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Influence of age on the development of immunological lung response in intrauterine undernourishment

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Abstract

Objective: We investigated the effect of intrauterine undernourishment on some features of asthma using a model of allergic lung inflammation in rats. The effects of age at which the rats were challenged (5 and 9 wk) were also evaluated.

Methods: Intrauterine undernourished offspring were obtained from dams that were fed 50% of the nourished diet of counterparts and were immunized at 5 and 9 wk of age. They were tested for immunoglobulin E anti-ova titers (by passive cutaneous anaphylaxis), cell count in the bronchoalveolar fluid, leukotriene concentration, airway reactivity, mucus production, and blood corticosterone and leptin concentrations 21 d after immunologic challenge.

Results: Intrauterine undernourishment significantly reduced the antigen-specific immunoglobulin E production, inflammatory cell infiltration into airways, mucus secretion, and production of leukotrienes B_4/C_4 in the lungs in both age groups compared with respective nourished rats. The increased reactivity to methacholine that follows antigen challenge was not affected by intrauterine undernourishment. Corticosterone levels increased with age in the undernourished rats' offspring, but not in the nourished rats' offspring. Undernourished offspring already presented high levels of corticosterone before inflammatory stimulus and were not modified by antigen challenge. Leptin levels increased with challenge in the nourished rats but not in the undernourished rats and could not be related to corticosterone levels in the undernourished rats.

Conclusion: Intrauterine undernourishment has a striking and age-dependent effect on the offspring, reducing lung allergic inflammation.

Keywords: Asthma; Intrauterine undernutrition; Inflammation; Corticosterone; Leptin

Introduction

Barker et al. [1] proposed that many chronic diseases arise from adaptations of the fetus when it is undernourished. According to this hypothesis, any human fetus has to adapt to a limited supply of nutrients. In doing so, it permanently changes its structure and metabolism. These "programmed" changes may affect the immune system.

It is commonly accepted that inadequate nutrition affects the immune response based on the observation that protein–calorie malnutrition is generally associated with increased susceptibility to infections [2]. Rothman et al. [3] demonstrated that acute starvation may place newborns at increased risk of infections and allergic disease and Favennec et al. [4] demonstrated an increased incidence of rhinitis in individuals with deficiency of zinc, vitamin C, and magnesium.

Epidemiologic studies have indicated that, in humans, the incidence of chronic lung disease and alterations of lung functions can be associated with birth weight and specifi-

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cally with maternal malnutrition [5,6]. However, with respect to lung allergic inflammation, the data are controversial. Although some studies have demonstrated no association between low birth weight and asthma [7,8], others have indicated that the asthma symptoms are inversely associated with birth weight [9,10]. However, a direct study of the effect of intrauterine malnutrition on asthma has not yet been performed.

Asthma is a chronic lung inflammation that is determined by the activation of T-helper type 2 lymphocytes, and the characteristic features of the disease are increased reactivity of the bronchi, excessive airway mucous production due to goblet-cell hyperplasia, and tissue and airway eosinophilia [11]. The lung allergic inflammation is a complex process that involves a number of cells and mediators. The cysteinyl leukotrienes (LTs; C_4, D_4, E_4) are known to play an important role in the physiopathology of asthma and they have multiple effects that contribute to the airways' obstruction, bronchoconstriction, and inflammation that characterize asthma [12]. These compounds, derived from arachidonic acid via the 5-lipoxygenase pathway, are produced by inflammatory cells such as eosinophils, mast cells, monocytes, and basophils [13].

Studies have demonstrated that malnutrition promotes alteration in the levels of circulating hormones, such as an increase in glucocorticoids [14,15] and a decrease in leptin [16]. Matarese [17] demonstrated that acute starvation, associated with decreased leptin levels, causes thymic atrophy and decreases the immune response in mice. However, in relation to the effect of intrauterine malnutrition on the offspring, the few data that are found in the literature are not conclusive. On the one hand, Lesage et al. [18] observed that food restriction of the pregnant rat during the last week of gestation induced, in the offspring, an increase in the circulating glucocorticoid levels. On the other hand, Nolan et al. [19] demonstrated that rats exposed to intrauterine protein malnutrition presented no difference in the circulating glucocorticoid levels when compared with nourished rats.

