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Attenuated allergic inflammatory response in the lungs during lactation☆

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ABSTRACT

Aims: To evaluate the influence of lactation on lung immune function during allergic inflammation.
Main methods: Female rats, 60–90 days old, were divided into three groups: no lung allergy virgins (N group), ovalbumin (OVA)-immunized and sensitized virgins (V group), and OVA-immunized and sensitized lactating females (L group). On gestation day (GD) 10, all animals in L group received a subcutaneous injection of 0.1 mg·kg^{-1} OVA plus aluminum hydroxide. On GD17, the L group received a subcutaneous booster injection of 10 μg OVA plus 10 mg aluminum hydroxide. After 7 days, an inhalatory challenge with 1% OVA was given in 15 min sessions for 3 consecutive days. Animals from the V group received the same treatment, meaning both tests and time intervals between OVA treatment and inhalatory challenge were the same as in the L group. Twenty-four hours after the last inhalation session, the animals were euthanized, and the following tests were performed: total and differential bronchoalveolar lavage (BAL) and femoral marrow lavage (FML) leukocyte counts, quantification of tumor necrosis factor α (TNF-α) and interferon γ (IFN-γ) levels in BAL fluid, and quantification of plasma corticosterone and catecholamine levels.
Key findings: The L group presented lower BAL total leukocyte counts and decreases in the number of eosinophils and macrophages compared with the V group. They also expressed higher BAL IFN-γ and lower plasma corticosterone levels. Plasma norepinephrine levels were higher in the L group than in the N and V groups.
Significance: Lactating female rats presented less intense allergic lung inflammation. Our findings suggest that lactation may protect females from asthmatic crises.

1. Introduction

Anecdotal reports suggest that asthmatic women experience improvements in the lung allergic response during lactation. Thus, lactation may favor a lower incidence of asthmatic crises. During lactation, various physiological changes occur, such as the attenuation of immune responses and stress, with regard to both physiological and physical stressors. One of the best examples of this attenuated response to stress is seen in rats during late pregnancy and lactation. During these reproductive phases, the hypothalamic-pituitary-adrenal axis requires minimal responsiveness to cope with situations that may threaten the rat's dynamic balance. This is reflected by decreases in adrenocorticotropic hormone and corticosterone secretion. This attenuated response is observed over parturition and lactation until weaning [29].

Modifications of the central nervous system (CNS) may influence the immune system, and the immune system can also alter the functioning of the CNS [3]. These two-way interactions play an important role in modulating the body's susceptibility and resistance to infectious and inflammatory diseases [40]. Thus, products that are regarded as immune system- or neuroendocrine system-specific coexist in lymphoid, endocrine, and nervous tissues. Endocrine and neural mediators can affect the immune system, and immune mediators can affect neural and endocrine structures [4]. Both the CNS and immune system actively participate in responses to stressors by modulating behavior and immune activity in accordance with the type, duration, and intensity of specific stressors. Responses that occur as a result of these stressors are generally adaptive in the short term [15,27,46].

Prolactin is a peptide hormone that is secreted by the anterior pituitary gland and is known to regulate stress, reproduction, and a wide variety of physiological processes [6,31]. The effects of prolactin on the

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immune system are complex [1,34,29]. In rodents, during pregnancy, plasma prolactin levels are high in the first half, decrease until late pregnancy, and then increase again in the postpartum period and throughout nursing [7]. Lactation is a state of physiological hyperprolactinemia. Emotional and physical stress can stimulate the secretion of prolactin, which could be an adaptation to ensure the competence of the immune system and physiological and behavioral responses to stress [1].

One curious aspect of prolactin biology is the modulation of autoimmune responses and inflammation. Several studies have reported the ability of prolactin to stimulate the proliferation and inflammatory activity of immune cells. These studies have not led to conclusive results [1,12,33,34].

Asthma is a chronic inflammatory disease of the airways, in which many cells, such as eosinophils, mast cells, neutrophils, dendritic cells, and T lymphocytes, play a critical role [41]. Asthma results from an inappropriate Th2 response to innocuous airborne antigens. Exposure to allergens is necessary for the development of asthma and onset of symptoms [45]. The infiltration of eosinophils and $CD4^+$ Th2 lymphocytes in airways is a main feature of asthma [11,41]. The hypersecretion of mucus in the bronchial walls is also characteristic of the disease [41, 49].

Previous studies were performed in mouse models to evaluate the prevention of asthma in offspring by deliberately exposing dams orally to aerosolized allergens during the lactation and nursing periods. The results suggested that breast milk can actively modulate the immune response in progeny by transferring the allergen through breast milk. During offspring development, such allergen exposure induced immune tolerance to allergic diseases, such as pulmonary allergic responses [45].

In the present study, we investigated the influence of lactation on immune function during allergic lung inflammation. Functional and biochemical parameters were evaluated in a model of experimental asthma.

