

## **Some Environmental and Socio-Economic Factors Associated to Asthma Establishment in Populations with Different Sanitary Conditions**

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### **Abstract**

*The objective of this research was to identify environmental factors that favor asthma incidence and also, analyze the development of inflammatory processes in the lung cells induced by bacterial population present in house air with and without reports of asthma in Puebla State, México. The role of bacterial products was analyzed using the pattern of asthma Ovalbumin Antigen (OVA) in mouse and the immune response in vivo through intratracheal inoculation of lipopolysaccharide (LPS). According to the data obtained, there were cases of asthma reported only in 33 of the 217 municipalities, finding a bigger tendency to develop it in less marginal municipalities. The results indicate that different bacterial groups and their products present in air, when they are in contact with people in early development phases, block the allergic hyper response induced by allergens. All of them could explain the low level of asthma incidence in extremely marginal municipalities.*

**Keywords:** lipopolysaccharide (LPS), Ovalbumin Antigen (OVA), endotoxin, intratracheal inoculation, Antigen (Ag)

### **Introduction**

The incidence of allergic disorders and the morbidity and mortality caused by these disorders, increased substantially, in developed countries during the second half of the twentieth century (Abbas & Janeway 2000; Lambrecht *et al.* 2000). There are probably multiple reasons for this increase, including improvements in vaccines, antibacterial drugs, and hygiene that decrease bacterial and parasitic infection and modify normal intestinal flora; changes in diet and activity; decreased family size; and a change in exposure to pollutants created by fuel combustion. Regarding to the last possibility, any increase of combustion-associated pollutants contributes to allergy. It may reflect a qualitative change in pollutant exposure caused by a shift from the use of wood and coal to the use of petroleum for energy, rather than a quantitative increase in exposure to combustion-related pollutants (Abbas & Janeway 2000; De Jong *et al.* 2002). Diesel exhaust particles (DEP) have been linked to the incidence of allergic disorders, particularly asthma, in epidemiological studies. Inhaled and injected DEP have been shown to be a potent adjuvant for the development of an allergy-related T helper type 2 cells (Th2) immune response in animal studies (Sparwasser *et al.* 1998; Magram *et al.* 1996). Atopic asthma is characterized by chronic eosinophilic airway inflammation, and occurs in individuals with strongly polarized Th2 recall responses to environmental allergen.

The incidence of asthma has greatly increased over recent years, concomitant with an improved hygienic status in the industrialized world, suggesting some form of environmental control over Th2 development (Jancovic et al. 2001; Jancovic *et al.* 2002). Th cell polarization of immune response in the lung, is influenced by the route of antigen (Ag) exposure, the subtype of cell presenting Ag, the dose of Ag, the genetic background of the host, and most importantly, by the nature of the Ag (Riedler *et al.* 2001). It is increasingly clear that different molecular patterns expressed on pathogens can fundamentally influence Th differentiation by signaling molecular pattern recognition receptors on cell (Tulic et al. 2000). Many of the microbial patterns such as bacterial LPS, peptidoglycan, induce the secretion of interleukin 12 (IL-12) by signaling through the Toll-like receptors (TLR) and the myeloid differentiation factor 88 signaling pathway, thus inducing polarization toward Th1 responses (Akira *et al.* 2001; Edwards *et al.* 2002).

Asthma is one of the most common chronic dysfunction in the world and it affects more than a hundred million of people of all ages. Between 10 and 15 percent of children in developed countries, suffer asthma and, in spite of the readiness of effective treatments, the incidence and the graveness of asthma is growing in a constant rhythm. The most probable causes of this increment have to be still determined, even though one possibility is that environmental factors play an important role.

### **Materials and methods**

#### **Morbidity Statistics Records**

The 1990-2010 Annual Morbidity Records of Puebla State in México country, according to the Services of Health (SSA) (Source: Sistema Único de Información Para la Vigilancia Epidemiológica, Dirección General Epidemiológica, SSA) have been reported by physicians and medical units weekly by law, and there are no variations respect to other sicknesses and the probability of underreporting of asthma is really low.

