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Short-term hyperprolactinemia decreases allergic inflammatory response of the lungs☆

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ABSTRACT

Aims: Prolactin is a major immunomodulator. The present study evaluated the effects of short-term hyperprolactinemia induced by domperidone before ovalbumin antigenic challenge on the lung's allergic inflammatory response.
Main methods: To induce hyperprolactinemia, domperidone was injected in rats at a dose of 5.1 $mg \cdot kg^{-1}$ per day, i.p., for 5 days from 10th to 14th day after OVA immunization. Total and differential leukocyte counts from bronchoalveolar lavage (BAL), femoral marrow lavage (FML), and blood were analyzed. The percentages of mucus and collagen production were evaluated. Levels of corticosterone and prolactin in serum, interleukin-4 (IL-4), IL-6, IL-10, tumor necrosis factor α (TNF-α) in lung explants supernatants were measured and interferon gamma (IFN-γ) in bronchiolar lavage cells suspensions (BAL) was measured.
Key findings: The rats that were subjected to short-term hyperprolactinemia exhibited a decrease in leukocyte counts in bronchoalveolar lavage, cellularity decrease in femoral marrow lavage fluid, a lower percentage of mucus, and an increase in lung IL-4, IL-6, IL-10, TNF-α and IFN-γ expression.
Significance: Hyperprolactinemia induced before antigenic challenge decreased allergic lung inflammation. These data suggest that prolactin may play a role in the pathophysiology of asthma. The present study demonstrates a prospective beneficial side effect of domperidone for asthmatic patients.

1. Introduction

Gastro-esophageal reflux disease (GERD) and asthma are frequently associated with each other. It is estimated that GERD is present in 60–80% of asthmatic adults and 50–60% of asthmatic children. There is considerable debate regarding the nature of this association, but GERD appears to be an important trigger for asthma and a risk factor for worsening symptoms, asthma-related hospitalization, and the requirement for oral steroids. Some studies have shown that anti-reflux therapy with domperidone improves asthma control in patients with symptomatic GERD [40]. Few studies of dopamine antagonist treatment for asthma have been conducted in humans, but case reports have described the use of domperidone in patients with GERD associated with asthma [21,42]. Several explanations have been made for the apparently paradoxical findings that medical antireflux therapy improves asthma symptoms, but not lung function [21]. However, improvements in lung function have been reported, attributable to the inhibition of bronchial hyperresponsiveness [22]. Thus, considering the possible therapeutic use of domperidone, the mechanisms by which it affects the lungs' allergic inflammatory response and moderates immune and inflammatory processes in asthma need to be explained.

Asthma is an inflammatory disease caused by repeated immediate-phase hypersensitivity and late-phase allergic reactions in the lungs, leading to the clinicopathologic triad of intermittent and reversible airway obstruction, chronic bronchial inflammation with eosinophils, bronchial smooth muscle cell hypertrophy, and hyperreactivity to bronchoconstrictors. The pathophysiologic sequence of atopic asthma is likely initiated by mast cell activation in response to allergen binding to immunoglobulin E (IgE) and Th2 cells that react to allergens [1] and produce inflammation. The inflammation is controlled by cytokines of the Th2 cluster. These cytokines include interleukin 4 (IL-4) and IL-13 (which regulates IgE production), IL-5 (which is heavily implicated in eosinophilia), and the counterregulatory cytokines IL-10 and

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transforming growth factor-β (TGF-β) that suppress T-cell function but activate other cells that contribute to the remodeling of airways [40]. Increased mucus secretion results from the action of cytokines, mainly IL-13, on bronchial epithelial cells [1].

Prolactin (PRL) is a peptide hormone that is secreted from the anterior pituitary gland under tonic inhibition by the hypothalamus via dopamine [5,36]. Prolactin is involved in more than 300 different functions, [6] and its functionality depends on the type of cells that express the PRL receptor. Based on its molecular and functional characteristics, it is a cytokine [36] and participates in innate and adaptive immune responses [23]. During an immune response, PRL promotes the proliferation and differentiation of T cells and influences the expression of CD69 and CD154 in CD4 T cells [6]. Prolactin possesses significant immunomodulatory properties because regulatory T cells constitutively express the PRL receptor [13]. This study was designed to test if hyperprolactinemia induced before antigenic challenge interferes with allergic lung inflammation.