2. Materials and methods

2.1. Animals

Female Wistar rats were obtained from the Department of Pathology Animal House, School of Veterinary Medicine, University of Sao Paulo. The animals were housed in rooms with ventilation at a constant temperature of 22–23 °C under a fixed 12 h/12 h light/dark cycle (lights on at 6:00 AM) with free access to food and water. All of the procedures were performed in strict accordance to the guidelines of the Colegio Brasileiro de Experimentacao Animal and National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Experimental design

Female Wistar rats, 60–90 days of age, were randomly divided into three groups: no lung allergy virgins (N group), ovalbumin (OVA)-immunized and -sensitized virgins (V group), and OVA-immunized and -sensitized lactating females (L group). After 1 month of cage habituation, the rats in the L group were mated with sexually experienced males, became pregnant, and generated offspring. When pregnancy was confirmed, each female was separated individually in a box. Naive and virgin females were also housed separately. Fig. 1 illustrates the timeline of the experiment.

On gestation day (GD) 10, all of the animals in L group received a subcutaneous injection of OVA (Egg Albumin Grade II, Sigma-Aldrich, St. Louis, MO, USA) at a dose of 0.1 $mg \cdot kg^{-1}$ plus 10 mg aluminum hydroxide (EMS Pharmaceuticals, São Paulo, Brazil) dissolved in phosphate-buffered saline (PBS) to actively sensitize the animals. On GD17, all of the animals in L group received a subcutaneous booster injection of 10 μg OVA plus 10 mg aluminum hydroxide dissolved in PBS at a dose of 0.1 $mg \cdot kg^{-1}$. Animals from the V group received the same treatment, meaning both tests and time intervals between OVA treatment and inhalatory challenge were the same as in the L group.

On GD21, all of the pups were born. Two days later, litter standardization was performed, in which litters were culled to eight pups per lactating female, with 4 female and 4 male pups whenever possible. On days 3, 4, and 5 after birth, all of the adult rats in the L and V groups were placed in groups of four animals each in an inhalation chamber that was connected to an ultrasonic nebulizer where they received aerosolized OVA (1% in PBS) for 15 min per day [14,25].

On day 6 after birth, 24 h after the last OVA challenge, all of the animals in the N, V, and L groups were weighed for anesthesia and euthanasia.

2.3. Sample collection

The animals were anesthetized with 5 $mg \cdot kg^{-1}$ of 2% xylazine hydrochloride (Konig; i.p.) and 30 $mg \cdot kg^{-1}$ of 5% ketamine (Ketalar; Konig; i.p.). The peritoneal cavity was opened, and blood was collected through the abdominal aorta in plastic syringes that contained 50 μl of 8% ethylenediaminetetraacetic acid (EDTA). Blood was set aside until clot formation and then immediately centrifuged for serum collection, which was stored at −80 °C. All blood collections were performed with the same schedule to avoid possible effects of circadian rhythm. Subsequently, the lungs were washed four times with 5.0 ml heparinized PBS (20 ml) through a polyethylene cannula (1 mm inner diameter) inserted by tracheotomy. Bronchoalveolar lavage (BAL) was performed according to a previous study [13]. Recovered BAL fluid was centrifuged at 170 × *g* for 10 min at 4 °C. The supernatant was

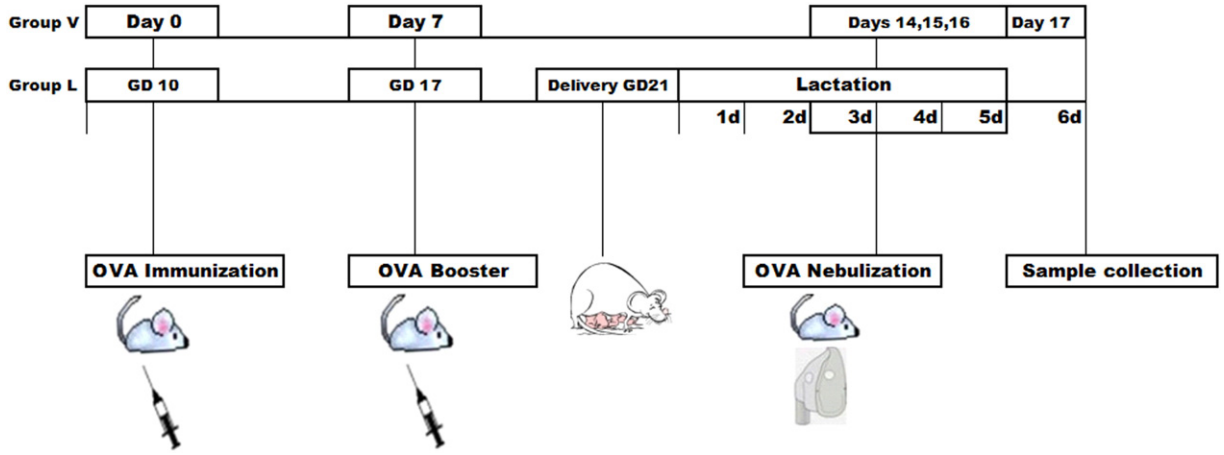

Fig. 1. Experimental timeline of observing the effects of lactation on allergic inflammation in the lungs induced by ovalbumin.