#### **Diagnostic's criteria of asthma**

Asthma patients in Hospital Regional ISSSTE Puebla, México were examined by a certified allergist and according to the Guidelines for the Diagnosis and Management of Asthma (GINA). He determined that the episodic symptoms of airflow obstruction were present; airflow obstruction was at least partially reversible, alternative diagnoses were excluded. He also carried out a detailed medical history, physical exams focusing on the upper respiratory tract, chest, and skin with Spirometry to demonstrate reversibility and chest X ray, also a laboratory proof finding high levels of immunoglobulin E (IgE). In the present study we used a questionnaire designed by the International Study of Asthma and Allergy in Childhood (ISAAC) to determine the prevalence of asthma, which allows users to find factors associated with asthma. ISAAC survey was validated and standardized in Spanish.

**Source:** Estimate of Consejo Nacional de Población y vivienda (CONAPO) according to Censo de Población y Vivienda 2010, and Encuesta Nacional de Ocupación y Empleo (ENOE) 2010, IV Trimestre.

#### **Antigen**

Chromatographically purified Ovalbumin (OVA) endotoxin-free, was obtained from Sigma Chemical Co., St Louis, MB, LPS (*Escherichia coli*, strain O26:B6) was purchased from Sigma-Aldrich (St. Louis, MO).

#### **Animals**

Male BALB/c mice (2, 4, 6, 8 and 24 weeks-old) and 8 weeks old Wistar Rats were purchased from the animal facility "Claude Bernard" of the Benemérita Universidad Autónoma de Puebla (BUAP). Mice and rats were housed in micro isolators under specific pathogen-free conditions.

#### **Animal asthma model**

Animals in all the experiments were immunized when they had 2, 4, 6, 8 and 24 weeks-old. The study was carried out in three groups of mice: Group I = OVA/OVA, Group II = LPS – OVA/OVA, Group III = OVA/OVA – LPS. Mice in Group I (OVA/OVA) was sensitized with 50 µg OVA intraperitoneal injection (ip) with 5 mg of the adjuvant Alum (Sigma) in 2 occasions with 5 days of difference. On day 10, animals were lightly anesthetized and a test of intratracheal instillation (i.t) with 50 µg of OVA was performed. The instillations were carried out daily during 4 serial days. On day 14, the allergic inflammatory response was evaluated, 24 h after the last instillation.

Mice in Group II (LPS - OVA/OVA) were challenged with intratracheal injection of 500 ng of LPS from day 0 to day 5; then mice were treated using the same protocol of the group OVA/OVA.

Mice in Group III (OVA/OVA-LPS) were sensitized in the same way that mice of the group OVA/OVA and later there were challenged with intratracheal injection of 500 ng of LPS on day 23, 24 h after the last instillation, any allergic inflammatory response was evaluated. Another group of mice were instilled in a same way, but only with phosphates salt solution (PBS) and were used as control group.

#### ***Bronchoalveolar lavage (BAL)***

Mice were weighed, anesthetized with ketamine (25 mg/kg ip) and xylazine (5 mg/kg ip), then intubated as described by Brown et. al (1999). Briefly, mice were suspended by their upper incisors using a rubber band on a 60° incline board. The trachea was transilluminated below the vocal cords to allow visualization of the trachea through the oral cavity. With the lower jaw held open and the tongue held out, a 2-cm length of PE-90 polyethylene tubing with a beveled tip (connected to a 20-gauge needle hub) was gently inserted into the tracheal opening. Mice were ventilated with 100% O<sub>2</sub> at 120 breaths/ min with a tidal volume of 200 µl for 3 min before BAL was performed. BAL was performed by slowly delivering 0.6 ml of PBS at 37°C through the tracheal tube. The fluid was slowly withdrawn by gentle suction immediately after delivery and stored on ice, then frozen to -70 °C until further processing. This procedure was repeated two additional times.

Cellular content was placed in PBS and the number of cells was determined throughout the quantification of an aliquot, using an automatic blood cell counter (Sysmex PDA-500, Sysmex Europe GMBH, Norderstedt, Germany).

#### ***Eosinophilia in BAL***

White blood cell slides were prepared using a cyto-centrifuge (Cytofuge Statspin, Iris Company, Norwood USA). Samples were stained for the detection of number of eosinophils via a histochemical technique that takes advantage of the resistance property to the cyanide of the eosinophil's peroxidase, using 3.3 diaminobenzidin tetrahydrochloride (DAB) as substratum. Slides were examined with optic microscope Zeiss (Zeiss KS400® Imaging system 3.0 Munich, Germany) with an x-40 magnificant in a session to simple blind. Differential quantification of the rest of cellular types was evaluated using May-Grunwald – Giemsa stain. At least there were 400 cells for sample and the final results were expressed in percentage.