The occurrence of some programmed disease in intrauterine undernourished rats could be related to age. At 8 wk of age, compared with nourished rats, intrauterine undernourished rats presented higher but not yet hypertensive levels; however, at 14 wk of age, they already presented hypertensive levels [20].

Considering the importance of intrauterine nutrition for adequate development and functioning of organs and tissues and the relation between airway allergic inflammation and nutrition [21], the aim of the present study was to investigate the effect of intrauterine undernourishment on the development of experimental asthma and the effect of the age at which the immunologic reaction is induced and that of growth. For this purpose, allergic lung inflammation was induced in 5- and 9-wk-old rats that were born to undernourished or nourished dams. The following parameters were evaluated: serum levels of ova-specific immunoglobulin E (IgE); neutrophil and eosinophil infiltrations; lung levels of LTB_4 and LTC_4; and reactivity of airways to methacholine and mucus production. The plasma levels of corticosterone and leptin were also measured.

Materials and methods

Animals

All procedures used in this study were approved and performed in accordance with guidelines established by the ethics committee of the Institute of Biomedical Sciences (041/2001), University of São Paulo. Wistar rats from our own colony (Hypertension and Inflammation Laboratory, Institute of Biomedical Sciences, University of São Paulo) were housed in a 22 ± 1°C environment at 60% humidity and were maintained on a 12-h light–dark cycle.

Protocol

Timed mating was carried out in age-matched (12- to 16-wk-old) female and male Wistar rats. To assess the stage of estrus of the females, vaginal smears were checked before the introduction of the males. Day 1 of the pregnancy was determined as the day on which spermatozoa were detected in the vaginal smear. After confirmation that mating had occurred, the rats were housed individually in standard rat cages. Female rats were randomly divided into two groups: nourished ad libitum (NR) and undernourished (UR). NR rats were fed a standard commercial rat diet (Nuvital, Nuvital Nutrientes S/A, PR, Brazil) containing protein (minimum 22%), carbohydrates (maximum 54%), fat (minimum 4.5%), cellulose (maximum 8%), minerals (maximum 10%), water (maximum 12.5%), and vitamins. UR rats were fed the same diet at 50% of the NR intake, as determined by the amount of food consumed by the control group from day 1 of pregnancy until day 23 (parturition). All rats were fed daily, in the morning, and consumption was determined 24 h later. After parturition, dams received food ad libitum; therefore, the pups differed only in prenatal dietary experience. To prevent variation in neonatal growth due to availability of milk during suckling, litter size was standardized to eight pups on day 1. After suckling, the undernourished dams were sacrificed. After weaning, the pups were fed with the same diet as the dams. At 5 and 9 wk of age, male offspring were used in experiments to evaluate the lung allergic inflammation.

Immunization protocol

Male rats 5 and 9 wk old from the UR and NR groups were sensitized on days 0 and 7 by intraperitoneal injection of a mixture containing 50 μg of ovalbumin (grade III, Sigma, St. Louis, MO, USA) and 1 mg of Al $(OH)_3$ (Rheis, Inc., Berkeley Heights, NJ, USA) in saline (total volume of

0.6 mL). At days 14 and 21 after first immunization, the rats were challenged by exposure to an aerosol of ovalbumin generated by an ultrasonic nebulizer (ICEL US-800, SP, Brazil) delivering particles of 0.5–10 μm in diameter at approximately 0.75 cc/min for 20 min. Therefore, the airway allergic response was evaluated when the rats were 8 and 12 wk of age. The concentration of ovalbumin in the nebulizer was 2.5% (wt/vol). These rats will constitute the experimental group and will be referred to as OVA/OVA throughout. The control group consisted of rats immunized, as referred earlier, and challenged with phosphate buffered saline (PBS) solution and will be referred to as OVA/PBS. To evaluate the efficiency of the immunization protocol, IgE anti-ova antibody titers were determined by passive cutaneous anaphylaxis reaction. It consists of the degranulation of skin mast cells induced by immunologic challenge as described by Mota and Wong [22].

Bronchoalveolar lavage

The rats were sacrificed by an overdose of anesthesia (sodium pentobarbital) 24 h after exposure to the second aerosol challenge. The bronchoalveolar lavage was collected and the total and differential cell counts were determined as described previously [23].

Determination of lung LTB_4 and LTC_4 concentration

The LTB_4 and LTC_4 concentrations were determined using commercially available enzyme immunoassay kits (Cayman Chemical Co., Ann Arbor, MI, USA), according to Pradelles and MacLouf [24]. Detection limits were 10 pg/mL for both mediators.

