minimum sample size based on the prevalence of *A. lumbricoides* in the rural and urban Venezuelan school population was performed. For this purpose, we used the Epi info program, version 3.3.2 (CDC, Atlanta, USA, 2005). Taking in account the overall prevalence (70%) of *A. lumbricoides* reported among the Venezuelan rural school population (República Bolivariana de Venezuela, Ministerio de Salud, 2005), to detect at least a prevalence of 60% with 95% of confidence, a minimum of 81 children would be require. Similarly, for the Venezuelan urban school children living in Caracas, a prevalence of 12% has been reported (República Bolivariana de Venezuela, Ministerio de Salud, 2005), thus, to detect a minimum prevalence of 6% with 95% of confidence, a sample size of 113 children would be needed. However, all those school children, whose parents signed the respective informed consent, were included in the study. Therefore, We evaluated 190 children attending the primary school from El Cardón, Coche Island, Nueva Esparta State (lat. 10.45 N, long. 63.55 W) and 160 from the primary school from La Salina, a small fishing community located at Sucre State (lat.10.34 N, long. 62,18 W), in northeastern Venezuela. Similarly, a group of 120 children attending the primary school located at the 23 de Enero slum area of Caracas (lat.10.33 N, long. 66. 55 W) was also studied. Age and sex were comparable between the different groups of children studied (Table 1).

2.2. Environmental and socio-economic factors

Socio-economic aspects that may influence the prevalence and intensity of helminthic infection, including sanitary conditions, level of poverty, and mother's level

Table 1
Socioeconomic and environmental factors in rural and urban Venezuelan school children

	Rural (350)	Urban (120)	Statistical significance	95% Confidence interval
Percentage of Illiterate mothers	28.5	15	$p = 0.0523$ Relative risk 1.276	1.033–1.576
Percentage with access to tap water	78.5	95	$p < 0.0001$ Relative risk 0.598	0.504–0.709
Percentage with latrine/toilet	86	97	$p = 0.001$ Relative risk 0.655	0.521–0.760
Percentage with extreme poverty	31	12	$p = 0.0024$ Relative risk 1.424	1.171–1.731
Percentage of children living in clay block open houses	83	17	$p < 0.0001$ Relative risk 3.468	2.621–4.856
Percentage of houses with positive house dust mites samples	23	86	$p < 0.0001$ Relative risk 0.174	0.115–0.264
Percentage of children using blankets and pillows	18	90	$p < 0.0001$ Relative risk 0.197	0.137–0.285

of education were determined in the different groups of children. Environmental factors influencing exposure to different aeroallergens such as type of houses, sleeping habits, use of blankets and pillows were also studied.

House dust samples were collected by our field research group in 60 randomly selected houses in each of the communities studied. Three different samples of house dust were taken using a standard vacuum cleaner with a new bag for each house, according to the recommendations of Platts Mills and De Weck (1989). The house dust samples were placed in 70% ethanol and stored at 4 °C. Samples were filtered several times and the presence of mites was determined by light microscopy (Colloff and Spieksma, 1992). Results were expressed as the percentages of houses with at least one positive sample in each community.

The degree of poverty of the families was determined according to criteria by the Venezuelan Ministry of Social Development (República Bolivariana de Venezuela, Gabinete Social, 2004). According to this classification, the total family incomes are compared to the cost of a "shopping basket" of basic food items, and to the cost of a basic food and services basket for a standard family. Families whose incomes were below the shopping basket of basic food items were considered as in extreme poverty.

2.3. Ethical considerations

Informed parental consent was obtained to participate in the study and the Ethical Committees of the Regional Department of the Ministry of Health and of the Institute of Biomedicine approved the protocol. During the study, medical assistance was provided to the children and they were treated for helminthic infections and respiratory diseases as well as for other common child diseases.

2.4. Parasite evaluation

Faeces samples were collected and direct examination of them was performed. The prevalence and intensity of *A. lumbricoides* infection was assessed using the method of Kato (Melvin and Brooke, 1989). The number of eggs per gram of faeces was calculated in all samples.

2.5. Clinical evaluation

Anthropometric measurements were determined according to the methods described by Gorstein et al. (1994). Standing height was assessed using a Harpender portable stadiometer and weight by a DETECTO balance (±0.1 kg). Weight/age, Height/age and Weight/Height were determined using the WHO standard curve. Anthropometric data was analyzed with EPI INFO (Public Domain Software from Epidemiology and Disease Surveillance, WHO, 1991).