#### ***Measurements of IgE total in serum and BAL***

Serum and BAL samples were obtained 24 hours after the last challenge and analyzed by enzyme linked immunosorbent assay (ELISA) with the sandwich technique. 96-well plates were incubated with purified monoclonal anti-IgE murine antibody (BD Pharmingen, San Diego, CA, USA) and for the detection, it was used an antibody anti-IgE murine biotinylated HRP-conjugated. Plates were developed with ABTS® solution (Boehringer Mannheim GmbH, Germany) and read at 415 nm. wavelength using a spectrophotometer. Each sample was determined in duplicate and in different concentrations. The IgE values were measured using a purified standard IgE murine solution. The lower limit on sensitivity of the assay was 15 ng/ml.

#### ***Measurements of IgE OVA specific in serum***

Plates were coated with OVA and the rest procedure was the same of IgE total. It was used as standard solution a mixture of sera with known concentrations of IgE OVA specific, according to Zuberi technique (2000).

#### ***Determination of sensibilization***

The IgE OVA specific titers of the sensitized mice were evaluated by a passive cutaneous anaphylaxis (PCA) proof. 8 weeks old Winstar rats were injected subcutaneously in 4 different places on their back, 0,05 ml of sensitized mice serum (OVA/OVA) in 1/40; 1/80; 1/160 y 1/320 dilutions, using as control, mice serum control. After 48 hours, the rats were injected via intravenous with 1 mg of OVA in 0.05 ml of PBS with Evan's Blue 1% and sacrificed 10 min after injection. The positive dilutions were those which cause blue blisters, 5mm or more of diameter.

### ***Identification of the bacterial population***

The present study was carried out in two municipalities of Puebla State, in houses of La Fragua community which does not count on asthma reports and in asthmatic patients' houses of Puebla City, which were diagnosed at Hospital Regional de Puebla ISSSTE, and several aspects were analyzed, among them: marginal status, asthma incidence, productive sector, housing condition, weather and geographic conditions (see Table 1).

To ensure that the selected houses represent the population adequately, a stratified sampling was developed in La Fragua, with a sample size of 154, a reliability level of 93% and a maximum mistake of 7%, for a total of 1894 houses.

The same procedure was done in the asthmatics' houses in Puebla City, with a sample size of 160, a reliability level of 93% and a maximum mistake of 7%, for a total of 1894 asthmatics' houses. Puebla City has a low marginalization index of -1.946 %. This municipality has 102 medical units, 2,714 physicians and 846591 inhabitant users; in addition, there are 10 health houses of SSA. It also has 34 private hospitals, 9 sanatoriums, 6 hospitals, 3 medical centers and 16 medical clinics.

La Fragua has 7767 inhabitants and there are 4 medical assistance units, 3 of them belong to the Instituto Mexicano del Seguro Social Solidaridad (IMSS) and the other one, to the Instituto Nacional Indigenista (INI). The IMSS units give service to a population of 3162 and it is given by 5 physicians and 2 nurses; the INI has 2 physicians and 2 nurses and also, 7 health houses.

Air sampling was performed using a M. Air T Sampler manufactured by Millipore Corporation with different culture media to obtain pure cultures. The sample was taken in the middle of the dining-room at a height 80 cm from the floor during 30 min, from May to July.

Identification was done according to the Bergey's Manual of Determinative Bacteriology. In all the cases a fresh culture after 18-24 hours was made, in culture media under optimal conditions. Quality control tests were done using a positive and a negative control for each culture media.

### ***Statistical analysis***

Statistical package InStat version II and Statview (Statview software, 4.51, Abacus concepts, Inc., Berkeley, CA, EE.UU.) were used. Difference between means were significant when  $p < 0.0001$ .

### ***Results and Discussion***

#### ***Incidence of asthma in Puebla State***

The data found in the 1990-2010 Annual Morbidity Records of SSA, showed that until 1995, asthma was not one of the 20 major causes of morbidity in the country and in Puebla state, however, since 1996 it appears as 10<sup>th</sup> national cause of morbidity with 223,376 reports and a rate of 239.72 per 100,000 inhabitants and, it became 20<sup>th</sup> in Puebla state with 3933 reports and a rate of 504.7 per 100,000 inhabitants. It was found in recent data (2010) 276,468 national reports and in Puebla State, 5962 reports reaching position 19 in state, and position 15 with 1995 cases in Puebla municipalities.