2. Materials and methods

2.1. Animals

Male Wistar rats were obtained from the Department of Pathology Animal House, School of Veterinary Medicine, University of São 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 Experimentação Animal and National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Allergic lung inflammation model in rats

The rats were sensitized with 10 μg ovalbumin (OVA; Egg Albumin Grade II, Sigma-Aldrich, St. Louis, MO, USA) [14,25] and 10 mg aluminum hydroxide (EMS Pharmaceuticals, Brazil) dissolved in phosphate-buffered saline (PBS) and administered subcutaneously on day 0 at a dose of 0.1 $mg \cdot kg^{-1}$. One week later (day 7), rats were boosted subcutaneously with the same treatment. For the challenge with OVA aerosol (1% in PBS), animals were individually placed in an inhalation chamber connected to an ultrasonic nebulizer for 15 min per day for 3 consecutive days (days 15, 16, and 17) according to previous studies [19,26].

2.3. Experimental hyperprolactinemia

Hyperprolactinemia was induced by the dopamine receptor blocker domperidone (Johnson & Johnson, Brazil). Although domperidone does not cross the blood–brain barrier,[28] it acts on hypophysis and increases PRL secretion [12,20,34]. Domperidone was administered at a dose of 1.7 mg/kg (i.p.) three times per day (6:30 AM, 2:00 PM, and 9:00 PM) for 5 consecutive days [34,35] from 10th to 14th day after OVA immunization. This treatment protocol has effectively produced stable hyperprolactinemia for more than 30 days [2].

2.4. Experimental outline

Male rats, 60–90 days of age, were randomly divided into four groups: naive group with no treatment and no lung allergy (N), control group (C), vehicle group (V), and domperidone group with induced lung allergy and respective treatments (D).

On day 0, the C, V, and D groups were injected with OVA and boosted on day 7. Between days 10 and 14, the D group was treated with domperidone as described above, whereas the V group was administered 0.9% NaCl. On days 15, 16, and 17, the C, V, and D groups received OVA in aerosol form to induce lung allergy. The experimental outline is depicted in Fig. 1.

2.5. Sample collection

On day 18, 24 h after the last OVA challenge, 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% ethylenediamine tetraacetic 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 circadian rhythms effects. 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 previous data [18]. Recovered BAL fluid was centrifuged at 170 × *g* for 10 min at 4 °C. The supernatant was discarded, and the resulting pellet was resuspended in PBS (1 ml). Rat femurs were removed, and a needle connected to a plastic syringe containing 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 [19,26] The adrenal glands were then removed and weighed. The relationship between the wet weight of the two adrenal glands (in milligrams) and body weight (in grams) was also analyzed, calculated, and compared among groups. Lung samples were also collected for explant culture and fixation for histochemistry.

2.6. Total and differential whole-blood counts

Samples were diluted 1:20 in Turk liquid (3% acetic acid) and counted in a Neubauer chamber. Blood smears were stained with May–Grünwald–Giemsa, and differential leukocytes were counted by light microscopy [19,26].

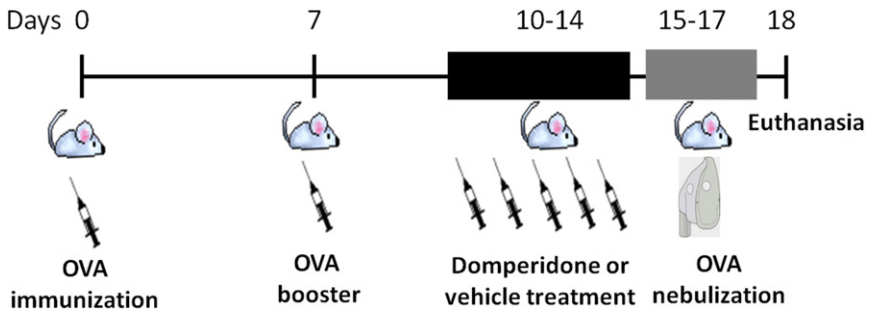

Fig. 1. Experimental outline.

2.7. Femoral myelogram

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

2.8. 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-Grünwald–Giemsa solution [19,26].