Asthma was characterized following the guidelines of the American Thoracic Society (The international Study of Asthma and allergies in childhood Steering Committee, 1998). Clinical respiratory symptoms such as cough and/or wheezing, as well as clinical antecedents of wheezing attacks, persistent cough, use of asthma

drugs, and the number of asthma crisis that needed hospitalization a year before the study, were evaluated in all the children studied.

Pulmonary function was measured using a Respyradine II solid state spirometer (Sherwood Medical, St. Louis, MO). The forced vital capacity (FVC), force vital capacity volume in one second (FEV1) were determined and expressed as percentages of the values predicted according to height and sex of the children, using normal values determined for a group of Spanish subjects (Cobos, 1979). Also, the FEV1/FVC ratio was calculated and expressed as percentages. Children with and FEV1/FVC ratio of less than 75% and current respiratory symptoms or at least two clinical antecedents of asthma were considered as asthmatic in this study.

Bronchial hyper reactivity was confirmed by bronchial provocation with histamine, in which 1 mL of histamine base (as dihydrochloride; Sigma Chemical Co., St. Louis MO) was nebulized in stepwise concentrations from 0.0081 to 5.0 mg/mL. FEV1 changes $\geq 20\%$ (PC_{20}) at a ≤ 2.5 mg/mL histamine concentration was considered as diagnostic for bronchial hyper reactivity.

2.6. Skin prick tests

Skin prick tests were performed with extracts of: *D. pteronyssinus*, mix of common moulds (*Aspergillus fumigatus*, *Alternaria* sp., *Candida albicans*), common insects (*Aedes communis*, *Culex pipens*) and *A. lumbricoides*. These allergenic extracts were prepared as described previously (Lynch et al., 1983) and were used at a 0.6 mg/mL concentration in a saline solution with 50% glycerol and 0.4% phenol. Control tests were performed with the diluent alone and with 1% (w/v) histamine dihydrochloride. Wheal diameters were measured after 20 min and diameters $\geq$3 mm were considered positive.

2.7. Specific IgE levels

Specific anti-*A. lumbricoides* IgE levels were measured by an ELISA developed in our Laboratory and standardized against the commercial RAST technique (Hagel et al., 2005; Hagel et al., 2006). *A. lumbricoides* adult worm antigen preparation (3 μg/well), or aeroallergen extract: *D. pteronyssinus*, common mix of moulds (*A. fumigatus, Alternaria* sp., *C. albicans*) or common mix of insects (*A. communis, C. pipens*) (1 μg/well), respectively, was coated onto 96-well microplates (Immunolon IV, Dynatech Laboratories Inc., Virginia USA) and incubated overnight at 4 °C. Excess antigen was washed off with PBS-T and plates were blocked for 2 h at 37 °C with 1% BSA. Undiluted test sera were plated and incubated for 1 h at 37 °C. After further washes with PBS-T, the plates were incubated with 1 μg/well murine monoclonal anti-human IgE (produced in the Institute for Child Research, Perth, Australia) for 1 h and then washed off with PBS-T, then the plates were incubated with peroxidase-conjugated anti-mouse IgG diluted 1:3000 (Sigma). The washing process was repeated and *o*-phenylenediamine (OPD) plus H_2O_2 was added. The O.D. was read at 490 nm. A standard titration curve using a pool of sera with high specific anti-*A. umbricoides* or anti-aeroallergen extracts (*D. pteronyssinus, moulds, insects*) IgE levels in the commercial PHADIA test was run with each assay, and the results were expressed as PHADIA RAST units (PRU/mL).

2.8. Lymphocyte surface markers

Peripheral blood samples were obtained by venipuncture, and collected into EDTA anticoagulant tubes. Flow cytometry assays were undertaken using a FACScan Flow Cytometer (Becton Dickinson, San Jose, California, USA). Dual colour (FITC-PE) immunofluorescent staining of cell surface antigens in unseparated blood was performed using monoclonal antibodies for the detection of the following cellular phenotypes: CD3+CD4+(FITC/PE), CD20+CD21+ (PE/FITC), CD20+CD23+ (FITC/PE). The monoclonal antibodies used were purchased by DAKO. Results were expressed as absolute number of lymphocytes/mm^3.

