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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

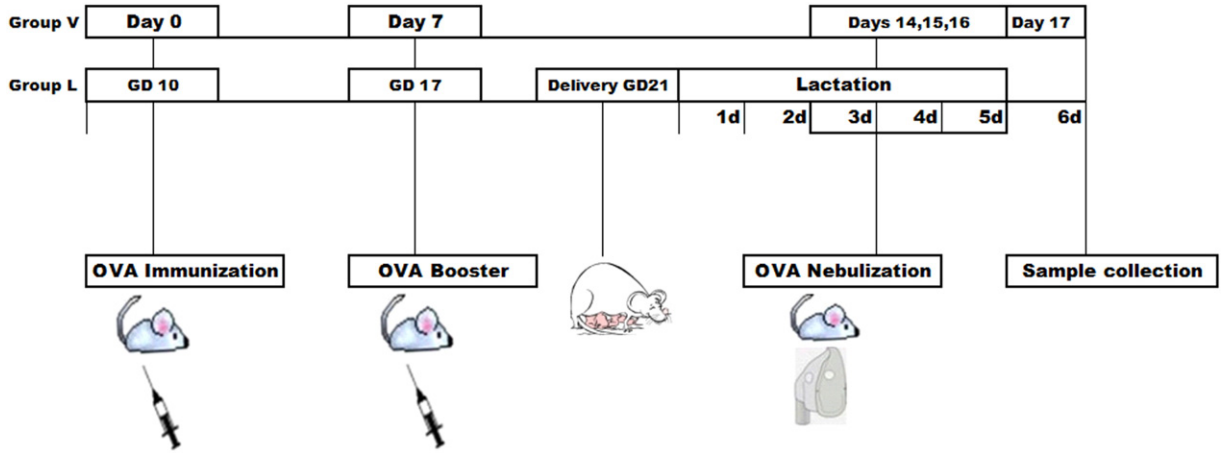


**Fig. 1.** Experimental timeline of observing the effects of lactation on allergic inflammation in the lungs induced by ovalbumin.