2.9. Prolactin and corticosterone measurements

Serum PRL levels were determined by radioimmunoassay [33]. The PRL assay has 0.19 ng/ml sensitivity and inter-assay and intra-assay coefficients of variation of 11.5% and 4.0%, respectively [9,10]. Serum corticosterone levels were determined using a commercial enzyme-linked immunoassay (ELISA) kit (Arbor Assays, #K014) according to the manufacturer's instructions, with 1:300 serum dilution.

2.10. Lung explant culture and medium cytokine analysis

Cytokine production (IL-4, IL-6, IL-10, and TNF-α) was determined in supernatant samples of lung explants maintained in culture of the naïve, control, vehicle e domperidone groups and stimulated with OVA. Lung samples of the four groups were cut randomly into four small pieces, then distributed into 24-well plates that were filled with 1 ml of Dulbecco's modified Eagle medium (DMEM) that contained 0.5% penicillin/streptomycin (10,000 IU, 10 $mg \cdot ml^{-1}$), and with 1 mg/ml OVA treatment, and incubated for 24 h at 37 °C with 5% CO_2. The results are expressed as picograms (pg) of cytokine produced per milligram dry weight of lung tissue. Cytokines were quantified using commercial ELISA kits (BD OptEIA rat cytokine ELISA kits, BD Biosciences) [19].

2.11. Cytokine production in BAL

The concentrations of cytokines and chemokines in BAL specimens were measured with an enzyme-linked immunosorbent assay (ELISA; R&D Systems, USA). The range of curve was: 39.10–2500 pg/ml.

2.12. Quantification of lung mucus and collagen

Lung samples were fixed in 10% formalin and processed by routine histological methods. Sections (3–4 mm thickness) were stained with hematoxylin and eosin (HE) and periodic acid Schiff (PAS) to detect mucus production and picrosirius to observe the presence of collagen fibers. An area was estimated between the inner and muscularis edge mucosa and five bronchioles in each animal using were imaged at 100× magnifications using a Nikon Eclipse 80i microscope, camera infinity and the software ImagePro Plus 5.0 software (Media Cybernetics, Silver Spring, MD, USA). The color threshold for picrosirius and periodic acid Schiff (PAS) were determined and the analyses were performed as follows: using the previously determined color threshold for mucus (PAS positive), the amount of PAS area was determined and results were expressed as the % of PAS area compared to the total epithelium; using the previously determined color threshold for picrosirius and the picrosirius-positive area was quantified. These results were expressed as the % of collagen (picrosirius positive) area compared to the total measured area [43].

2.13. Statistical analysis

Data distribution was verified using Kolmogorov–Smirnov test. Parametric data were analyzed using ANOVA followed by Student–Newman–Keuls *post hoc* test. Kruskal–Wallis test was applied for non-parametric data, and comparisons were made using Mann's U test. All of the statistical analyses were performed using GraphPad Instat 5.01 software.

3. Results

3.1. Domperidone increases prolactin serum levels

The experimental outline is depicted in Fig. 1. Serum PRL levels are presented in Fig. 2A. Prolactin levels in domperidone-treated animals were approximately nine-times higher than in animals from the other groups ($p < 0.01$).

Animals treated with domperidone exhibited lower serum corticosterone levels compared with the C group ($p = 0.01$), but no significant difference was found between the V and D groups ($p = 0.96$; Fig. 2B). The C group had higher corticosterone levels than the V group ($p = 0.01$, $U = 4.00$) and D group ($p < 0.01$).

