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Ovariectomy Modifies TH2, and TH17 Balance in BALB/C Allergic Mice

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ABSTRACT

Asthma is a chronic inflammation of the airways affecting over 300 million people worldwide. As in the autoimmune diseases, it is well described that women are the most affected by asthma. The higher number of women presenting this pathology suggests the involvement of female sex hormones in the construction of the allergic immune response.

Female Balb/c mice were used for the experiments. Thirty-eight animals were separated into four groups: OVX-Ova; Sham-Ova; OVX-Sal; Sham-Sal. Then animals underwent acute allergic induction protocol by Ovalbulmin (OVA). Ovariectomized animals showed greater number of leukocytes in bronchoalveolar lavage (BAL) and elevated white blood cells recruitment to the lung environment observed by histological analysis.

There was a significant increase of eosinophils and mast cells in inflammatory sites at pulmonary tissue. The relative uterine and body weight were lower in ovariectomized animals and higher in Sham mice, respectively. Moreover, the lack of the sex hormones induced an increase in interleukin (IL)-4 and titers of immunoglobulin G1 (IgG1) antibodies. However, increased production of IL-17A was only observed in Sham animals.

Altogether, data this study suggest that ovariectomy induces the formation of a stronger Th_2 response in allergic animal. However, the immune processes involved in the allergic response in females currently remain unclear.

Keywords: Airway inflammation; Asthma; IL-17A; Ovariectomy; Sex hormones

INTRODUCTION

Asthma is a chronic inflammatory disease of the airways characterized by reversible airflow obstruction, goblet cell and smooth muscle hyperplasia, increased mucus production, hyperactivity and ultrastructural

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remodeling of the airways.[1] Worldwide, approximately 300 million people have asthma and it is believed that this number may be underestimated due to similarity of symptoms with other airways diseases.[2]

The prevalence of asthma is higher in developed and industrialized countries. It is believed that the process of urbanization and westernized life style favors to the development of chronic airway inflammation.[3] The limited exposure to microbial agents in childhood could be responsible for this difference in prevalence asthma between urban and rural areas.[4]

Asthma affects male and female differently. Before puberty men are more prevalent and after this stage, are women.[5,6] It has been suggested that there is an influence of female sex hormones in the modulation of inflammatory airway components.[7] Thus, changes in lung function have been observed in relation to the phases of the ovulatory cycle.[8] Moreover, it has been reported a relative decrease in the respiratory capacity during periods near the end of the menstrual cycle condition known as premenstrual asthma.[9,10]

Allergic inflammatory response occurs through immune dysregulation of pulmonary structures, residing and recruited cells against the allergen, such conditions responsible for triggering an asthmatic response. The imbalance in the ratio of $CD4^+$ T cell lines - Th_1, Th_2, regulatory cells (T_{reg}) and Th_{17} - directs the immune response in asthma.[11] Accordingly, allergic airway inflammation has not been associated with Th_1 cells[12] and has been usually described as a disorder of predominantly Th_2 lymphocytes, eosinophils and mast cells.[13] The major cytokines secreted by this profile are interleukin (IL)-4, IL-5 and IL-13, eosinophils are protagonists in this scenario of inflammation.

More recently, Th_{17} cells have also been associated with immunopathology of asthma and were related to the main features as hyperresponsiveness and ultrastructural remodeling airways, in addition to pharmacological therapy resistance with corticosteroids.[14] About 10% of asthma patients have the severe form of the disease. Generally, this group of patients resistant to traditional drug treatment are mainly composed of females.[15]

The profiles of T_{reg} and Th_{17} are mutually regulated.[16] The lower presence of T_{reg} cells in asthma has been related to increased allergic inflammatory response.[17] The induction of Th_{17} profile abrogates tolerance to OVA in non-sensitized mice mediated by T_{reg} cells in chronic allergic airway inflammation.[18] It is well described that females have a more intense Th_2 response compared to males.[19] This has been also observed in studies with murine models.[20] This condition favors a greater number of asthmatics event, since as this morbidity is classically characterized by an immune response consisting predominantly of lymphocytes, cytokines and chemokines of profile Th_2.[21,22]

Studies in animal models using the ovariectomy protocol have been used to describe the mechanisms involved in the hormonal modulation. Antunes and coworkers[23] pointed out that sham female mice exhibit greater infiltration of inflammatory cells, ultrastructural airway remodeling, with significant reduction of respiratory function. In this line, ovariectomy decreased these characteristics, suggesting estrogen (E_2) as a potent stimulant of airway inflammation. However, Draijer and coworkers[24] observed the opposite, with E_2 having a protective role in airway inflammation.