discarded, and the resulting pellet was resuspended in PBS (1 ml). Rat femurs were removed, and a needle connected to a plastic syringe that contained 5 ml PBS was inserted into each femoral marrow to allow cell collection by flushing. The femoral marrow lavage (FML) fluid was centrifuged at 170 ×*g* for 10 min, and the cell pellet was resuspended, processed, and analyzed for total leukocyte counts [14,25].

2.4. Total and differential whole-blood counts

The samples were diluted 1:20 in Turk liquid (3% acetic acid) and counted in a Neubauer chamber. Blood smears were stained with May-Grunwald-Giemsa, and differential leukocytes were counted by light microscopy [14,25].

2.5. Femoral myelogram

The total number of bone marrow cells was quantified in FML fluid using Turk solution in a Neubauer chamber.

2.6. Bronchoalveolar lavage leukocyte counts

Bronchoalveolar lavage cell suspensions were diluted 1:20 with Turk solution, and total leukocytes were counted in a Neubauer chamber. Differential cell counts were performed using cytocentrifuge preparations (Cytospin, Fanem, Brazil) and stained with May-Grunwald-Giemsa solution [14,25].

2.7. Corticosterone measurements

Plasma corticosterone levels were determined using a commercial enzyme-linked immunoassay (ELISA) kit (Arbor Assays, #K014) according to the manufacturer's instructions, with 1:300 plasma dilution.

2.8. Cytokine production in BAL fluid

The concentrations of cytokines in BAL specimens were quantified using commercial ELISA kits (TNF-α, BD OptEIA rat cytokine ELISA kit, BD Biosciences; IFN-γ, Rat IFN-γ Quantikine ELISA Kit, R&D Systems).

2.9. Measurement of plasma catecholamines

We applied a previously described method [16] with relevant changes for our sample volumes and equipment. The measurement of catecholamines was performed using high-performance liquid chromatography (HPLC) coupled with electrochemical detection (HPLC-ED; Shimadzu Model 20A) in reverse phase with ion pairing (heptane sulfonic acid). The HPLC-ED automatically injects 70 μl per sample. The system maintained an isocratic flow of 1 $ml \cdot min^{-1}$, and the oven temperature was maintained at 50 °C. We used a 15 × 4.6 mm column and 5 μm particle size (C-18 Shimpack). The electrical potential in the electrochemical detector (Altec-Decade) was 800 mV. The mobile phase consisted of phosphate-citrate buffer (3 mM heptanesulfonic acid, 20 mM anhydrous sodium phosphate, 23 mM sodium citrate, 0.1 mM EDTA, 3% methanol [v/v], and 1% acetonitrile [v/v]), pH 3.18. The mobile phase was filtered through a membrane with 0.45 μm porosity and deaerated with the aid of an ultrasonic bath. For the extraction of plasma catecholamines, we used a liquid-liquid extraction technique and 3,4-dihydroxybenzylamine (DHBA) as the internal standard. Thus, 500 μl of plasma was transferred to a 2-ml microtube together with 300 μl of 0.2 M perchloric acid solution, 30 mg aluminum oxide, and 1 $ng \cdot ml^{-1}$ of the internal standard. This solution was subjected to 30 min of agitation on an orbital platform and centrifuged under refrigeration for 10 min at 2000 ×*g*. The supernatant was discarded, and the remainder was washed three times with 1 ml of ice-cold deionized water each time and centrifuged for 10 min at 2000 ×*g*. After washing, analytes were eluted with 150 μl of perchloric acid solution, vortexed for 5 min, and subjected to the same centrifugation procedure. The supernatant was recovered and analyzed.

2.10. Statistical analysis

Bartlett's test was used to verify that the variances were equivalent. The data distribution was verified using the Kolmogorov-Smirnov test for the assessment of BAL and FML total and differential counts, total and differential blood cellularity, and cell counts. Parametric data that had a normal distribution were analyzed using analysis of variance (ANOVA) followed by the Student-Newman-Keuls or Tukey multiple-comparison post hoc test. The Kruskal-Wallis test was applied for non-parametric data, and comparisons were made using Dunn's post hoc test or Mann-Whitney *U* test. All of the statistical analyses were performed using GraphPad Instat 5.01 software, and the level of significance was $p < 0.05$.