2.9. Statistical Analysis

Statistical analysis was performed using a Graph-Pad Instat version 3.00 for Windows 95, GraphPad Software, San Diego California USA.

The number of *A. lumbricoides* eggs/g of faeces was logarithmically transformed and the mean and standard deviations (S.D.) calculated. Results were expressed as geometric means and geometric mean + 1 S.D. The mean ± S.D. of specific anti-*A. lumbricoides*, anti-*D. pteronyssinus, anti-moulds and anti-insects* IgE values was also determined. The proportion of positive responder children (IgE levels > 0.7 UI/mL) for both *A. lumbricoides* and the different aeroallergen extracts was determined according to the International PHADIA RAST (PHARMACIA) classification.

Mean values of the different B and T cell subpopulations were compared using unpaired Welch's *t*-test. Comparison of the proportion of parasitized children as well as the percentages of positive skin prick test in the

different groups was done using Fischer's exact test. Relative Risk and 95% Confidence interval were calculated. Pearson's rank correlation between different variables was also determined. The Spearman rank correlation was used to establish a possible association between the percentage of predicted FEV1 values and specific IgE levels in serum samples.

3. Results

3.1. Environmental and socio-economic factors

We did not find any significant difference between socioeconomic factors among the two rural groups of children. Both communities showed a lack in sanitary facilities being the percentage with access to tap water 76% in La Salina, Sucre State and 81% in El Cardón, Nueva Esparta State. Also, the proportion of children living in houses with latrine/toilette was very similar (85% and 86%, respectively). The percentage of children living in extreme poverty conditions was also comparable between the rural communities, being of 26% in La Salina and of 35% in El Cardón.

Table 1 shows that access to sanitary facilities was significantly ($p < 0.0001$) deficient in the groups of children living in rural areas compared to their urban counterparts. The proportion of children living in extreme poverty and with illiterate mothers was also significantly higher ($p = 0.0523$ and 0.0024, respectively) in the rural group.

There were differences in the type of houses between the urban and rural communities. Whilst the great majority of rural children (80% in La Salina and 85% in El Cardón) live in clay block bungalows, near the sea (fishing communities from the east coast of Venezuela), children from the urban slum, live in small reinforced concrete houses or small apartments in the main city (Table 1). Sleeping habits were also found to be different between rural and urban groups. The proportion of children using pillows and blankets was significantly higher ($p < 0.0001$) in the urban group, whereas most of the rural children sleep in swing hammock. Only, 24% of the children from La Salina and 12% from El Cardón used blankets and pillows. In addition, the percentages of houses or bungalows with positive house dust mites samples (12% in La Salina and 17% in El Cardón) was significantly lower ($p < 0.0001$) among the rural communities compared to the urban slum (Table 1).

3.2. Prevalence and intensity of A. lumbricoides infection

The prevalence of intestinal helminthic infection was very similar among the rural groups of children in which sanitary conditions and poverty levels were comparable (65% of *A. lumbricoides* in La Salina and 72% in El Cardón, 70% of *T. trichiura* in La Salina and 68% in El Cardón). A higher prevalence ($p < 0.0001$) of *A. lumbricoides and T. trichiura* ($p < 0.0001$) was observed among

Table 2
Clinical parameters and prevalence of helminthic infection in rural and urban groups of Venezuelan school children

	Rural (350)	Urban (120)	Statistical significance	95% Confidence interval
Median age	9 years (6–12)	9 years (7–12)	Mann–Whitney 1059.5	-
Male/female	93/84	90/70	$p = 0.0573$	
Percentage of respiratory symptoms	24.6	15.8	$p = 0.175$ Relative risk 1.118	0.948–1.448
Percentage of asthmatic children	17.9	12	$p = 0{,}1002$ Relative risk 1.255	0.999–1.576
Percentage of height/age < 10th percentile	20	15	$p = 0.521$ Relative risk 1.09	0.852–1.412
Percentage of weight/age < 10th percentile	14.5	8	$p = 0.428$ Relative risk 1.116	0.876–1.552
Percentage of height/weight < 10th percentile	6	3	$p = 0.406$ Relative risk 1.267	0.894–1.794
Prevalence of *Ascaris lumbricoides*	68.5	8.7	$p < 0.0001$ Relative risk 2.704	2.169–3.372
Prevalence of *Trichuris trichiura*	69	5	$p < 0.0001$ Relative risk 3.203	2.517–4.076

the whole rural group of children compared to the urban group.