Results of studies involving animal models are still very conflicting.[23-25] Thus, it is noted that the role of female sex hormones and the mechanism by which it acts on the immune system remains undefined, becoming the subject of intense debate and controversy among researchers.[26,27] In this way, the present study aimed to investigate the influence of ovariectomy in modulating of allergic immune response airways in the earliest times of inflammation in Balb/c mice.

MATERIALS AND METHODS

Animals and Ethical Aspects

Female BALB/c mice, 8-10 weeks of age, from the Instituto Multidisciplinar em Saúde facilities were used throughout the experiment. They were housed in boxes in a light-and-temperature-controlled room (12:12-h light-dark cycle, 21±2°C) with free access to food and water. Animal Studies were approved by the Ethics Committee on Animal Use - CEUA, IMS/CAT/UFBA. Protocol n^o 012/2014.

Formation of the Groups

A total of 38 animals underwent the surgical procedure and separated into four groups: The group 1 was ovariectomized and received ovalbumin (OVA) (OVX-Ova); The group 2 passed through the sham surgery and received Ova (Sham-Ova); The group 3 was ovariectomized and received saline (OVX-Sal);

The group 4 passed through the sham surgery and received saline (Sham-Sal).

Ovariectomy Protocol

Vaginal lavages were performed and the mice that were in the reproductive stages of their estrous cycle - pro estrus and estrus - underwent the surgical procedure. Bilateral ovariectomy was held a week before the experiments. The animals were anesthetized with an intraperitoneal injection (i.p) of the anesthetic solution consisting of xylazine (5mg / kg) and ketamine (20 mg / kg). Incisions were performed on both sides (just below the rib) after the identification and subsequent removal of the ovaries adhering tissue, skin and muscle were sutured. The animals received prophylactic antibiotic therapy with enrofloxacin– (Flotril 2.5%, Schering-Plough, Brazil). A similar manipulation was performed in another group of mice, with the aim of promoting a similar stress due to surgery, but without removal of the ovaries. The components of this group were termed sham mice.

Sensitization and Antigen Challenge

OVX-Ova groups and Sham-Ova were subjected to sensitization with OVA by subcutaneous injection at dose of 2 mg, adsorbed into 4 mg/mL of aluminum hydroxide dissolved in saline solution on days 7 and 14 after surgical procedure of ovariectomy or sham surgery. Seven days after the last sensitization animals were challenged one time with 100µg OVA dissolved in 30µL of saline solution by endotracheal route, adapted from [28]. Euthanasia was performed 12 and 24 hours after challenge by i.p deepening anesthesia (xylazine-50mg/kg; ketamina-500mg/kg).

Collection of Peripheral Blood

Blood samples were collected after euthanasia of animals. Blood was centrifuged at 300g for 10 minutes at room temperature to separate serum, which was stored at - 20 ^{0}C in 1 mL microtubes to evaluate the production of IgG antibodies by ELISA.

Weight of Animals and Relative Mass of the Uterus

On the day of euthanasia, all animals had their body weight measured. After euthanasia, the uterus of all animals were collected and weighted. The uterus relative weight was performed as follows: Uterine relative weight = uterus weight / mice weight X 100.

Bronchoalveolar Lavage

Bronchoalveolar lavage (BAL) was colleced in mice. For this purpose, a total volume of 1.5 ml (3 times, 0.5 ml) of saline was injected into the lungs by tracheal route. The BAL fluid was collected and centrifuged (300 g, 10 min-4 ^{0}C). The cellular pellet was resuspended in 500 uL of saline and the total leukocyte count was performed using a Neubauer chamber. Supernatants were used for cytokine assays.

Histological Analysis

Mouse lungs were fixed in 10% formaline, and lung tissues were embedded in paraffin blocks. Tissue sections, 5 µm thick, were affixed to microscope slides and deparaffinized. The slides were stained with haematoxylin–eosin for inflammatory cell infiltration, periodic acid Schiff for identification of goblet cells, toluidine blue for recruitment of the mast cells and they were examined under light microscopy. Beyond, images were taken from a light microscope coupled to a photographic lens (Olympus BX51, Japan) through software analySIS getIT(Olympus Soft Imaging Solutions GmbH, Japan) through the evaluation 20 fields per slide for analysis of pulmonary structures and cell count.

Measurement of Serum Antibodies

OVA-specific immunoglobulin G1 (IgG1) and immunoglobulin G2a (IgG2a) levels were measured by enzyme-linked-immunosorbent assay (ELISA) 12 h and 24 h after the challenge according to the manufacturer's suggested protocol (INVITROGEN^{TM} - LIFE TECHNOLOGIES).

IL-4 and IL-17 Assay

The dosage of IL-17A and IL-4 was performed by ELISA according to the manufacturer's suggested protocol (INVITROGEN^{TM}- LIFE TECHNOLOGIES).