Evaluation of airway reactivity

Rats were immunized and submitted to two antigen aerosol exposures as described previously. Twenty-four hours after the second antigen challenge, increase in perfusion pressure (centimeters of H_2O) versus dose (micrograms of methacholine) was measured for the entire observation period, areas under the curve were calculated, and the results expressed as mean area under the curve (square millimeters) as described previously [23].

Mucus production

Lungs were removed after bronchoalveolar lavage collection; tissues were sliced into 5-μ sections and stained with hematoxylin/eosin for examination using light microscopy or with periodic acid-Schiff/hematoxylin for evaluation of mucus-producing cells. The intensity of mucus production was evaluated in each preparation and scores from 0 to 3 were assigned: 0 when none of the bronchi showed any sign of mucus; 1, 2, or 3 when 25%, 50%, or >50% of the bronchi epithelium was covered by mucus. Values represent the sum of 10 bronchi scored randomly at 250× magnification [23].

Corticosterone and leptin assay

The rats from groups UR and NR were decapitated at 0800 h, the blood was collected, and the corticosterone and leptin plasma levels were determined using enzyme immunoassay (Cayman Chemical Co., Ann Arbor, MI, USA), according to Pradelles and MacLouf [24]. Detection limits were 25 and 100 pg/mL, respectively.

Statistical analysis

Data are expressed as means ± standard errors of the mean. Statistical evaluation of the data was carried out by analysis of variance, and sequential analysis of differences among means was done by Tukey's contrast analysis. $P < 0.05$ was considered statistically significant.

Results

Characteristics of offspring

Maternal undernutrition resulted in fetal growth retardation, which was reflected by a clear reduction in birth weight in the offspring exposed to intrauterine undernutrition (3.7 ± 0.1 versus 5.6 ± 0.1 g, $n = 22$, $P < 0.001$). However, no difference was observed in mean litter size at birth in control (9.1 ± 0.3, $n = 10$) and nutritionally restricted (8.4 ± 0.8, $n = 10$) dams, indicating that the reproductive ability was unaffected. UR offspring had greater body weights than NR offspring at 5 wk of age (130.0 ± 5.1 versus 110.0 ± 3.9 g). At 9 wk of age, male UR offspring had a body weight (255.0 ± 6.1 g) similar to that of male NR offspring (259.1 ± 3.6 g).

Effect of maternal undernutrition on allergic lung inflammation

Serum IgE anti-OVA antibody levels

As expected, in the OVA/PBS groups, no IgE anti-ova antibody was detected; therefore, NR OVA/OVA presented a high titer of IgE anti-OVA antibody (1:128 in both age groups), indicating the efficiency of the immunization protocol, whereas UR OVA/OVA presented a reduced production of the anaphylactic antibodies when compared with NR OVA/OVA (1:64 versus 1:128 and 1:32 versus 1:128, in 8- and 12-wk-old offspring, respectively). Moreover, UR OVA/OVA at 12 wk of age presented lesser production of the anaphylactic antibodies than UR OVA/OVA at 8 wk of age (1:64 and 1:32, in 8- and 12-wk-old offspring, respectively).

Cells in bronchoalveolar lavage fluid

The UR and NR OVA/OVA rats presented increased total cell infiltration when compared with respective OVA/PBS groups at both ages (8 and 12 wk old). No difference was observed in total cell infiltration when UR and NR OVA/OVA were compared at 8 wk of age. At 12 wk of age, however, UR presented reduced total leukocyte infiltration. UR and NR OVA/OVA presented a marked increase in eosinophils and neutrophils in the bronchoalveolar lavage fluid when compared with UR and NR OVA/PBS, indicating that they responded to the immunologic stimuli. Although UR OVA/OVA at 8 wk of age did not differ from NR at the same age, eosinophil infiltration in UR OVA/OVA (12 wk old) was lower than that in NR OVA/OVA (12 wk old; Fig. 1).