According to the WHO classification (Montresor et al., 1998), the intensity of *A. lumbricoides* infection was found to be moderate (under 7000 egg/g faeces) among the rural children population and not clinically significant (under 3000 egg/g faeces) among its urban counterpart (Table 2). The prevalence of other helminths such as *Strongyloides stercoralis* and *Ancylostoma duodenale* were considered as sub-clinic (less than 18%) in the rural groups. These parasites were not detected among the urban children.

3.3. Clinical evaluation and pulmonary function

We did not found statistically significant differences between the percentages of asthmatic children (17.3% in La Salina, 18.4% in El Cardón and 12% in the urban group). The same pattern was observed in the prevalence of respiratory symptoms like wheezing and persistent cough among the different groups evaluated in this study (22.4% in La Salina, 26.7% in El Cardón and 15.8% in the urban group). The proportion of children with anthropometric deficiencies: height/age, weight/age and weight/height under 10th percentile, according to the WHO criteria were also comparable between the different groups evaluated and there were no statistical differences between urban and rural children (Table 2). However, the mean predicted percentages of FEV1 were significantly lower (Welch *t*-test: 30.090; $p < 0.0001$) in the rural children (75 ± 3) compared to the urban group (87 ± 4). Also, PC_{20} fall in FEV1 was significantly higher ($p = 0.0058$; relative risk 2.192) in the rural group. Therefore, bronchial hyper reactivity was demonstrated in 27% rural and 14% urban children.

3.4. Specific IgE levels and skin allergic reactivity

Table 3 shows that bronchial hyper reactivity in rural children was associated to specific anti-IgE levels ($p > 0.0001$) and skin hypersensitivity against *A. lumbricoides* antigen ($p < 0.0001$). A significant inverse correlation (Spearman rank -0.6724, $p < 0.0001$) was found between the percentage of predictive FVE1 values and anti-*A. lumbricoides* IgE levels among these children. In contrast, in the urban group bronchial hyper reactivity was associated to an elevated skin hypersensitivity to *D. pteronyssinus* ($p = 0.003$) and high specific anti-IgE levels against this allergen ($p = 0.0089$) (Table 4). We did not found any associations between bronchial hyper reactivity and allergic reactivity to other aeroallergens such as moulds or insects.

Table 3
Ascaris lumbricoides infection, IgE levels and skin hyper reactivity against *A. lumbricoides* according to bronchial hyper reactivity in rural and urban Venezuelan school children

	Rural				Urban				
	Bronchial hyper-reactivity (93)	Asymptomatic (257)	Statistical significances	95% Confidence interval	Bronchial reactivity (17)	hyper-	Asymptomatic (103)	Statistical significances	95% Confidence interval
Prevalence of *A. lumbricoides* (%)	72	67	$p = 0{,}4355$ Relative risk 1.197	0.8079–1.773	10		8	$p = 0.6324$ Relative risk 1.467	0.3894–5.524
Geometric mean Number of *Ascaris* eggs/g faeces (Geometric mean + 1 S.D.)	4850 (6300)	5060 (6800)	$t = 0.164$ $p = 0.8730$	–	1305 (1826)		980 (1050)	$t = 0.1908$ $p = 0.8511$	–
Percentage of children with specific anti-*Ascaris* IgE levels > 0.7 UI/mL (mean ± S.D.)	63.4 (3.75 ± 0.66)	26.5 (0.8 ± 0.45)	$p < 0.0001$ Relative risk 3.047	2.123–4.374	12.5 (0.65 ± 0.19)		8.5 (0.43 ± 0.20)	$p = 0.6545$ Relative risk 1.321	0.3140–4.672
Percentage of children with positive *Ascaris* skin prick test (wheal > 3 mm)	55	16	$p < 0.0001$ Relative risk 3.405	2.443–4.746	7.2		6.5	$p = 0.3742$ Relative risk 0.8750	0.1335–5.839

Table 4
Serum IgE levels and skin allergic hyper reactivity according to bronchial hyper reactivity in rural and urban Venezuelan school children