The data found in the Annual 2010 of Morbidity Records of Puebla State according to the Services of Health, showed a total of 5962 cases of asthma in 33 of the 217 municipalities of Puebla State (Figure 1). Censo de Población y Vivienda 2010, shows that in Puebla State there are 5,779, 829 inhabitants, in 217 municipalities. 70.5 % of municipalities show high and very high marginalization, with a population of 35.4 % of all state; 21.2 % of municipalities show medium marginalization with a population of 18.2 % of all state; 6.5% of municipalities show low marginalization with a population of 1.8 % of all state; and finally 1.8% of municipalities show very low marginalization with a population of 30% of all state.

Those municipalities in which asthma patients were reported possess certain common characteristic, when analyzing the data of the National Institute for Statistics of Mexico (INEGI). These municipalities in general showed good sanitary conditions as the presence of drinkable water, drainage and cement floor and the economical activity was preponderantly tertiary (industry); on the contrary, the other 184 municipalities in which there were no reports of asthma patients, in general, they have not good housing conditions due to, in some cases, a lack of services like drainage,

and even some houses have soil as a floor, also INEGI's data indicate that the residents of these communities defecate outdoors or in latrines, increasing the exposition to pathogens and their toxins. Several aspects were analyzed among them; marginal status was an important factor in asthma incidence ( $P=0.0001$ ). This data showed that very low marginal position increases the tendency to develop asthma and decreases when the marginalization is higher (Graphic 1).

Also it was analyzed the economical activities in the different municipalities of the State of Puebla in order to search for any association between the type of preponderant productive sector and asthma incidence. The analysis of the data provided by the INEGI in its 2010 census finds connection among the type of economic activities in the municipalities and the asthma incidence ( $P < 0.0001$ ). A high incidence of asthma in the tertiary sector was observed meanwhile a low incidence of asthma in the primary sector was present (Graphic 2). When analyzing the association among the services we do not find any significant differences (see Table 2).

Analysis of the type of housing conditions, we find out that there was a high degree of correlation between the housing conditions and asthma incidence. Good housing conditions were associated with asthma ( $P=0.0001$ ) compared with those with lower housing conditions in which no asthma patients were reported. (Tables 2, 3 and Graphic 3).

#### ***Analysis of the bacterial population present in the air***

La Fragua air sampling analysis showed that the species preponderantly present in the air of these homes were: *Escherichia coli*, *Shigella*, *Salmonella*, and *Klebsiella* which were identified by biochemical analysis of the strains. There were about 60 colonies per box, 50% *E. coli*, 30% *Shigella*, 10% *Salmonella* y 10% *Klebsiella*. Microbiological air monitoring of asthmatic patients homes did not show *Escherichia coli*, *Shigella*, *Salmonella*, or *Klebsiella*.

#### ***Analysis of the lung mice by BAL to determine the type of cellular infiltrates in normal conditions***

We developed asthma model in mice Balb/c 2,4,6,8 and 24 weeks old. In order to analyze the role of the bacterial products in early stadiums of asthma development and thus, to understand the way the immune response could be modulated. We found in 6, 8 and 24 weeks mice, an infiltrate rich in eosinophils typical of hyper response of allergic type, when analyzing bronchoalveolar lavages (BAL) of mice after the allergenic challenge with OVA. In control group mice the predominant cellular type in the lung was resident macrophages.

To determine the mice asthmatic response, the IgE anti-OVA titers were evaluated in sensitized mice, where we found  $450 \pm 50$  ng/ml levels for IgE OVA specific and  $2250 \pm 200$  for IgE OVA total. The activity of the IgE serum OVA specific was determined by PCA proof. We found a positive reaction in those rats challenged with OVA sensitized mice serum (OVA/OVA), for all dilutions. The development of an IgE-mediated response to common aeroallergens is the strongest identifiable predisposing factor for developing asthma (GINA). Analyzing the response to LPS in 2, 4, 6 and 8 weeks old mice we did not find response, only a lightly one in 24 weeks old mice, with an infiltrate rich in neutrophils. Indicating that the timing in which the lung cells could be activated is very important for the development of future immune response in lung (Table 4).