Statistical Analysis

Statistical analysis of data was performed using the Mann-Whitney-U-test using the GraphPadPrism program (version 5.0, GraphPad Inc., San Diego, CA, USA) and all data are expressed as median±standard deviation (SD). The *p*-value for significance was set at $p<0.05$, with a 95% confidence interval.

RESULTS

Ovariectomized Mice Exhibit Higher Body Weight and Lower Relative Weight of the Uterus

In order to assess the total weight of the animals and their uterus, body and uterus mass were weighed before and after euthanasia, respectively. The mice that underwent the surgical procedure of bilateral ovariectomy showed a higher body weight and lower relative weight of the uterus, regardless of whether received OVA or saline (Figure 1). This result was correlated with vaginal lavage five days after surgery, in which the animals showed no classic features regarding the ovulatory phases of the estrous cycle (data not shown).

The Ovariectomy Increases the Leukocyte Recruitment to the BAL

The total count of white blood cells in the BAL was measured twelve and twenty four hours after challenge. Ovariectomized OVA challenged animals showed increased cell recruitment in relation the sham mice, in earliest moments after challenge (Figure 2). Furthermore, in systemic level, OVA-challenged animals present eosinophilia in peripheral blood (data not show). There was also a difference between the groups OVX-Ova and OVX-Sal, Sham-Ova and Sham-Sal (Figure 2).

Ovariectomy Induces Increased Eosinophil Recruitment to Inflammatory Site

With the aim to evaluate the possible differences in modulating the recruitment of white blood cells to the inflammatory site, the relative counts were performed on slides from cytospin. In Figure 2, it is observed that the animals that underwent the surgical procedure of ovariectomy and sensitized/challenged with OVA showed a higher recruitment of eosinophils. However, the presence of female sex hormones did not affect the quantitative values of eosinophils. The difference was only observed when the comparison was performed with the controls.

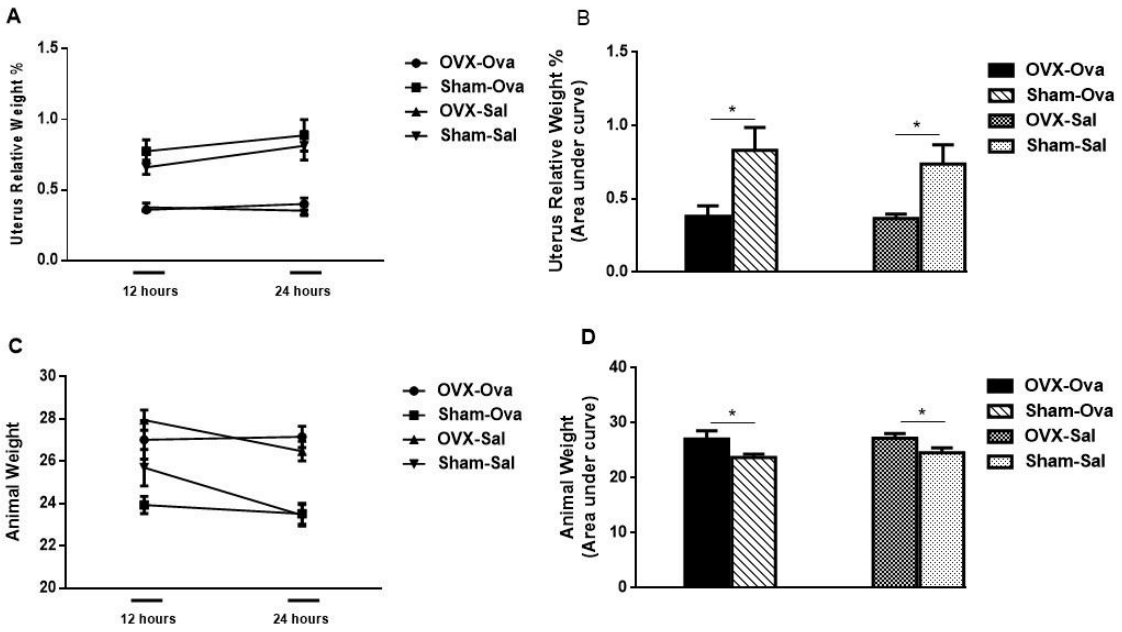

Figure 1. Determination of body and uterus relative weight. Ovariectomy induced the gain of body mass with reduction of relative weight of the uterus (%). Body mass and uterus relative weight were compared between groups ovariectomized sensitized/challenged with ovalbumin (Ova) (OVX-Ova) vs sham-operated sensitized/challenged with Ova (Sham-Ova) and ovariectomized sensitized/challenged with saline (OVX-Sal) vs sham-operated sensitized/challenged with saline (Sham-Sal). Body mass of animals was measured just before euthanasia and during the experiment the uterus were removed and their mass were determined. Results for each group are expressed as median±SD. n=4-5. *p<0.05. A- Uterus relative weight; B- Uterus relative weight (Area under curve); C- Animal bodyweight; D- Animal bodyweight (Area under curve).