Percentage of children	Rural				Urban			
	Bronchial hyper-reactivity (93)	Asymptomatic (257)	Statistical significances	95% Confidence interval	Bronchial hyper-reactivity (17)	Asymptomatic (103)	Statistical significances	95% Confidence Interval
Specific anti-*Dermatophagoides* IgE levels > 0.7 UI/mL (mean ± S.D.)	21 (0.65 ± 0.32)	16 (0.45 ± 0.34)	$p = 0.202$ Relative risk 1.382	0.8536–2.237	56 (1.70 ± 0.78)	25 (0.7 ± 0.33)	$p = 0.0089$ Relative risk 3.33	1.378–8.066
Positive *Dermatophagoides* skin prick test (wheal > 3 mm)	16	12	$p = 0.370$ Relative risk 1.271	0.804–2.012	46	12	$p = 0.003$ Relative risk 5.304	1.952–10.119
Specific anti-moulds IgE levels > 0.7 UI/mL (mean ± S.D.)	17 (0.35 ± 0.23)	12	$p = 0.217$ Relative risk 1.340	0.861–2.085	21 (0.55 ± 0.22)	10	$p = 0.112$ Relative risk 2.330	0.880–6.161
Positive anti-moulds skin prick test (wheal > 3 mm)	14	9	$p = 0.256$ Relative risk 1.293	0.882–2.277	18	7	$p = 0.150$ Relative risk 2.357	0.811–6.845
Specific anti-insects IgE levels > 0.7 UI/mL (mean ± S.D.)	20 (0.66 ± 0.42)	15	$p = 0.256$ Relative risk 1.293	0.851–1.964	17 (0.45 ± 0.12)	11	$p = 0.417$ Relative risk 1.622	0.5316–4.951
Positive insects skin prick test (wheal > 3 mm)	18	11	$p = 0.1063$ Relative risk 1.478	0.966–2.261	14	9	$p = 0.654$ Relative risk 1.321	0.3463–5.041

Statistical comparisons between rural and urban groups, showed that the percentages of rural children with clinically significant specific anti-*A. lumbricoides* IgE levels (45%) and *A. lumbricoides* skin positivity (36%) were significantly elevated compared to their urban counterparts (10.6% and 7%, respectively) ($p < 0.0001$; relative risk 1.436 for anti-*A. lumbricoides* IgE levels and $p < 0.0001$; relative risk 1.721 for *A. lumbricoides* skin positivity). In contrast, specific anti-*D. pteronysinnus* IgE levels were significantly lower ($p < 0.0001$; relative risk 0.712) in the rural children (19%) compared to the urban group (41%). The same pattern was observed for skin prick test positivity, 14% in the rural children and 29% in the urban group ($p < 0.0001$; relative risk 0.7481).

3.5. Circulating T cell subpopulations

We observed a significant increase (Welch *t*-test 15.526; $p < 0.0001$) on the mean number of circulating CD3+T lymphocytes subpopulations among the rural children (2650 ± 830 lymphocytes/mm^3) compared to the urban group (1700 ± 320 lymphocytes/mm^3). A similar pattern (Welch *t*-test 27.532; $p < 0.0001$) was observed for circulating CD3+CD4+ T cells (1600 ± 360 lymphocytes/mm^3 in the rural children and 820 ± 245 lymphocytes/mm^3 in the urban group).

Table 5 shows statistical comparisons of the different lymphocytes subpopulations according to the presence of bronchial hyper reactivity among rural and urban children. A significant elevation on the mean number of CD3+ T cells was associated to bronchial hyper reactivity in both rural ($p < 0.0001$) and urban ($p = 0.032$) children. Also, the mean number of circulating CD3+CD4+ T cells was significantly higher in those rural children with bronchial hyper reactivity ($p < 0.0001$) compared to their asymptomatic counterparts, whereas there was no significant difference among the urban group. The mean number of circulating CD3+CD8+ was significantly elevated ($p < 0.0001$) in urban children with bronchial hyper reactivity compared to those asymptomatic (Table 5).