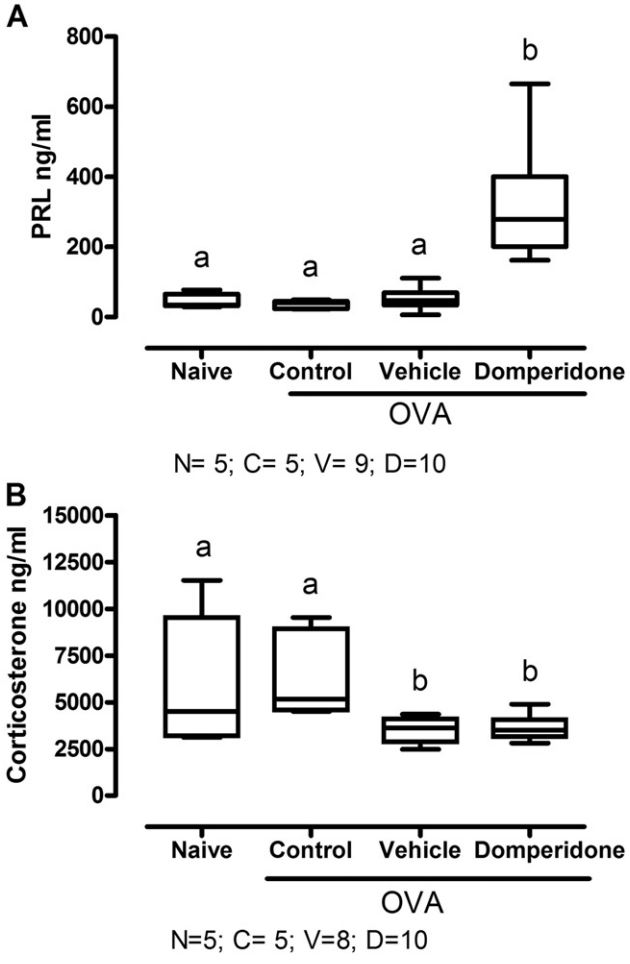

Fig. 2. Domperidone increases prolactin serum levels. Effects of domperidone before ovalbumin (OVA) inhalation on serum prolactin levels (A), and serum corticosterone levels (B). N, naive group; C, control group; V, vehicle group; D, domperidone group. Different letters above the columns indicate significant differences. The data are expressed as median ± SE, interquartile intervals, and maximum and minimum values. Prolactin: $p < 0.01$ (ANOVA and Newman–Keuls multiple-comparison test). Corticosterone: $p < 0.05$ (Kruskal–Wallis test followed by Mann–Whitney test). Different letters above the columns indicate significant differences.

The absolute weight of the adrenal gland and ratio between the weight of the adrenal gland and body weight ($mg \cdot g^{-1}$) were not significantly different between groups (data not shown).

3.2. Domperidone inhibits OVA/induced increase in BAL inflammatory cells

Fig. 3A shows that the C group exhibited a greater number of cells ($p < 0.05$) in BAL fluid compared with the N and D groups, but no difference was found between the C and V groups. The total cell number in BAL fluid was significantly higher in the V group than in the N group, but no significant difference was found between the N and D groups ($p > 0.05$). Differential cell analyses in BAL fluid revealed that macrophage counts in the C group were higher than in the N group ($p < 0.05$; Fig. 3B). With regard to lymphocytes, the highest lung migration was observed in the C group ($p < 0.05$) compared with the N group ($p = 0.02$), but the C group was not significantly different from the D or V group. A significant difference in lymphocyte counts was found between the N and V groups ($p = 0.01$), but no difference was found between the N and D groups. Eosinophil lung migration was the highest in the C group, followed by significantly lower counts in the V and D groups, with significant differences between groups ($p < 0.05$).

No significant differences in total leukocyte blood cellularity were found between groups (Fig. 3C). The C group had different circulating cell counts from the other three groups, but this did not reach statistical