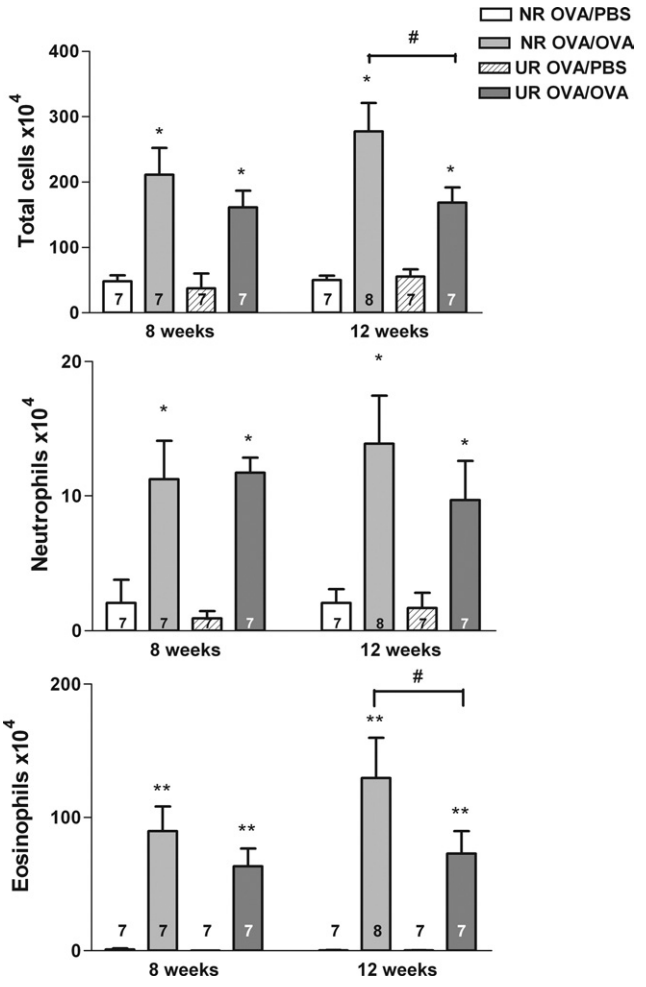

Fig. 1. Cells in the bronchoalveolar lavage. Eosinophil and neutrophil counts were determined on hematoxylin/eosin-stained cytocentrifuge preparations. Results are presented as mean ± SEM. Numbers inside the bars indicate the number of rats used. $^{*}P < 0.05$, $^{**}P < 0.01$ versus the respective OVA/PBS group; $^{\#}P < 0.05$ versus NR OVA/OVA group. NR, nourished ad libitum; OVA/OVA, challenged with ovalbumin (experimental group); OVA/PBS, challenged with phosphate buffered saline (control group); UR, undernourished.

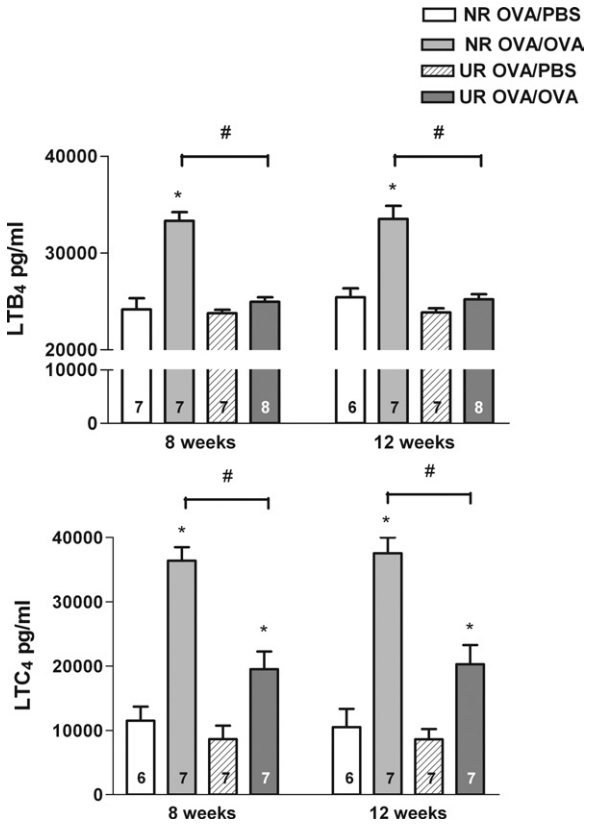

Fig. 2. The LTB_4 and LTC_4 concentrations were determined by enzyme immunoassay. Results are expressed as mean ± SEM. Numbers inside the bars indicate the number of rats used. $^{*}P < 0.05$ versus respective NR OVA/PBS group; $^{\#}P < 0.05$ versus respective NR OVA/OVA group. NR, nourished ad libitum; OVA/OVA, challenged with ovalbumin (experimental group); OVA/PBS, challenged with phosphate buffered saline (control group); LT, leukotriene; UR, undernourished.