3.6. Circulating B cell subpopulations

The mean number of total B lymphocyte circulating sub-populations (CD20+) was found to be significantly elevated (Welch *t*-test 11.028; $p < 0$, 0001) among rural children (625 ± 130 lymphocytes/mm^3) compared to the urban group (450 ± 120 lymphocytes/mm^3). The same pattern (Welch *t*-test 27.532; $p < 0.0001$) was observed for B

Table 5
Absolute number of circulating lymphocytes in rural and urban Venezuelan school children according to the presence of bronchial hyper reactivity

Lymphocytes/mm^3	Rural				Urban			
	Bronchial hyper-reactivity (93)	Asymptomatic (257)	Statistical significances	95% Confidence interval of the difference	Bronchial hyper-reactivity (17)	Asymptomatic (103)	Statistical significances	95% Confidence interval of the difference
CD3+	2836 ± 608.4	1965 ± 820	$t = 9.297$ $p < 0.0001$	−1031.9 to −710.5	1820.2 ± 322.5	1630 ± 266.5	$t = 2.306$ $p = 0.0325$	−357.21 to −22.72
CD3+CD4+	1846 ± 388	1120 ± 340	$t = 5.940$ $p < 0.0001$	−816.15 to −635.85	801.3 ± 115.4	897 ± 246	$t = 2.598$ $p = 0.0126$	21.57 to 170.42
CD3+CD8+	990 ± 256	923 ± 243	$t = 2.184$ $p = 0.0304$	−127.59 to −6.41	1019 ± 122	733 ± 123	$t = 8.944$ $p < 0.0001$	−352.49 to −219,51
CD20+	622 ± 243	625 ± 133	$t = 0.1087$ $p = 0.9136$	−49.62 to−35.62	430.5 ± 127.2	470.9 ± 121.4	$t = 1.211$ $p = 0.239$	−28.685 to 108.79
CD20+CD21+	600 ± 145	585 ± 112	$t = 0.8442$ $p = 0.3998$	−47.83 to −17.87	335.5 ± 132.5	345 ± 112.3	$t = 0.2953$ $p = 0.7710$	−60.87 to 80.87
CD20+CD23+	285 ± 126.4	130 ± 65	$t = 10.936$ $p < 0.0001$	−182.1 to 127.86	182.6 ± 63.8	72.4 ± 43.6	$t = 6.862$ $p < 0.0001$	−143.31 to −76.68

activated lymphocytes sub populations (CD20+CD21+) being 620 ± 130 lymphocytes/mm^3 among the rural children and 340 ± 110 lymphocytes/mm^3 in the urban group.

The mean number of B cells subpopulations (CD20+and CD20+CD21+) was not associated to bronchial hyper reactivity among the children studied (Table 5). Although, the expression of the low affinity IgE receptor (CD23) on circulating B cells was more elevated in those children with bronchial hyper reactivity ($p < 0.0001$) compared to their asymptomatic counterparts. The number of CD20+CD23+ B cells correlated positively with specific anti-*A. lumbricoides* IgE serum levels (Pearson rank 0.675; $p < 0.0001$) and with number of circulating CD4+ T cells (Pearson rank 0.4562; $p < 0.005$).

4. Discussion

It is well known that *A. lumbricoides* infection is involved in asthma and bronchial hyper responsiveness modulation (Weiss, 2000). It has been proposed that the burden and chronicity of the infection are important variables in determining whether these parasites act as a risk factor or confer protection against these diseases. In areas where geo-helminthic transmission is mild or seasonal, an acute allergic enhancing phenotype, due to the effect of migrating larvae derived antigens or by allergenic stimulation targeted to non-parasite allergens, may predominate (Cooper, 2002). In contrast, in areas in which transmission is intense and continuous, more chronic and allergic suppressing infections, characterized by their capacity to stimulate anti-inflammatory cytokines and blocking antibodies by parasites derived mediators would be expected (Yazdanbakhsh et al., 2001). Probably due to an improvement of de-worming programs in rural communities in Venezuela, *A. lumbricoides* infection among the rural groups studied may behave as "seasonal", and moderate worm burden were also found. This could be related to the increased prevalence of asthma observed in this study compared to previous reports in rural children from Venezuela (Hagel et al., 1993b; Lynch et al., 1983). In addition, a study performed in China showed that light *A. lumbricoides* infections were associated with an increased asthma risk in children from rural areas (Palmer et al., 2002). Also, asthmatic children from a Venezuelan endemic area improved clinical symptoms of asthma after deworming (Lynch et al., 1997).