3. Results

Confirming previous data that validated the present model [14,26,35], Fig. 2A shows that the V group had a significantly higher total number of cells ($p < 0.0001$, ANOVA followed by Tukey's test) in BAL fluid compared with the N and L groups. For differential cell counts in BAL fluid, the number of lymphocytes and neutrophils was not significantly different between the L group and N and V groups ($p > 0.05$, Kruskal-Wallis test followed by Dunn's test). The group with the highest migration to lung macrophages was the V group, which was significantly different from the N group ($p = 0.0404$, Kruskal-Wallis test followed by Dunn's test). A significant decrease in the migration of eosinophils to the lungs was observed in the L group compared with the V group ($p < 0.0001$, ANOVA followed by Neuman-Keuls test; Fig. 2B).

Not significant differences in total blood leukocyte cellularity were found between groups ($p > 0.05$, ANOVA followed by Neuman-Keuls test). Differential counts of neutrophils, lymphocytes, monocytes, and eosinophils in blood were also not significantly different between groups ($p > 0.05$, ANOVA followed by Neuman-Keuls test; Fig. 2C and D, respectively).

Total cell counts in bone marrow were significantly lower in the L group compared with the V group ($p = 0.0071$, Kruskal-Wallis test followed by Dunn's test; Fig. 2E).

We also quantified the cytokines TNF-α and IFN-γ in BAL supernatants. No significant differences in TNF-α were found ($p > 0.05$, Kruskal-Wallis test followed by Dunn's test). The L group had higher BAL IFN-γ levels than the N group ($p = 0.0496$, ANOVA followed by Neuman-Keuls test). The cytokine results are shown in Fig. 3.

Plasma corticosterone levels were significantly lower in the L group than in the V group ($p = 0.0096$, $U = 111$, ANOVA followed by Mann-Whitney *U* test; Fig. 4A). No significant differences were found in epinephrine levels between groups ($p > 0.05$, ANOVA followed by Neuman-Keuls test). However, the L group exhibited significantly higher norepinephrine production than the N and V groups ($p = 0.0026$, ANOVA followed by Neuman-Keuls test; Fig. 4C).

4. Discussion

The present study evaluated the influence of lactation on immune function in allergic lung inflammation. Lactation decreased pulmonary allergic inflammation in rats in early lactation. Lactating rats that were exposed to the same antigen presented less cell migration to the lungs, especially macrophages and eosinophils. These results suggest that lactation plays an important role in the pathophysiology of asthma. The present results were obtained with a physiologically relevant experimental model in rats. The results are consistent with previous data from our laboratory that utilized a pharmacological experimental model that evaluated the effects of short-term domperidone-induced hyperprolactinemia before inhalatory