Lung LTB_4 and LTC_4 levels

Although LTB_4 production was increased in NR OVA/OVA (8 and 12 wk old) when compared with NR OVA/PBS of the same age, no difference was found when UR OVA/OVA (8 and 12 wk old) were compared with UR OVA/PBS of the same age. UR OVA/OVA (8 and 12 wk old) presented reduced LTB_4 production when compared with NR OVA/OVA (8 and 12 wk old, respectively). Increased LTC_4 levels were observed in NR and UR OVA/OVA (8 and 12 wk old) when compared with NR and UR OVA/PBS (8 and 12 wk old), respectively; in addition, in UR OVA/OVA, LTC_4 production was reduced when compared with NR OVA/OVA of the same age (Fig. 2).

Reactivity of airways to methacholine

Bolus injection of increasing doses of methacholine (0.1–100 μg) caused bronchoconstriction, measured as an increase in perfusion pressure. Figure 4 shows that, in the UR and NR OVA/OVA (8 and 12 wk old), the reactivity of the airways to methacholine was significantly higher than that of the UR and NR OVA/PBS; however, no difference was observed when UR and NR OVA/OVA were com-

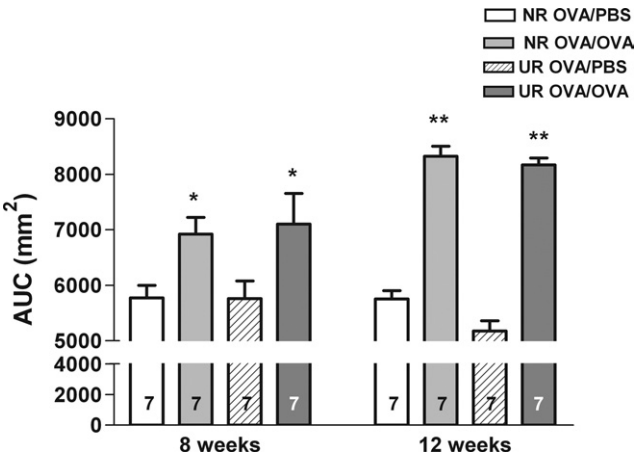

Fig. 3. Airway reactivity to methacholine. The increase in perfusion pressure to methacholine was recorded in lungs. Results are expressed as mean AUC (square millimeters) ± SEM. Numbers inside the bars indicate the number of rats used. $^{*}P < 0.05$; $^{**}P < 0.001$ versus the respective OVA/PBS group. AUC, area under the curve; NR, nourished ad libitum; OVA/OVA, challenged with ovalbumin (experimental group); OVA/PBS, challenged with phosphate buffered saline (control group); UR, undernourished.

pared. At 12 wk of age, the NR OVA/OVA presented higher reactivity of the airways to methacholine than did the younger NR OVA/OVA (8 wk old); however, in UR OVA/OVA, no increase was observed in the reactivity of the airways to methacholine when rats at 8 and 12 wk of age were compared (Fig. 3).

Mucus production

In the OVA/PBS group, none of the bronchi contained mucus (score 0). At 8 and 12 wk of age, UR OVA/OVA presented a reduction in mucus production when compared with NR OVA/OVA at the same age. In addition, mucus production was reduced in UR OVA/OVA at 12 wk of age when compared with UR OVA/OVA at 8 wk of age (Fig. 4).

Corticosterone and leptin levels

When corticosterone levels of 8-wk-old UR and NR (OVA/OVA and OVA/PBS) were compared, it was found that the levels did not differ. At 12 wk of age, UR OVA/OVA presented higher corticosterone levels than did NR OVA/OVA. Similar results were also observed when UR and NR OVA/PBS were compared at 12 wk of age. At 12 wk of age, UR OVA/OVA and UR OVA/PBS presented higher corticosterone production when compared with UR OVA/OVA and UR OVA/PBS at 8 wk of age, respectively (Fig. 5A). In relation to the leptin levels, Figure 5B shows that in the NR rats, induction of allergic inflammation increased leptin levels in 8- and 12-wk-old rats. In UR at both ages, the immunization and challenge with ovalbumin did not alter the leptin levels. Therefore, in relation to NR, they presented lower leptin levels (Fig. 5B).