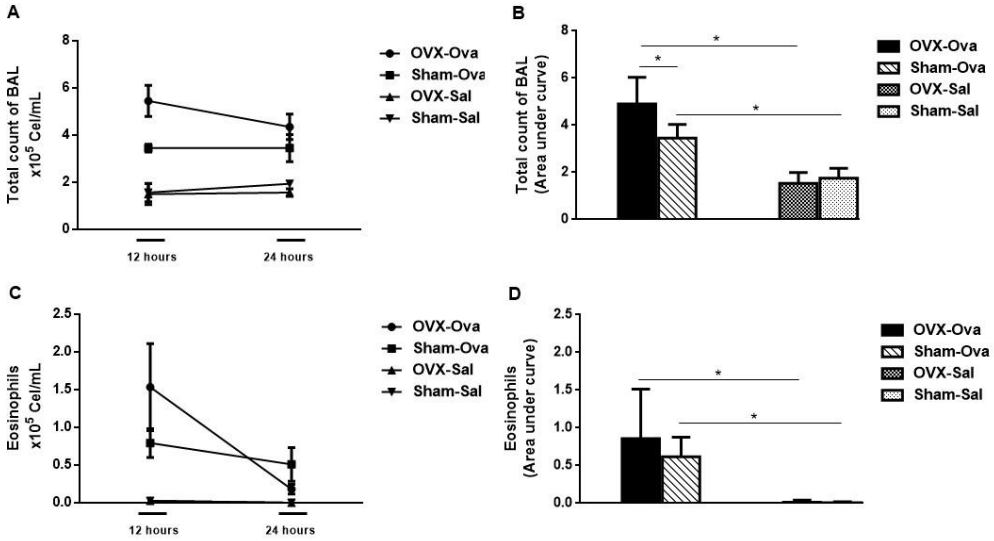

Figure 2. Total and differential cell counts in the bronchoalveolar lavage (BAL). Ovarian hormones depletion increased the recruitment of white cells to BAL. Total and differential cell numbers in BAL were compared between groups ovariectomized sensitized/challenged with ovalbumin (Ova) (OVX-Ova) vs sham-operated sensitized/challenged with Ova (Sham-Ova) and control groups respectively. Quantification of total and differential cells in BAL was performed by light microscopy in Neubauer chamber and slides made by citospin, respectively. Results for each group are expressed as median±SD. n=4-5. *p<0.05. A- Total cell counts in the BAL; B- Total cell counts in the BAL (Area under curve); C- Number of eosinophils in the BAL; D- Number of eosinophils in the BAL (Area under curve).

Ovariectomy Induces An Increased Recruitment of Inflammatory Cells and Eosinophils and Mast Cells Into Lung Tissue

Collection of the lungs were performed 12 and 24 hours after challenge to assess whether the recruitment of inflammatory cells into the lung environment after the antigenic challenge with OVA was changed after the depletion of female sex hormones. It was observed a higher inflammatory cell infiltrate in the ovariectomized animals, with the presence of a large number of eosinophils and mast cells (Figure 3). However, the pattern of globet cells was not modified by surgical procedure (data not show). No inflammatory changes were observed in the groups receiving saline solution with or without presence of female sex hormones.

Depletion of the Ovarian Hormones Induces An Increased Production of IgG Antibodies in the Serum of Animals

In order to evaluate the correlation of the surgical procedure with the production of antibodies, the titles of antibodies of anti-OVA IgG subclass were measured by ELISA in the serum of animals. There was an increased production of IgG1 and IgG2a ova-anti in ovariectomized animals in both times, 12 and 24 hours (Figure 4). All groups that received OVA showed increased in IgG1 in relation to control groups. However, only the group that did not have the female sex hormones presented difference from the control group for IgG2a (Figure 4).

Ovariectomy Alters the Production of Cytokines IL-4 and IL-17A

To assess whether the presence of cytokines in the inflammatory site would be influenced by the surgical procedure, BAL were collected and their supernatant concentration of cytokines were measured by ELISA. It was observed that the ovariectomized-OVA mice increased their production of IL-4, particularly in earlier times, 12 hours after challenge. However, IL-17A was only related to the presence of the ovarian hormones, showing a higher production in the Sham-Ova animals (Figure 5). For IL-4, all OVA groups showed difference in relation the control groups but, for IL-17A, this scenario only was observed in presence of female sex hormones.