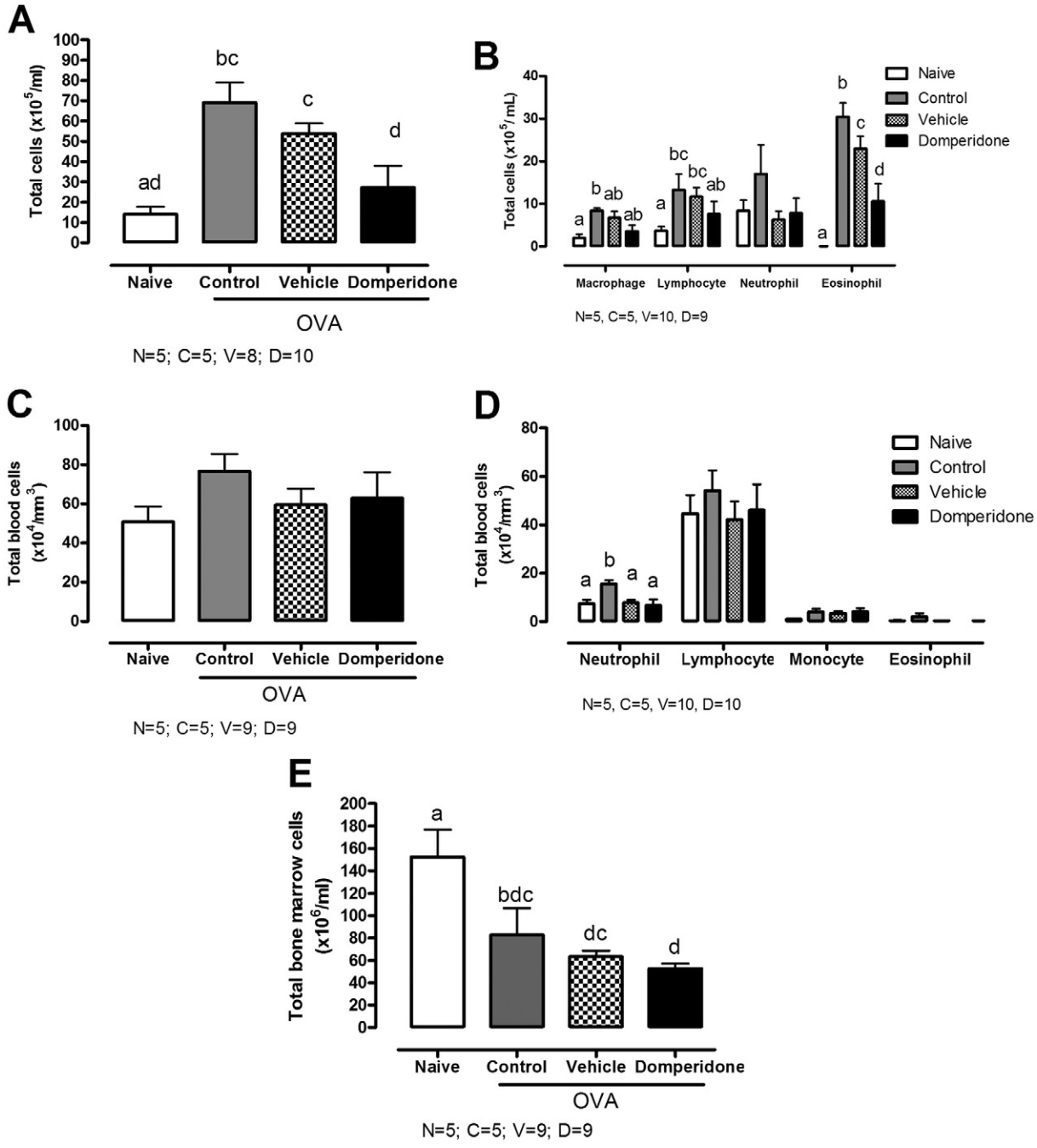

Fig. 3. Domperidone inhibits OVA-induced increase in BAL inflammatory cells. Effects of domperidone treatment before OVA inhalation on the total number of cells in BAL fluid (A), differential leukocyte counts in BAL fluid (B), the total number of cells in whole blood (C), differential leukocyte counts in whole blood (D), the total number of cells in bone marrow (E). N, naive group; C, control group; V, vehicle group; D, domperidone group. Different letters above the columns indicate significant differences. The data are expressed as mean ± SE. Total BAL: $p < 0.005$. Differential BAL: $p < 0.05$. Total whole blood: $p > 0.05$. Differential whole blood: $p < 0.05$. Total bone marrow: $p < 0.005$ (ANOVA and Newman–Keuls multiple-comparison test).

significance. With regard to differential blood cell counts (Fig. 3D), the number of neutrophils was significantly different for the C group ($p < 0.05$), which had a higher number of neutrophils compared with the N group ($p < 0.01$); and V group ($p < 0.01$). Blood eosinophil counts were higher in C group when compared numerically between D group, but this did not reach statistical significance ($p > 0.05$). No significant differences in blood lymphocyte and monocyte counts were found between groups.

FML cellularity was not significantly different between the D group and C and V groups (Fig. 3E). Compared with the N group, the D, C, and V groups presented significantly lower FML cellularity ($p < 0.05$).