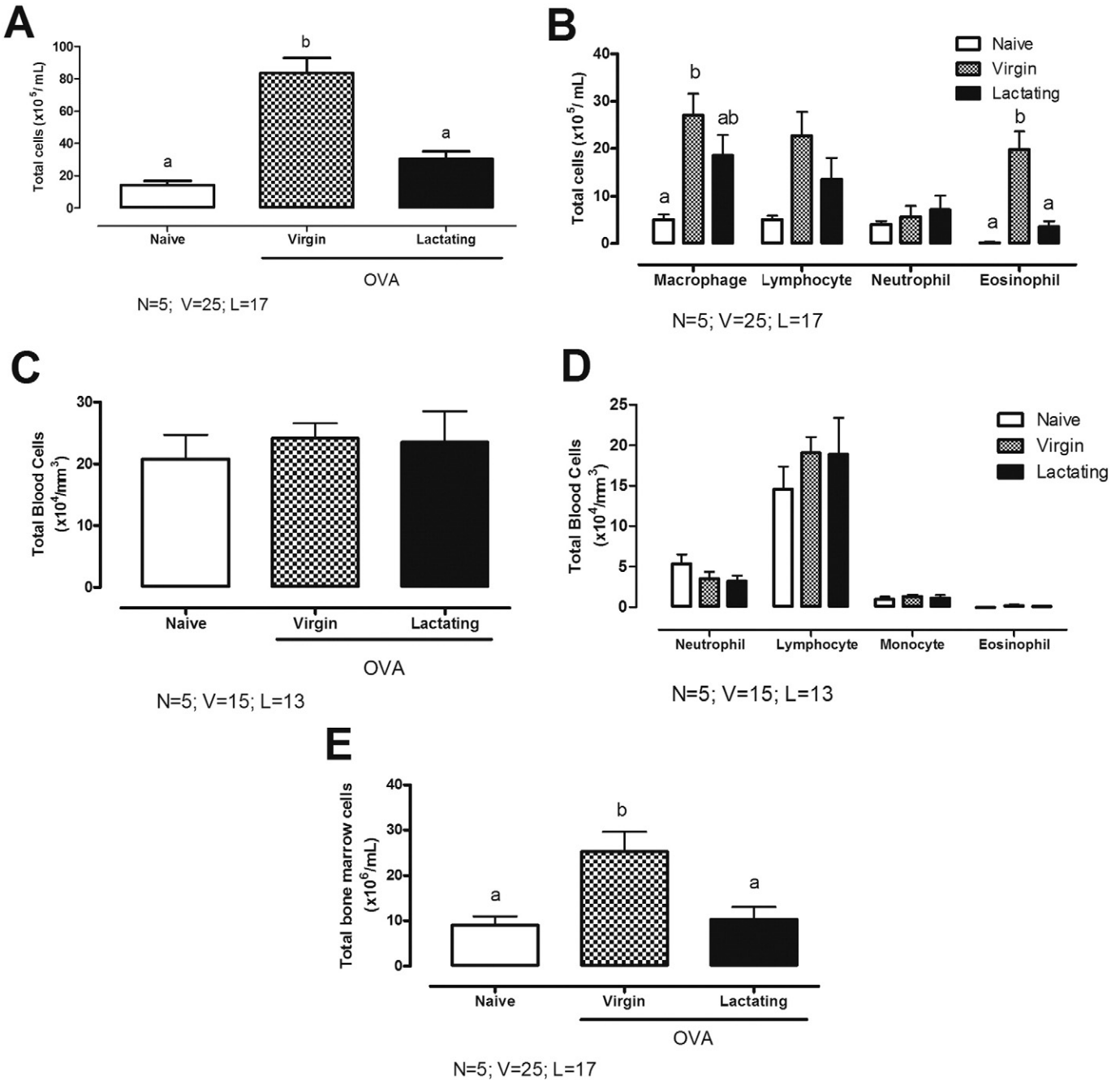

Fig. 2. Lactation inhibits cellular migration in bronchoalveolar lavage (BAL) fluid and reduces ovalbumin-induced allergic inflammation in the lungs. (A) Total cell count in BAL fluid. (B) Differential leukocyte count in BAL fluid. (C) Total leukocyte count in blood. (D) Differential leukocyte count in blood. (E) Total bone marrow cells. N, naive (non-lactating rats not immunized and not challenged with OVA); V, virgins (non-lactating rats immunized and challenged with OVA); L, lactating (lactating rats immunized and challenged with OVA). Different letters above the columns show significant differences. The results are expressed as mean ± SEM. $p < 0.0001$ (BAL), $p = 0.0404$ (macrophages), $p = 0.0010$ (eosinophils), $p > 0.05$ (total blood cells), and $p = 0.0071$ (FML).

antigen challenge with OVA on pulmonary allergic inflammation [35]. Reproductive experience (i.e., pregnancy and lactation) induces physiological changes in mammals. A previous reproductive experience can modulate the activity of dopaminergic hypothalamic systems while decreasing serum prolactin (PRL) levels and oxidative burst activity in peritoneal macrophages. Dopamine receptor antagonists increase serum PRL levels, and both PRL and dopamine receptors might be involved in the modulation of macrophage activity, providing a means of communication between the nervous and immune systems [8,9] Macrophages during a second pregnancy become more sensitive to the phagocytotic effects of prolactin.[9]. Enhanced cognition and reduced stress have been reported in parous rats. Thus, meaningful behavioral adaptations occur that promote the survival of the mother and her infants [20].

Notably, the concentration of prolactin is an important modulatory factor of the inflammatory response. Lactating females have higher levels of plasma prolactin, and this hormone emerges as a modulating factor in the allergic response in lactating females [28,39]. Nursing is a natural model of neuroprotection, with the participation of prolactin [29].

In both the domperidone pharmacological treatment and during lactation we have observed a significant reduction in total number of cells and percentage of eosinophils in the BAL. The BAL results in the group of allergic virgin rats showed that OVA treatment effectively induced

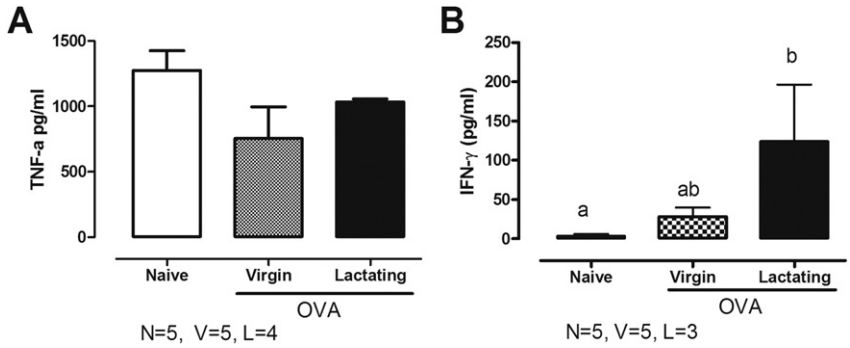

Fig. 3. Lactation increases IFN-γ levels in allergic rats. Quantification (in $pg \cdot ml^{-1}$) of the cytokines TNF-α (A) and IFN-γ (B) in BAL fluid in naive rats and virgin and lactating allergic rats. N, naive (non-lactating rats not immunized and not challenged with OVA); V, virgins (non-lactating rats immunized and challenged with OVA); L, lactating (lactating rats immunized and challenged with OVA). Different letters above the columns show significant differences. The results are expressed as mean ± SEM. $p > 0.05$ (A) and $p < 0.05$ (B).