In order to determine the polarization of the immune response, we analyzed this aspect through pre-inoculated mice with LPS, which is a typical abettor of Th1 responses; after the OVA pattern was induced, finding that for 2 and 4 weeks mice, pre-inoculated intratracheally with LPS and later on sensitized with OVA, there was not hyper-response of allergic type. Contrary to this in 6, 8 and 24 weeks mice the response was only of 80-90%, these data indicate that the age and the previous establishment of a response Th1 is important for the development of the hyper-response of allergic type (Table 4 and Graphic 4). When we analyzed the response to LPS in 2, 4, 6, 8 and 24 weeks old mice (OVA/OVA) we found in the BAL, an increase of the response, with an infiltrate rich in eosinophils, typical of an inflammatory response (Table 4).

#### ***Discussion***

Bronchial asthma has become one of the illnesses with a very high incidence which results in high costs in public health as well as in the family economy. The role of allergic sensitization inside the pathogenesis of asthma has been broadly studied but there are still some environmental factors to unveil in order to know how they unchain many cellular and molecular mechanisms. In Mexico, asthma has turned into a national epidemic, because it is among the 10 main causes of morbidity.

The data collected on the incidence of asthma in different municipalities from the state of Puebla, showed that 33 out of 217 of them, have reported cases of asthma. The analysis concerning the relationship of the incidence of asthma and sanitary conditions of different municipalities from Puebla State, reveals that, the municipalities with low marginality that have potable water, drainage, electrification, as well as a major tertiary activity of the population, the tendency of developing asthma is higher than in those that present high marginality, that do not count with these tertiary activities services. These data agree with the percentage gathered in industrialized countries where the proportion of asthmatic people fluctuates between the 26% and 35%, whereas in the underdeveloped countries it does not reach 5% (Nolte 2001). This kind of results could have several explanations; we think that between other, this increase in the prevalence of asthma, especially in the developed countries could be attributed to the improvements of the sanitary conditions.

Analysis of the productive activities of the different municipalities from Puebla State showed a high degree of correlation between the productive sector and the asthma incidence. We found that the preponderant activity of the municipalities without asthma is primary kind, whereas, the ones with asthma, is tertiary (see Graphic 2).

This information indicates that the municipalities with better houses and a more accentuated urbanization present a higher number of asthma reports, due to the presence of some environmental factors that modulate positively the incidence of asthma, whereas the sanitary conditions of the municipalities with higher degree of marginalization negatively modulate the development of asthma. On the one hand, our data showed the presence of Gram negative bacteria at houses of people without asthma and, on the other hand, Gram negative bacteria were not found at samplings made at houses of asthmatic people from Puebla City. So, these differences in the exposition to Gram negative bacteria could be an important factor that is involved in a specific immune response as a result of a particular route of exhibition and the type of antigen (Flores et al. 2005), the genetic background of host (Michel 2006), the presenting cell, the dose and the time in which this interaction with the antigen occurs. These facts suggest that exposure to bacterial products originated by outdoors defecation, generates a protective effect against the development of hyper response of allergic type like asthma so, that is why reports of asthma in these municipalities do not exist or are very rare.

We used an experimental animal model in order to reproduce some of the conditions presented in asthmatic patients and analyzed the role of LPS in the establishment or inhibition of an allergic response like asthma. We found that mice maintained under sterile conditions, aged 2, 4, 6, 8 and 24 weeks when being challenged with an allergen like the OVA, presented a similar allergic response with infiltrate rich in eosinophils and high levels of IgE OVA specific, typical of airway inflammation of asthmatic response. Although mouse models rarely reproduce completely all the features of human disease, after sensitization and respiratory tract challenges with antigen, mice develop a clinical syndrome that closely resembles allergic asthma, characterized by eosinophilic lung inflammation, airway hyper responsiveness (AHR), increased IgE, mucus hyper secretion, and eventually, airway remodeling. Our results agree with those obtained by Sagar et al (2013). However, observations from mouse models of allergic asthma support many existing paradigms in patients.