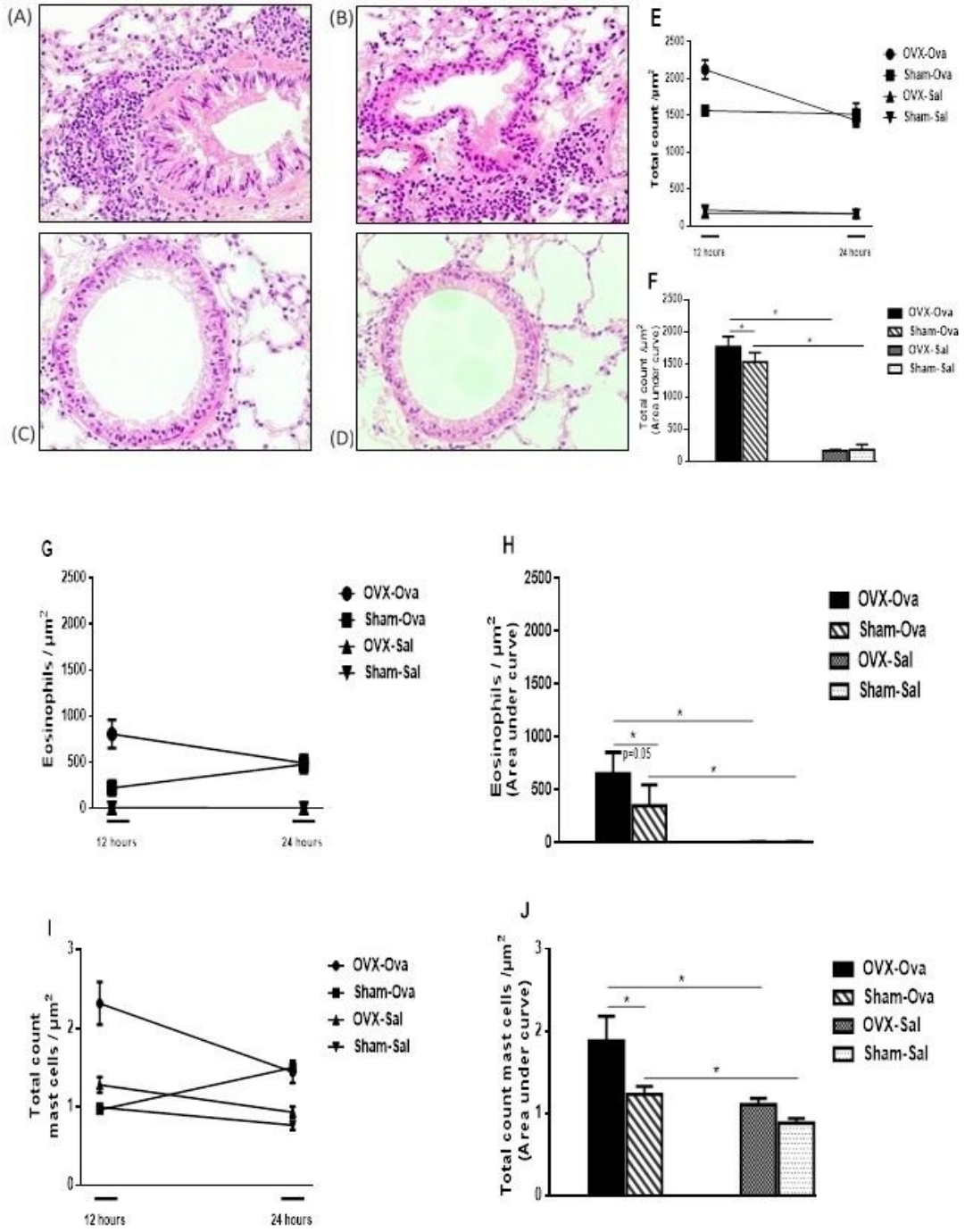

Figure 3. Analysis of recruitment of inflammatory cells to lungs by histological evaluation. Inflammatory cell accumulation was higher in animals underwent ovariectomy. Recruitment of white cells, eosinophils and mast cells to lung tissue was compared between groups ovariectomized sensitized/challenged with ovalbumin (Ova) (OVX-Ova) vs sham-operated sensitized/challenged with Ova (Sham-Ova) and control groups respectively. Histopathological slides were prepared from the collected lungs at the time of euthanasia, stained with hematoxylin–eosin or toluidine blue, and analyzed by light microscopy for total and differential cell counts. Results for each group are expressed as median±SD. n=4-5. *p<0.05. Histopathological changes in lung tissue are seen in (A)- OVX-OVA; (B) Sham-Ova; (C) OVX-Sal; (D) Sham-Sal; E- Total cell counts of inflammatory cells; F- Total cell counts of inflammatory cells (Area under curve); G- Number of eosinophils; H- Number of eosinophils (Area under curve); I- Total count of mast cells; J- Total count of mast cells (Area under curve).