pulmonary allergic inflammation. Compared with naive rats, the allergic virgin group developed greater cell migration in the lungs, with increases in the migration of macrophages and eosinophils. Interestingly, eosinophil migration to the lungs did not increase in lactating dams. This suggests that lactation and perhaps physiological hyperprolactinemia itself effectively reduce pulmonary eosinophil migration.

Likewise, it was shown a decrease in bone marrow cell counts of hyperprolactinemic and lactating groups, a possible consequence of prolactin action on bone marrow receptors [37,44]. Consistently, hyperprolactinemia induced five days before the inhalation challenge was able to reduce lung cell influx, alter cell migration profile in the bone marrow and in the blood, decreasing the bone marrow cellularity and migration of eosinophils to the lung [35]. With regard to the cellularity of FML fluid, lactating animals presented significantly lower counts than allergic virgins. The reduction of the cellularity of FML during lactation may be attributable to a lower demand on the bone marrow in these animals. Previous studies from our and other laboratories showed that this is associated with physiological hyperprolactinemia [35,36,48].

The immune balance controlled by T helper 1 (Th1) and T helper 2 (Th2) is crucial for immunoregulation and its imbalance causes various immune diseases including allergic asthma. Therefore, diagnosis of Th1/Th2 balance in autoimmune diseases including asthma is essential for the application of immune balance regulating drugs. Th1/Th2 balance is not only controlled by Th1 cells and Th2 cells, but also by various regulatory factors including regulatory T cells, sexual factors, chemokines, transcription factors, signal transduction pathway (STAT6), among others [23]. As Th1 cells secrete IL-2 and IFN-γ [2], they act by inhibiting bronchial asthma. Several studies have revealed incapacity of Th1 cells to suppress bronchial hyperresponsiveness induced by Th2 cells. A combination of Th1 and Th2 cells and their products increased airway inflammation and bronchial hyperresponsiveness [19]. Recruitment and migration of eosinophils inside the inflamed area after the antigenic challenge is controlled by cytokines, chemokines and inflammatory mediators. Allergic immune responses are united to the Th1/Th2 unbalance with an increase in Th2 cytokines profile [18]. TNF-α function in the pathogenesis asthma is unclear [38]. TNF-α is upregulated in the airways of asthmatics after allergen challenge [11] and induces the expression of epithelial cytokines, such as IFN-γ [24].

Th1 cells also appear to play a role in allergic inflammation in local tissues, failing to counter balance Th2 responses in airways inflammation. The role of Th1-type cytokine IFN-γ in asthma is still a matter of debate: in an earlier study Krug and coworkers [22] described an increased frequency of IFN-γ^+ T cells in bronchoalveolar lavage fluid from asthmatic compared with control subjects. However, other investigators have shown an inhibitory effect of IFN-γ on pulmonary allergic responses. Other steroid hormones, such as cortisol or dehydroepiandrosterone (DHEA) are produced by the adrenal gland. And these hormones may affect leukocyte function directly [23]. Studies of pregnancy have suggested that sex steroids may drive the balance toward Th1 or Th2 cytokine responses [23]. There is a dramatic reduction in the responsiveness of the hypothalamo-pituitary-adrenal (HPA) axis to various physical or emotional stimuli in both pregnant and lactating rats [32]. This appears to be due to changes throughout the HPA axis. Prolactin reverses the effects of corticosteroids and increases the Th1-type cellular response [10]. Also, the Th1 response in asthma model can be anti-inflammatory. These literature data are consistent with the observations made in this and other studies [5,10,28]. Mice with high levels of prolactin present increased Th1 and antigen specific T lymphocytes activity. Th1 cells secrete IFN-γ which inhibits bronchial asthma [2]. Accordingly, it is possible that prolactin, promoting the Th1 immune response which is associated with the activation of cellular responses and macrophage activation in the synthesis of IFN-γ in NK cells and T lymphocytes [28,35] and acts as anti-inflammatory in group L. With regard to IFNγ cytokine it was increased both in the hyperprolactinemic and lactating groups. As a Th1 cytokine, IFNγ could have balanced Th1/Th2 profiles, which would lead to a decrease in allergic airway inflammation we observed in these animals.