Although, the prevalence of asthma and the frequency of respiratory symptoms were comparable between the urban and rural groups, the manifestation of bronchial

hyper reactivity was significantly higher among the rural children. This is consistent with previous findings showing a basal state of bronchoconstriction in asthmatic (Lynch et al., 1999) and non-asthmatic (Lynch et al., 1992a,b) Venezuelan parasitized children. Regardless the prevalence and intensity of *A. lumbricoides* infection, bronchial hyper reactivity in rural children was found to be associated to the capacity to develop a strong specific IgE response and a high allergic skin hypersensitivity against *A. lumbricoides*. In this respect, we have previously reported that an underlying genetic component may influence the production of specific IgE responses against parasite antigens in children from Venezuelan endemic areas (Lynch et al., 1998; Le Souef et al., 2000). These results are in agreement with recent studies carried out in Brazilian adolescents, with asthma or allergic rhinitis, living in helminthic endemic areas that have shown elevated levels of specific anti-*Ascaris* IgE (Medeiros et al., 2006). Also, *Ascaris*-specific IgE levels have been positively associated with atopic manifestations in South African children chronically exposed to these parasites (Obihara et al., 2006).

Allergic reactivity against common aeroallergens was not clinically significant among these rural children. Probably, due to life style conditions and other environmental factors (Perdomo de Ponce et al., 1991), exposure to the major allergen *D. ptenonyssinus*, was low in these children. Thus, exposure to parasite antigens seems to be the predominant risk factor in the promotion of bronchial inflammation among these children. However, the possible influence of other local sources of allergens such as domestic animal epithelia or the presence of dry fish and shellfish remains, commonly seen in rural fishing communities should be investigate.

Previous studies in human populations have shown that Th2 cytokines are up-regulated in individuals infected with *A. lumbricoides* compared with those from non-endemic areas (Cooper et al., 2000). Studies performed in allergic individuals have highlighted the role of the low affinity IgE receptor (CD23) in the stimulation of the IgE response against environmental allergens (Aberle et al., 1997; van Neerven et al., 2004). Venezuelan slum pre school children infected with *A. lumbricoides* have shown elevated levels of circulating CD20+CD23+ B cells compared to uninfected children (Hagel et al., 2003) and a strong correlation between CD20+CD23+ B cells and specific IgE against *A. lumbricoides* antigens has been observed in Warao Amerindian parasitized children (Hagel et al., 2006). In this work, we observed that the capacity to produce high levels of specific IgE against parasite antigens was associated with an increased number of circulating CD4+ helper T cells and CD20+CD23+ B cells. This could reflect the preferential stimulation of Th2 type responses by *A. lumbricoides* derived antigens.

In contrast, in the urban group of children, bronchial hyper-reactivity was associated to skin hypersensitivity and specific anti-IgE against *D. pteronyssynus* rather than to parasite antigens. The significant increase in the number of CD23 positive B cells in children with bronchial hyper reactivity suggests a role of this receptor in the isotopic differentiation of specific anti-*D. pteronyssynus* IgE B cells. Thus, in the absence of helminths the capacity to develop Th2 derived IgE responses may be directed to other common allergens. Therefore, exposure to mites may be the main cause of respiratory disorders such as asthma among urban children. On the other hand, a significant increase in the number of CD3+CD8+ T cells was associated to the occurrence of bronchial hyper reactivity in the urban group. This could be attributed to local immune responses against rhinovirus and other viral infections (O'Sullivan et al., 2001; Papadopoulos et al., 2004) that are frequent in urban children from low socio-economic levels.

Our results suggest that the intrinsic capacity to develop strong specific IgE responses, possible associated to a predominant TH2 immune profile evolved to improve resistance to helminthic infection in tropical populations, could render these children more susceptible to allergic hyper sensitivity and bronchial inflammation. In the other hand, improvement of life standards particularly those associated to access to tap water and adequate sanitary conditions may decline parasitic infections, shifting the cause of bronchial hyper reactivity from an association with IgE responses against mild *A. lumbricoides* infection to an association with IgE to airborne allergens. However, the importance of genetic and environmental factors influencing the increase on the prevalence of bronchial hyper reactivity and asthma in rural Venezuelan children needs further studies.

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