Our results showed that previous challenge with an inductor of Th1 response, modifies the further immune response induced by an allergen, in the experiments that were carried out with intratracheal inoculation of LPS and after mice were challenged with OVA, we did not find response. Only in cases of 6, 8 and 24 weeks old mice, they presented an increased percentage of eosinophilia, when being challenged first with LPS and later with OVA, indicating that the time in which the lung cells are activated is significant in the development of immune response in lung when are challenged with LPS.

It is clear that the age is a very important fact to modulate the immune response in front of bacterial peptides. Our results indicate that previous exhibition to bacterial peptides during the early stages of development, inhibits the allergic hyper response induced by allergens. According to these results Janahi I.A. (2006), also find that the prevalence rate of asthma decreased with age.

These results also show that the exhibition to LPS in mature phase does not regulate negatively the hyper response induced by the chronic exposure to the allergens or generates a protective effect against them.

On the contrary, it exacerbates the severity increasing the inflammation of airways (Michel et al. 1996), the data shown previously indicate that when the asthma is established, the presence of LPS exacerbates the asthmatic onset but LPS is not capable to induce the asthma onset by itself (Braun-Fahrlander et al. 2002, Ci et al. 2012). Our results, as well as Vargas ones, suggest a dismissed of asthma cases as the hygiene hypothesis indicates (Vargas 2006).

These data show us how important could be the role of selecting the appropriate moment of the immune response in the lung against the specific antigens Th1 and the way in which they could provide a protective effect against an allergic response. In contrast, the exposure to LPS in children in their early years of life can diminish the asthma incidence in later stages of their lives. This protection is related to the increment in number and activity of regulatory T cells that regulates the immune response. The molecular base of the immune sensitization indicates that the polarization of immune response to Th1 or Th2 is mutually inhibitory, and this polarization cause that T cells produce different patterns of cytokines that once initiated it is difficult to revert (Anderson 2002, Tsuchiya et.al 2012).

Our results show that exposition to LPS and other TLR ligands in early childhood may decrease the incidence of asthma later in life. This observation is result of obtained data in houses of asthmatic people from Puebla City and people exposed to pathogens or their products. The outcome is critically dependent on the timing between exposure to microbial antigen and allergen sensitization.

When microbial antigens develop a Th1 immune response in naive individual before the allergen can mount a Th2 inflammatory response, the first Th1 immune response cannot be modify later on. In contrast, an encounter with a bacterial antigen in an already Th2 inflamed tissue of an asthmatic individual, usually aggravates the allergic conditions.

### **Conclusions**

Our results show an inverse correlation between the asthma incidence and the degree of marginality of the municipalities of Puebla State, since the housing conditions influence the establishment of asthma. Drainage and latrines lack, force the population to defecate outdoors, this facilitates the chronic contact with Gram (-) bacteria since childhood to adulthood, generating a protective effect that diminish the risk of suffering asthma. This means that a previous contact with bacterial products instead of allergens induces a protective effect against the development of allergic response, as asthma. The experimental data suggest that a single or transient exposition is not sufficient to direct the immune response toward a strong Th1 pattern that could prevent the development to asthma later in life, only frequent and repeated exposure to a variety of microbial antigen may prevent the development of asthma. However, once the allergic response is established, it cannot be modified by the presence of bacterial products, it only can be exacerbate. This indicates that the time, the exhibition route and dose of antigen are preponderant in the modulation of the immune response and establishment of asthma.

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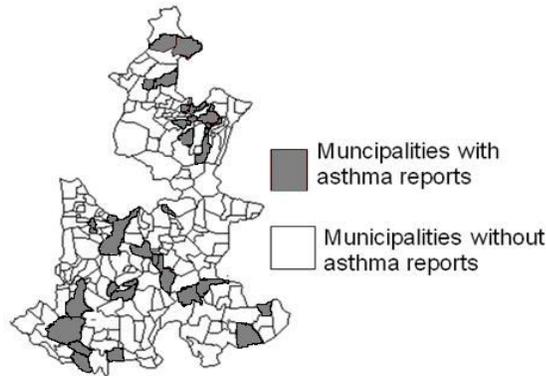
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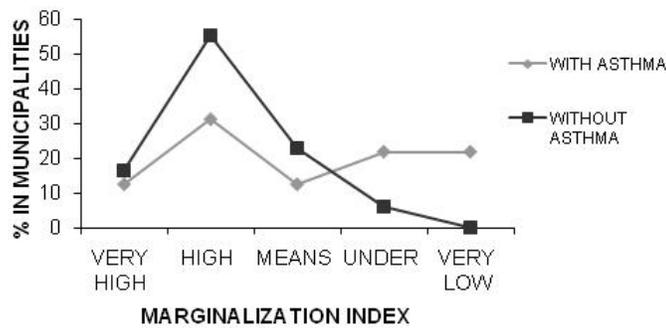
**Table and Figures**