Physiological levels of glucocorticoids are immunomodulatory, causing cytokines to shift from proinflammatory to antiinflammatory actions [40]. Corticosterone levels in the L group were significantly lower than in the V group. This may be attributable to a decrease in the intensity of pulmonary allergic inflammation in lactating animals. Because the allergic response was reduced during lactation, a compensatory increase in corticosterone levels was no longer necessary. Protein hormones, such as prolactin, are produced by in the anterior pituitary. Binding sites for sex steroids are present on lymphocytes and they can be metabolized in immunocompetent cells, suggesting that sex steroids may affect leukocyte activity [23]. Moreover, a reversal of corticosterone levels might reduce the allergic response. One possibility is that endogenously produced corticosteroids may have reduced eosinophil inflammation in lactating animals before the moment at which corticosterone measurements were made. Endogenous corticosterone increases the pathophysiological response of the adrenal glands, possibly to protect the lungs during eosinophilic allergic inflammation [30]. Sex steroids may produce different modulatory effects depending on the cytokine profile of the cells [23]. Asthma is a state characterized by Th2-driven immunity [18]. The Th2 pattern of immunity is characterized by the production of IL-4, IL-6 and IL-10 and is associated with a humoral immune response or the production of antibodies [40]. Hormonal fluctuation during lactation exerts a strong influence, inducing neuroplasticity in the hypothalamus and extra hypothalamic regions, and diminishing the stress and inflammatory responses [29]. In addition to altered endocrine responses during lactation, stress also changes immune function in comparison to non-lactating animals, suggesting that bi-directional communication between the immune and endocrine systems is also

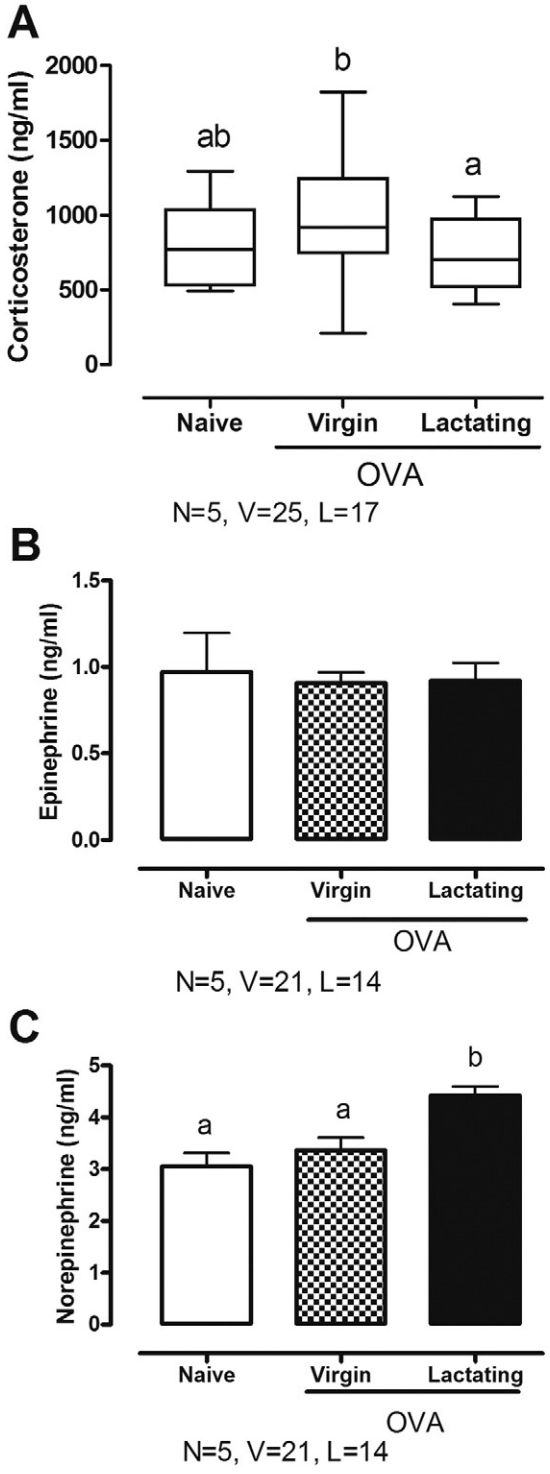

Fig. 4. Lactation reduces plasma levels (in $ng \cdot ml^{-1}$) of corticosterone and increases the levels of norepinephrine in allergic rats. (A) Plasma corticosterone levels. (B) Plasma epinephrine levels. (C) Plasma norepinephrine levels. N, naive (non-lactating rats not immunized and not challenged with OVA); V, virgins (non-lactating rats immunized and challenged with OVA); L, lactating (lactating rats immunized and challenged with OVA). Different letters above the columns show significant differences. For corticosterone, the data are presented as median, interquartile range, and maximum and minimum values ($p < 0.05$). For epinephrine and norepinephrine, the data are expressed as mean ± SEM (epinephrine, $p > 0.05$; norepinephrine, $p < 0.005$).