Eosinophils are central effector cells in allergic lung inflammation and are responsible for airway inflammation. We evaluated the correlation between plasma PRL levels and eosinophil chemotaxis and migration to the lung in allergic animals. Allergic animals that had high PRL levels due to domperidone treatment presented lower eosinophil migration. A correlation coefficient of $r = -0.18$, confidence interval of -0.73 to 0.51, and $p = 0.62$ were considered an inverse association (Fig. 4).

The percentage of collagen among the different treatment groups (C, V, and D) was not significantly different ($p > 0.05$), but the C, V, and D groups presented higher collagen deposition than the N group ($p < 0.05$; Fig. 5A, B).

3.3. Domperidone inhibits OVA-induced increase in peribronchiolar mucus deposition

Mucus production in the D group was significantly lower than in the V group ($p < 0.01$; Fig. 6A, B). Mucus production was higher in the D group than in the N group ($p < 0.01$) but not significantly different between the D and C groups. Mucus production was not significantly different between the C and V groups, whereas the C group had higher mucus production than the N group ($p < 0.01$).

3.4. Domperidone increases IL-6 and IL-10 levels

To investigate changes in cell migration to the lung, we evaluated the effects of PRL on the production of IL-4, IL-6, IL-10, TNF-α in supernatant samples of lung explants in culture, and IFNg in bronchiolar lavage cells suspensions (BAL), in allergic rats (Fig. 7). The concentrations of IL-4 ($p = 0.03$), IL-6 ($p < 0.01$), and IL-10 ($p < 0.01$) in the supernatants in domperidone-treated rat lung explants were significantly higher than in the supernatants of lung explants obtained from the N, V, and C groups that were challenged again with OVA in vitro. Significant differences in TNF-α were found between the D and V groups ($p = 0.01$). Group D exhibited higher IFN-g concentration in the BAL when compared to vehicle and naïve groups ($p < 0.01$).

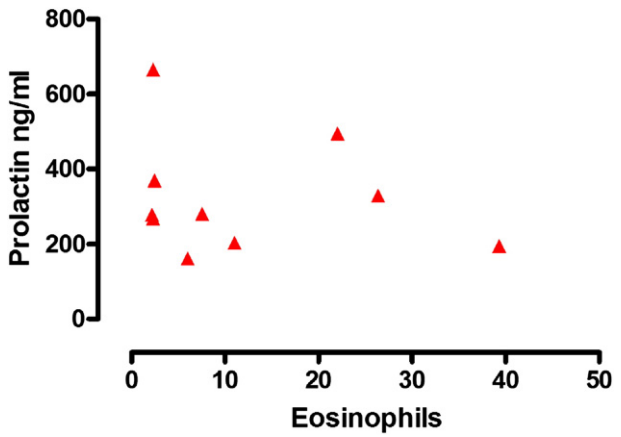

Fig. 4. Correlation between prolactin levels and eosinophil chemotaxis to the lung in sensitized rats challenged with OVA in the domperidone group. Data points are expressed as mean values.

4. Discussion

The present study evaluated the effects of short-term hyperprolactinemia induced by domperidone before ovalbumin antigenic challenge on the lung's allergic inflammatory response. Hyperprolactinemia induced before antigenic challenge decreased allergic lung inflammation. These data suggest that prolactin may play a role in the pathophysiology of asthma.

Domperidone blocks dopamine D_2 receptors, particularly within the tuberoinfundibular dopaminergic system, and induces hyperprolactinemia [2,17,20,32,34]. The present results confirm this effect of domperidone and are consistent with previous findings from our laboratory [11,32,34,35].

Much of what we know about the pathogenesis and pathophysiology of asthma has been derived from human and animal studies of allergic asthma, but some caution is needed in extrapolating the data from these models to better understand what occurs in nonatopic asthma. Asthma does not occur in rodents or other nonhuman animals, and particular care is needed when applying data from animal models of asthma, most of which would be more accurately described as models of airway inflammation caused by allergic sensitization [40]. Nevertheless, this asthma model was successfully applied in the present study.

The results of the BAL analysis in the asthmatic groups (C, V, and D) demonstrated that the animals had higher cell migration to the lungs as a response to the allergic inflammatory process induced by OVA compared with the N group, which represented baseline lung cellularity.