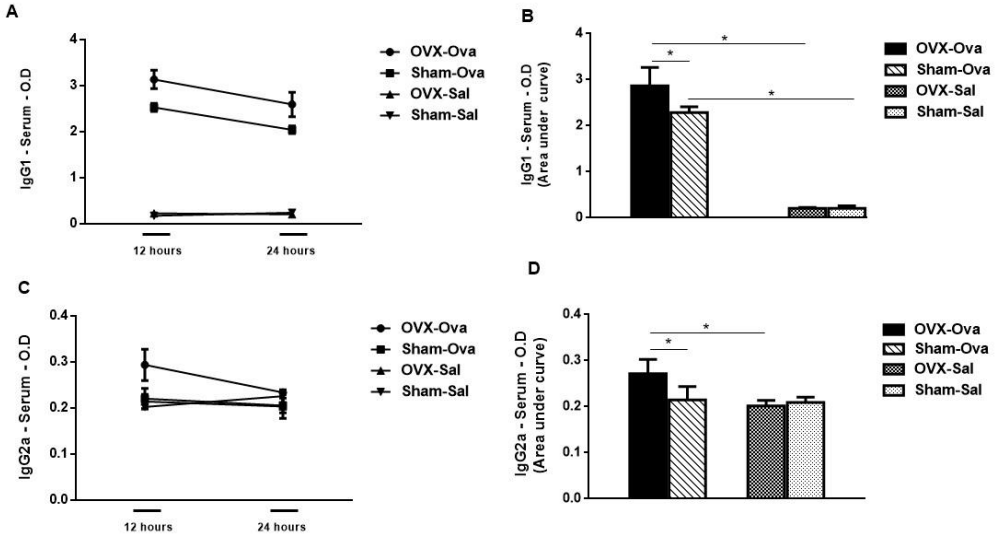

Figure 4. Serum Concentrations of IgG1 and IgG2a measured by optical density. The lack of ovarian hormones was associated with higher serum concentration both IgG1 and IgG2a. Comparison was performed between groups ovariectomized sensitized/challenged with ovalbumin (Ova) (OVX-Ova) vs sham-operated sensitized/challenged with Ova (Sham-Ova) and control groups respectively. Blood samples were collected from the animals and centrifuged to obtain the serum. Serum concentrations of IgG1 and IgG2a were determined by ELISA. Results for each group are expressed as median±SD. n=4-5. *p<0.05. A- IgG1 serum; B- IgG1 serum– (Area under curve); C- IgG2a serum; D- IgG2a serum (Area under curve).

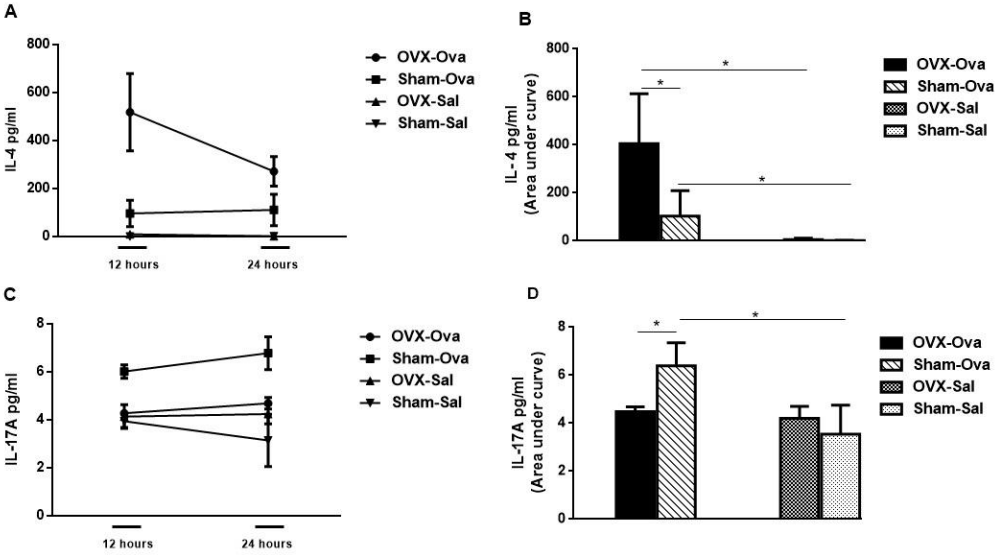

Figure 5. Quantification of IL-4 and IL-17A in asthmatic mice. The concentration of IL-4 was higher in ovariectomized mice, however, for IL-17A, the result was in the opposite direction. Comparison was performed between groups ovariectomized sensitized/challenged with ovalbumin (Ova) (OVX-Ova) vs sham-operated sensitized/challenged with Ova (Sham-Ova) and control groups respectively. IL-4 and IL-17A concentrations were determined from supernatants of bronchoalveolar lavage (BAL) by ELISA. Results for each group are expressed as median±SD. n=4-5. *p<0.05. A- Quantification IL-4; B- Quantification IL-4 (Area under curve); C- Quantification IL-17A; D- Quantification IL-17A (Area under curve).