**Table 1: Weather and Geographic conditions.**

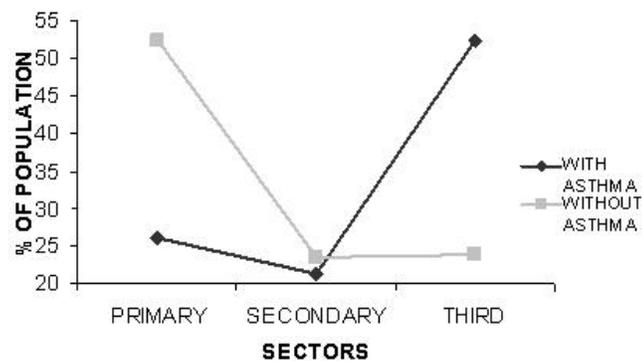
	Municipalities	
	La Fragua	Puebla city
Heigth	2140 meters high	2,460 meters high
Temperate Subhumid weather	X	X
Semidry temperate weather	X	
Semicold subhumid weather	X	X
Cold weather		X



**Figure 1: Municipalities in Puebla State with and without asthma.**



**Graphic 1: Marginalization index of the municipalities of Puebla State with and without asthma.**



**Graphic 2: Economic activity of the municipalities of Puebla State with and without asthma, according to the productive sectors.**

**Table 2: Services in the municipalities of Puebla State with and without asthma.**

	Services		
	Drinkable Water	Drainage	Electrification
With Asthma	74.99 %	53.66 %	91.94 %
Without Asthma	71.28 %	42.21 %	86.6 %

**Table 3: Diagnose of the municipality La Fragua.**

Total housings	1894
Roof of sheet	39.01 %
Without drainage neither toilet	10.34 %
Without electrification	2.04%
Without drinking water	9.95%
Earth floor	27.95%
Over- crowding	65.88%

Diagnose of the population's profile of the municipality La Fragua.



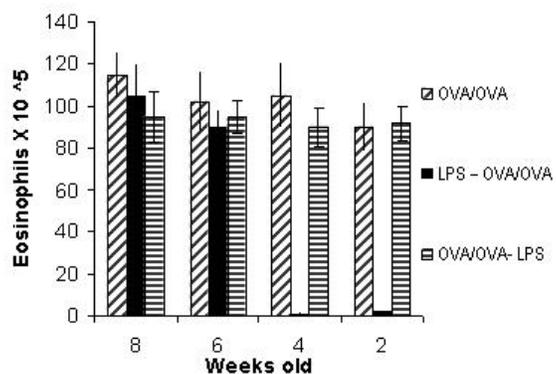
**Graphic 3: Housing conditions of the municipalities of Puebla.**

**Table 4: Number of cells and eosinophils recovered from BAL fluid (BAL= bronchoalveolar lavage).**

AGE WEEKS	EOSINOPHILS x 10 <sup>5</sup>			TOTAL CELLS x 10 <sup>5</sup>
	OVA/OVA	LPS OVA / OVA	OVA / OVA + LPS	
24	103 ± 14	95 ± 13	119 ± 13	224 ± 4
8	95 ± 12	88 ± 15	115 ± 11	220 ± 3
6	105 ± 15	80 ± 8	102 ± 14	225 ± 3
4	90 ± 9	2 ± 1	105 ± 15	225 ± 3
2	92 (8)	1 ± 1	85 ± 11	222 ± 3
CONTROL				170 ± 2

Values are means ± S.D. of 3 independent experiments each one with a n=4. Mice were sensitized and challenged as described in materials and methods, and bronchoalveolar lavage (BAL) fluid was obtained from the same groups described. \*p< 0.05 for non-treated vs. treated.

**Graphic 4: Quantitation of eosinophils recovered from BAL fluid**



Effect of LPS in the modulation of the allergic response. Cells were counted and values are means  $\pm$  SD. The data are representative of 3 test independent with n=4.