Differential counts in BAL fluid in the V group were significantly different from the C group, suggesting that vehicle treatment was not completely innocuous. The C group exhibited a greater number of cells in BAL fluid, possibly because of an increase in the migration of bone marrow cells in response to allergic pulmonary inflammation induced by OVA. The D group had a decreased population of eosinophils compared with the C and V groups. The D group had higher PRL levels and lower eosinophil migration. Therefore, this might suggest a causal relationship, in which hyperprolactinemia reduces migrating eosinophils.

When we analyzed the total cell number in bone marrow, the hyperprolactinemic D group exhibited a smaller number of total cells compared with the N group, which represented baseline bone marrow cellularity. The reduction of bone marrow cells in the D group was associated with the lowest number of cells in BAL fluid and blood, which may have been caused by hyperprolactinemia. Thus, domperidone treatment before the challenge had anti-inflammatory effects in the lungs in allergic rats. These effects may be attributable to the effect of short-term domperidone-induced hyperprolactinemia on lymphopoiesis [48]. These authors studied the ability of recombinant human prolactin (rhPRL) to stimulate the growth of murine hematopoietic progenitor cells in vitro. In this study, the authors confirmed that the observations made in mouse cells can be extended to humans. Prolactin failed to increase the granulocyte-macrophage progenitor (CFU-GM) and growth of committed erythroid progenitors (BFU-E) when PRL was added to the cultures at various concentrations (1–1000 ng/ml) or to unfractionated bone marrow [49]. There is a correlation between PRL levels and the number of CD4 + T lymphocytes and B lymphocytes, but prolactin is not essential for lymphopoiesis [36,48]. Prolactin might act directly on its own receptors on progenitor cells in bone marrow. The interaction between PRL with PRL receptors activates the JAK family of kinases. This pathway phosphorylates STAT5 latent proteins, conferring DNA binding capacity to STAT and resulting in the transcriptional activation of certain genes, such as STAT1, STAT3, and STAT5b. This pathway also activates the transcription of key genes in the development of the Th1 response, such as T-bet factor. The transcription of this gene is activated even by low concentrations of PRL