DISCUSSION

Asthma is considered a serious public health problem of global proportions, consisting of the most prevalent and studied respiratory disease worldwide.[29] Responses to chronic inflammatory airway vary according to sex, in this respect, the female sex is the most affected group by these morbidities.[30] Puberty is a crucial point. From the beginning of reproductive age women become more prevalent in relation to asthma. It is believed that there is an influence of female sex hormones, although the mechanisms are not clear by which this hormone component modulates inflammation.[20,31]

In order to understand the mechanisms involved between allergic airway inflammation and ovarian hormones in their earlier moments, this study used an experimental allergic induction model associated with the bilateral ovariectomy protocol. The main parameter used to evaluate the concentrations of female sex hormones and the effectiveness of the surgical procedure was the relative weight of the uterus.[32] When there is the cessation of hormone concentrations in the systemic circulation, the uterus begins to atrophy causing the reduction of the relative uterus weight in around 50% in the ovariectomized animals (Figure 1). This pattern occurred regardless of the type of solution used for sensitization / challenge, related only to the presence or not of the ovarian hormones.

Reduction of the uterine weight between ovariectomized and sham animals in all groups was accompanied by a weight gain in animals which did not have the ovaries, this increase corresponds to mean of about 12% in OVX compared to sham group (Figure 1). These results are consonant with Robinson and coworkers[33]535 in which ovariectomized animals receiving E_2 showed higher uterine weight and lower variation in body weight compared to placebo treated ovariectomized animals. In this line, Chen and coworkers[34] found that obesity in Balb/c mice enhances Th_2, response in an experimental model of OVA-induced asthma by NKT cells.

Depletion of female sex hormones has been related to increased body weight, especially among women aged between 55 and 65 years.[35] Studies in animal models corroborate this trend.[36] Johnston and coworkers[37] reported a greater hyperresponsiveness of airways and higher serum IgE production using obese mice to evaluate the allergic response induced by OVA.

Regarding the cells present in the BAL, the OVX-OVA group showed a higher total leukocyte count compared to Sham-OVA and control groups, especially in time of 12 hours (Figure 2). Although it shows a lower recruitment of white cells in the BAL, the Sham-OVA group was significant in relation to its control group. No changes were observed between control groups. This suggests that removal of the ovaries shows a pro-inflammatory effect after challenge with OVA in earlier times and an increased inflammation by the recruitment of white cells to the lung environment, specifically 42% higher in relation to Sham-OVA group. This study becomes unprecedented to evaluate the pattern of allergic response in such early periods. The most of works only evaluates the inflammatory response 24 hours after challenge or later.

When assessing the function of the estrogen from the slow release pellets in increasing concentrations inserted during surgical ovariectomy. Dimitropoulou and coworkers[38] observed that the white cell counts in BAL were inversely proportional to the concentrations of estrogen. Reaching baseline levels in higher concentrations, annulling the inflammatory process triggered by the OVA. In another study, ovariectomized female mice exhibit increased airway hyperresponsiveness, condition reverted after exogenous administration of the E_2.[39] These data suggest the anti-inflammatory role of estrogen in line with our results.

Among the main types of inflammatory cells present in the BAL, highlights the eosinophils, with higher concentrations in ovariectomized animals.[38,40] In our study, there was a peak in the recruitment of eosinophils 12 hours after challenge. Despite an increase of 39% in recruitment of OVX-OVA group this difference was not significant (Figure 2). This situation is in a very controversial scenario, because other studies have reported the opposite, with the E_2 acting as pro-inflammatory and thus recruiting more eosinophils to the lung environment in sham animals.[23,41] In present study, significance only was observed between the allergic groups and their respective controls.

In our study, the ovariectomy induced higher recruitment of inflammatory cells *in situ* observed in lung. These animals showed higher total counts of white and mast cells, an increase of 86% and 52% respectively in compared to Sham- OVA groups. (Figure 3). Eosinophils are associated with the Th_2

response. The PAS-staining showed no hyperplasia/ hypertrophy of goblet cells, finding likely related to protocol of induction allergic, the acute type (data not shown). Control groups did not present any inflammatory process.

In the study performed by Carey and coworkers[42] ER-α deletion did not affect the inflammatory parameters, as well as, BAL cell recruitment and cytokine production. However, Shah and coworkers,[43] treated a group of animals with 17β-estradiol and another group with a selective antagonist for the RE-α and observed that animals treated with E_2 showed higher inflammation. This condition was reduced in animals treated with an antagonist drug.