altered during lactation [29]. In addition, activity of the hypothalamic–pituitary–adrenal axis (HPA) and associated corticosterone or cortisol release is elevated during the peripartum period. Associated with gestation and/or parturition, there also occur important changes in the neurohormones, oxytocin and β-endorphins as well as in the neurotransmitters, dopamine, norepinephrine, glutamate and GABA in rat [31]. Corticosteroids can prevent airway inflammation, by reducing the synthesis and release of pro-inflammatory mediators by bronchial epithelial cells [47]. Corticosterone levels are high at parturition [31], and this event also promotes immunological adjustments that favor Th1 immune phenotype, that may be mediated by oxytocin. Oxytocin has been implicated in the control of the axis at this time, but the inhibitory action of central oxytocin on ACTH or corticosterone secretion seen in virgin female rats is not evident during pregnancy and lactation [32]. However, central oxytocin is involved in the regulation of emotionality at this time. In addition to its anxiolytic effect, prolactin, acting at brain prolactin receptors, seems to exert an inhibitory effect on HPA axis responsiveness. CRH synthesis in the PVN and CRH binding in the adenohypophysis reported in late pregnancy can, at least in part, account for the reduced release of ACTH and consequently of corticosterone into blood in response to an external stimulus in both pregnancy and lactation [43], as we have observed in the results of lactating females. Numerous studies have demonstrated alterations in T cell reactivity after allergen immunotherapy, with reduction in Th2 cytokine expression upon allergen stimulation often accompanied by increased expression of the Th1-associated cytokine IFN-γ [23]. Other neuropeptides like prolactin and oxytocin that are significantly involved in reproductive functions, have also been shown to exert effects on the reactivity of the HPA axis. These physiological functions of the brain prolactin system are especially relevant in the peripartum period, as an attenuation of behavioral and neuroendocrine stress responses has been described during pregnancy and lactation [42]. However, effects of HPA axis hormones (corticosterone in the rat) are bimodal, depending on the reason for their elevation and the context in which HPA system is being activated [31]. In humans, the early postpartum period has been associated with up-regulated inflammatory responses and a relapse of autoimmune disorders such as rheumatoid arthritis and multiple sclerosis, often interpreted as a flare-up due to the rebound of the immune system after pregnancy [29].

In the present study, lactating animals exhibited higher concentrations of norepinephrine compared with the N and V groups. Thus, our results may be attributable to the direct participation of norepinephrine in the decrease in pulmonary allergy that was observed in lactating dams. Adrenergic stimulation increases the frequency and volume of breathing by relaxing the bronchial muscles through the activation of β2 receptors [17]. In fact, recent studies showed that leukocytes express beta2 adrenergic receptors [21]. Specifically, all immunological parameters are considered to originate from leukocytes, and a particularly important part of the immune response stems from catecholamine release. The nervous system regulates the immune system regionally through the innervation of immune organs by the sympathetic nervous system and in locations where inflammation occurs via sympathetic nerves [40]. The local neural regulation of immune responses occurs through sympathetic nervous system activation and the effects of neurotransmitters (e.g., norepinephrine) on immune cells in the spleen and lymph nodes [40]. Norepinephrine levels are increased during moderate asthma in humans, in such a way that epinephrine levels remained normal. This is consistent with our findings that indicate norepinephrine was released by the sympathetic nervous fibers of the L group, as a pathophysiological response to protect lactating rats' lungs. This could be a mechanism to suppress allergic airway inflammation since immune cells like mastocytes, eosinophils, neutrophils and lymphocytes possess 2AR receptors [30]. β agonists are effective in diminishing bronchoconstriction caused by adenosine. Moreover, they attenuate TNF-α production by monocytes and the release of leukotrienes LTC4 by eosinophils. In such a way that interactions between β receptors and inflammatory cells could contribute to the efficacy of β agonists in asthma therapy [17]. Thus, the decrease in pulmonary allergy that was observed

in lactating dams may be attributable to a prolactin-induced increase in plasma norepinephrine concentrations.

One possibility is that endogenous lactogenic hormones exert actions throughout pregnancy and lactation to mobilize modulatory signaling pathways, leading to a reduction of the expression of pulmonary allergic responses during lactation. In the context of asthma during lactation, prolactin may act by inhibiting inflammatory processes, promoting adrenergic activation through β2 adrenergic receptors, and promoting bronchodilation. Our results emphasize the importance of studying asthma in the context of specific physiological states. For various mammalian species, parturition occurs during the spring. Pollen in the air might be an abundant allergenic factor during this season. Thus, protecting lactating females against such allergens might have high adaptive value.

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