Discussion

It is unquestionable that undernutrition during pregnancy has lifelong consequences on the offspring. Animal experiments have shown that undernutrition in utero leads to persistent changes in blood pressure, cholesterol metabolism, insulin response to glucose, and a range of other metabolic, endocrine, and immune functions known to be important in human disease [25,26]. In our study, the protocol of intrauterine undernutrition led to low–birth-weight offspring as we described in previous studies [20,27] and fulfilled our criteria for the study.

The sensitization seems to be the initial process in the development of lung allergic inflammation. IgE plays a pivotal role in allergic disorders. The protocol we used was effective in increasing IgE anti-ova antibody in UR or NR at 8 and 12 wk of age. However, compared with NR at the same age, lower levels of IgE anti-ova antibody were found in UR OVA/OVA at 8 and 12 wk of age, which may lead to an attenuated allergic response. Corroborating these findings, in malnourished children, decreased serum IgE levels were found when compared with well-nourished children [28]. Moreover, because the IgE anti-ova production was lower at 12 than at 8 wk of age, we suggest that the age at which the rats are challenged, along with growth, is important in determining the intensity of the response.

Airway inflammation characterized by peribronchial eosinophil infiltration is a constant feature in most patients with bronchial asthma. Increased numbers of eosinophils are found in the bronchoalveolar lavage fluid and in bronchial biopsy samples obtained from asthmatic patients [29] and in experimental models of asthma [11]. Also, the eo-

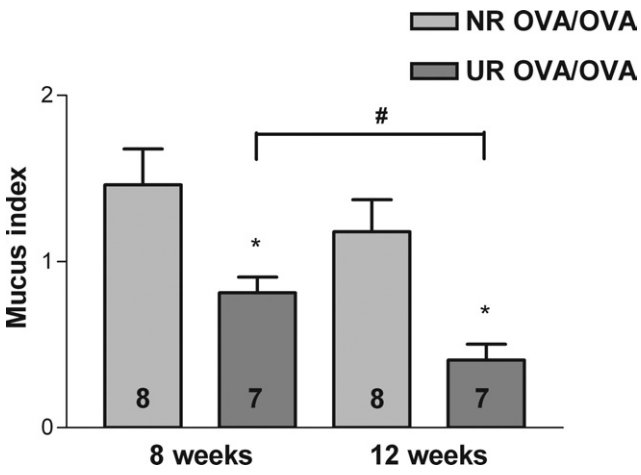

Fig. 4. The intensity of mucus production was evaluated by the presence of mucus and scores from 0 to 3 were assigned: 0 when none of the bronchi show any sign of mucus; 1, 2, and 3 when 25%, 50%, and >50% of the bronchi epithelium shows signs of mucus. Results are presented as mean of the scores ± SEM. Numbers inside the bars indicate the number of rats used. $^{*}P < 0.05$ versus the NR OVA/OVA (12-wk-old) group; $^{\#}P < 0.05$ versus the 8-wk-old UR OVA/OVA group. NR, nourished ad libitum; OVA/OVA, challenged with ovalbumin (experimental group); UR, undernourished.