The involvement of immunoglobulins in the formation and maintenance of the allergic response is essential for degranulation and chemotaxis of inflammatory cells. In our study, the titer of IgG1 antibodies showed higher concentrations in earlier times after the antigenic challenge in ovariectomized animals. OVX-OVA group exhibited a production of antibodies of IgG1 25% higher compared to allergic group with presence of female sex hormones (Figure 4). Furthermore, all allergic groups showed a higher IgG1 production in relation to the basal group. The increase of these titers has been correlated to a potentiation of the Th_2 response in experimental models of asthma induced by OVA.[44]

To evaluate the influence of gender in the production of immunoglobulins in allergic immune response in BALB / c mice, Takeda and coworkers[27] found that female animals showed higher titers of IgE and IgA. With the model of ovariectomy in our work, besides increased IgG1, also observed greater production of IgG2a (Figure 4) although with less weight it compares to IgG1. Our results suggest that the absence of ovarian hormone components enhance the production of antibodies of the IgG class.

Th_2 response in our study was considered as the association between IL-4 production and IgG1 antibodies. Depletion of female sex hormones induces higher IL-4 concentrations especially in earlier times (Figure 5). Our data are in line with other published studies, in which the presence of ovarian hormones or treatment of these animals with the E_2 is associated with an anti-inflammatory role.[38,40,45]

The use of an agonist for the bound E_2 receptor to G protein, also known as fast-acting receptor, in Balb/c and C57BL/6 mice reduced all the inflammatory parameters of asthma. In the same study, the anti-inflammatory effect was lost in mice deficient in IL-10, suggesting that this reaction is mediated by IL-10 and $Foxp3^+$ T $CD4^+$ cells.[46] However, in the other way Oliveira and coworkers[25] observed that mice underwent surgery to remove the ovaries showed less inflammation, sustained by increased IL-10 expression and $Foxp3^+$ T $CD4^+$ cells in peripheral lymphoid organ. These studies present conflicting results, due to difficulty in determining the role of female sex hormones in airways inflammation.

According to Robinson and coworkers[33] the dual role of ovarian hormones in inflammatory responses are associated with cycle phase and the hormonal concentration at the time that occurs exposure to antigen. The proportions between the female sex hormones are essential in this moment because it is believed that the focus of immune response and their intensities vary depending on which hormone is overlapping each other at the time of exposure. Thus, this characteristic may explain the reason for such conflicting results on the interaction between hormonal component and the inflammatory axis.

Despite the Th_2 response is considered to be the classical pathway of allergic immune response, the results obtained from researches on this type of response have not been translated into significant alternative therapy for severe refractory asthma.[18] However, new studies have associated the severe refractory asthma with the profile Th_{17} allergic airway inflammation [47]. The primary cell line of this cytokine is IL-17A, produced mainly by lymphocytes Th_{17}, γδ T cells and CLI3s.[48] In our study, the presence of ovarian hormones was correlated with an increased production of IL-17A compared to the ovariectomized group (Figure 5). No difference was observed between the ovariectomized groups on the production of IL-17A.

Maintenance and secretion of cytokines Th_{17} profile has been related to the presence of cytokine IL-23.[49] To evaluate the influence of female sex hormones in modulating these cells, Newcomb and coworkers [48] pointed out that ovarian hormones negatively regulate microRNA *let-7f* expression, a regulator of IL-23R expression on Th_{17} cells. These data suggest that the presence of ovaries enhances the establishment of Th_{17} cell line and their cytokines. The use of knockout animals for IL-17 by means of a mutation in the IL-6 receptor annulled remodeling in a murine model of

chronic asthma. Therefore, these results indicate the involvement of Th17 cells in the process.[18]

In this way, using a murine model of experimental asthma, Choy and coworkers[50] found a mutual regulation between the profile Th_2 and Th_{17}. To treating the animals with *anti*-IL-4 and anti-IL-4/IL-13 there was a potentiation of IL-17A expression. This result suggests that an inhibition of Th_2 cytokine stimulates the expression of the proteins Th_{17} profile. This finding is consistent with the findings in our study, because the animals of Sham-Ova group showed a higher IL-17A did not express significant way of IL-4, representative in classic Th_2 pathway.

Therefore, our study showed inflammatory changes at the earliest times after antigen challenge with OVA, responsible for generating higher leukocyte recruitment in BAL and in the lung tissue, production of antibodies of IgG1 and IgG2a and cytokines after removal of the ovaries. We observed an association between IL-17A and female sex hormones and a possible modulation between profiles Th_2/Th_{17} of immune response. However, the mechanisms involving female sex hormones and their modulation between the axis of the allergic inflammatory response remain unknown. Thus, further studies are needed to identify these pathways and thus build new therapeutic approaches.

This study had limitations such as the lack of an intact group and using animal lineage and route for sensitization and antigenic challenge. The lack of definition of the circulating concentrations of the female sex hormones in moments of sensitization and challenge, may have influenced the immune modulation in the present study. Therefore, the results presented here must be carefully interpreted.

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