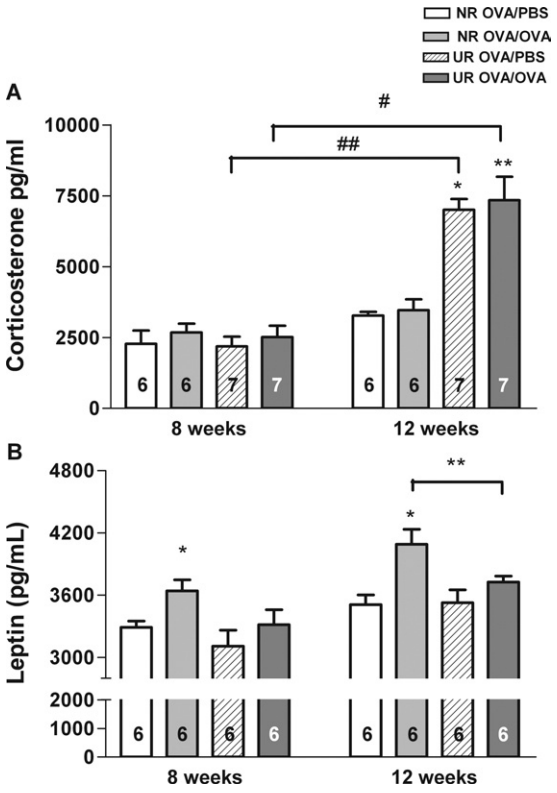

Fig. 5. (A) Corticosterone levels (picograms per milliliter) in sera as determined by enzyme immunoassay. Results are expressed as mean ± SEM. Numbers inside the bars indicate the number of rats used. $^{*}P < 0.05$ versus the respective NR OVA/PBS group; $^{**}P < 0.05$ versus the respective NR OVA/PBS group; $^{\#}P < 0.05$ versus the UR OVA/OVA (8-wk-old) group; $^{\#\#}P < 0.05$ versus the 8-wk-old UR OVA/PBS group. (B) Leptin levels (picograms per milliliter) in sera was determined by enzyme immunoassay. Results are expressed as mean ± SEM. Numbers inside the bars indicate the number of rats used. $^{*}P < 0.05$ versus the respective OVA/PBS group; $^{**}P < 0.01$ versus the NR OVA/OVA (12-wk-old) group. NR, nourished ad libitum; OVA/OVA, challenged with ovalbumin (experimental group); OVA/PBS, challenged with phosphate buffered saline (control group); UR, undernourished.

sinophil number was demonstrated to correlate with the severity of the disease [11,30]. This happens because eosinophils have the potential to cause damage to the airway mucosa and associated nerves through the release of granule-associated basic proteins (which damage nerves and epithelial cells), lipid mediators (which cause bronchoconstriction and mucus hypersecretion), and reactive oxygen species (potentially able to injure mucosal cells) [11]. In our study, although we could not find a reduction in the 8-wk-old group, in the 12-wk-old group eosinophil infiltration was reduced as compared with NR rats of the same age, indicating that intrauterine undernourishment affected the capacity of inflammatory cells to react to an immunologic stimulus. Corroborating this finding, we recently demonstrated that UR rats present reduced migratory capacity to respond to chemotactic agents [27]. The lack of alteration at 8 wk may indicate that the age at which the organism is challenged by an immunologic stimulus might influence the response, probably by a better defense at early stages of development.

Eosinophil infiltration seems to increase with growth in NR but not in UR rats because in NR rats the number of eosinophils was higher at 12 than at 8 wk of age. The same did not occur in UR rats. The reason for that is not easily explained by our data.

The mechanism involved in the reduced eosinophil infiltration in UR rats might be related to the release of inflammatory mediators. The cysteinyl leukotrienes (C_4, D_4, E_4) are potent inflammatory mediators that play an important role in the physiopathology of allergic lung disease, and their levels are elevated in the airways in response to an allergen challenge [31,32]. In asthma, bronchoconstriction, bronchial hyper-reactivity induction, the increase of vascular permeability, mucus secretion, and smooth muscle cell proliferation are related to leukotriene activity [33]. LTB_4 is a potent chemotaxis agent of neutrophils and eosinophils [34]. Therefore, the reduced LTB_4 production in UR rats might explain, at least in part, the reduced infiltration at 12 wk of age. However, regarding UR rats at 8 wk of age, the reduction of LTB_4 probably was not sufficient in altering the eosinophil infiltration that was not different from that of NR rats at the same age or other mediators compensated for the reduction, maintaining the eosinophil infiltration near values found in NR rats.

Increased airway reactivity in response to stimuli, a characteristic feature of allergic lung inflammation, is represented by the response to bronchoconstrictor agents elicited by doses that would have little or any effect in healthy individuals [35]. In fact, we demonstrated hyper-reactivity to methacholine in 8- and 12-wk-old UR rats, despite the reduced LTB_4 and LTC_4 production found in UR rats. This indicates that the lung tissue is preserved to respond to bronchoconstrictor stimuli. Although LTB_4 has no direct action on airway smooth muscle [36], it may contribute to bronchoconstriction by increasing vascular permeability and mucus secretion [37,38]. Because LTB_4 and LTC_4 levels were reduced in UR rats, we suggest that other substances, such as cytokines (interleukin-1, interleukin-5, tumor necrosis factor-α), prostaglandins D_2 and $F_{2\alpha}$ and oxygen reactive species (superoxide anion and hydrogen peroxide) [39], could be related to the airway hyper-reactivity in the UR rats. Increased release of reactive oxygen species, such as superoxide anion and hydrogen peroxide, has been reported in exhaled breath condensates, in circulating granulocytes, and in the bronchoalveolar lavage cells of patients with asthma [40]. Indeed, our group demonstrated that, in this model of intrauterine undernutrition, the rats presented an increase in superoxide anion production [20,41]. Thus, we hypothesize that the increase in the superoxide anion production presented by UR rats might be one of the factors related to airway hyper-reactivity observed in this group.

It is suggested that eosinophils contribute to airway hyper-responsiveness in humans and in experimental models, increasing the severity of the disease; however, the involve-

ment of eosinophils in bronchial hyper-reactivity response is controversial [11]. We could not demonstrate an association between eosinophil infiltration and hyper-reactivity because the reduced eosinophil infiltration was not accompanied by reduced hyper-reactivity, at least at 12 wk of age. In addition, it seems that growth increased the capacity of the NR rats to respond to bronchoconstrictor stimuli because a greater response to methacholine was found in 12- compared with 8-wk-old NR rats, whereas in UR rats similar responses at 8 and 12 wk were found.

Mucus hypersecretion with decreased mucociliary clearance are important factors contributing to airway obstruction and correlate symptomatically with inflammatory changes in allergic lung inflammation in humans and in experimental models [42]. UR affected mucus production because UR OVA/OVA produced less mucus than did NR OVA/OVA at both ages. The fact that at 12 wk of age UR OVA/OVA presented less mucus than UR OVA/OVA at 8 wk of age reinforces the hypothesis that the age at which the organism is challenged, along with growth, is determinant in conditioning the intensity of the immunologic response.

It is well established that food availability influences the rhythmicity of the hypothalamic-pituitary-adrenal axis. Indeed, starvation and food restriction increase the activity of the hypothalamic-pituitary-adrenal axis in humans [43] and rats [18]. This can lead to adrenal hypertrophy and increased circulating corticosterone levels. This alteration, in addition to affecting the metabolic homeostasis, can compromise the mechanisms of innate defense. High levels of glucocorticoid inhibit the action of various inflammatory mediators, reduce the synthesis of chemokines and cytokines, and downregulate the activation of leukocytes, thereby attenuating the inflammatory process [14,42]. On the one hand, Lesage et al. [18] observed that, in newborns of dams that received 50% food during the last week of gestation, the corticosterone level showed an increase when compared with nourished rats. On the other hand, Langley-Evans et al. [44] observed, in progeny of protein-restricted rats, an unaltered basal corticosterone concentration but an increase in hippocampal glucocorticoid receptor binding, a result that suggests an increase in glucocorticoid feedback sensitivity. In the present study, the glucocorticoid levels of UR OVA/OVA at 8 wk of age did not differ from those of NR OVA/OVA at the same age. However, in UR rats at 12 wk of age, the level of glucocorticoids showed an increase. Therefore, an increase in glucocorticoid levels can be the contributing factor to the impaired allergic lung inflammation in UR rats observed at 12 but not at 8 wk of age. The reason for this difference between ages is not easily explained by our present data because at both ages a reduced lung allergic inflammation was found, although of a lower magnitude in 8-wk-old UR rats.

We investigated the possible role of leptin because this agent is a pleiotropic cytokine involved in different metabolic and endocrine functions. It plays a key role in the regulation of body weight and exerts other biological functions modulating hematopoiesis, angiogenesis, and the immune response, increasing the phagocytic activity, cytokine production, and reactive oxygen species production [16,17]. In addition, leptin levels were found elevated in experimental models of infection and inflammation [45,46]. Accordingly NR rats in both age groups presented increase in leptin level after immunization and challenge with ovalbumin. However, in UR OVA/OVA, no increase in leptin levels was found. This might help to explain the impairment of allergic lung inflammation in UR rats.

In summary, we found that intrauterine undernourishment reduces allergic lung inflammation in the offspring. The intensity of the response depends on the age at which the organism is challenged. The decrease in the allergic inflammatory response observed at 12 but not at 8 wk of age correlates with the corticosterone levels in our model. Lack of an increase in leptin levels in UR rats might also have contributed to the impairment of the response.

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