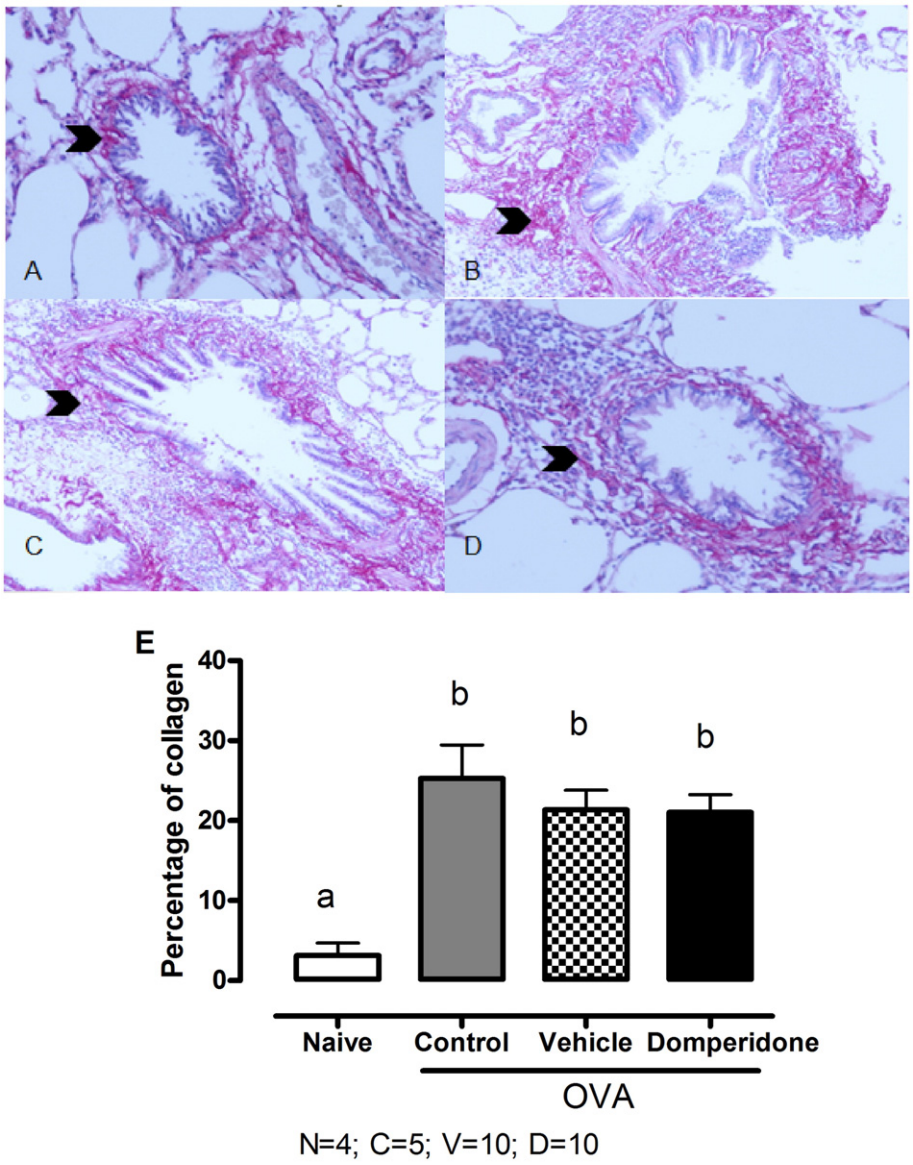

Fig. 5. Lack of evidence of domperidone effect on OVA-induced increase peribronchiolar collagen deposition. Representative images of collagen staining with picrosirius in each treatment group (A, naïve; B, control; C, vehicle; D, domperidone). The arrows indicate positive picrosirius collagen staining. Effects of domperidone treatment before OVA inhalation on the percentage of collagen in the inflammatory response of the lungs (E). N, naive group; C, control group; V, vehicle group; D, domperidone group. Different letters above the columns indicate significant differences. The data are expressed as mean ± SE. $p < 0.05$ (ANOVA and Newman–Keuls multiple-comparison test).

and inhibited by high concentrations of prolactin CD4+ lymphocytes [6,23]. Low doses of PRL elicit a pro-inflammatory response and promote antibody production, whereas high doses suppress these responses. Growth hormone, insulin-like growth factor 1, and PRL could promote growth effects on immune response cellular function through cytokine release [48].

IL-4 has a central function in the promotion of eosinophilic inflammatory reaction in allergic diseases, through the activation of IgE isotype [37]. The D group presented higher IL-4 concentrations than only the N group and higher TNF-α expression than the V group ($p < 0.05$). Prolactin promotes pro-inflammatory immune responses via NFκB and interferon regulatory factor (IRF-1). These cited factors are known to induce TNF-α increase, considered essential inflammation mediator [35], which can affect pathophysiological processes during hyperprolactinemic conditions [8]. TNF-α contributes to a dysregulated inflammatory response in the airways of asthmatics, in part by inducing the epithelial expression of pro-inflammatory cytokines and adhesion molecules [24], which was observed in the hyperprolactinemic group with increases in IL-6, IL-10 and IFN-g secreting cells. IFN-g production in BAL is higher on the hyperprolactinemic group.

Prolactin induces the transcription of interferon regulatory factor (IRF-1) that is a multifunctional transcription factor, and an important regulatory factor of the differentiation and maturation of the lymphocytes T and B [30]. In T lymphocytes, IRF-1 is critical for the development and Th1 response, as well as their proliferation and apoptosis. In Nb2 T cells, IRF-1 is induced by prolactin stimulation as part of the initial G1 activation [50]. IRF-1 regulates the expression of important genes that mediate the immune response, host defense, cell cycle progression, tumoral suppression and apoptosis [47]. Consistently, prolactin promotes the Th1 immunology response, which is associated with activation of the cellular response, with macrophages activation, and with IFNg syntheses in the NK cells and T lymphocytes [30].

TNF-α stimulates ACTH, growth hormone, thyroid-stimulating hormone, and PRL secretion in rat pituitary cells in vitro. In contrast, TNF-α was found to inhibit the release of ACTH and other hormones in response to hypothalamic factors by acting directly on pituitary cells [39].

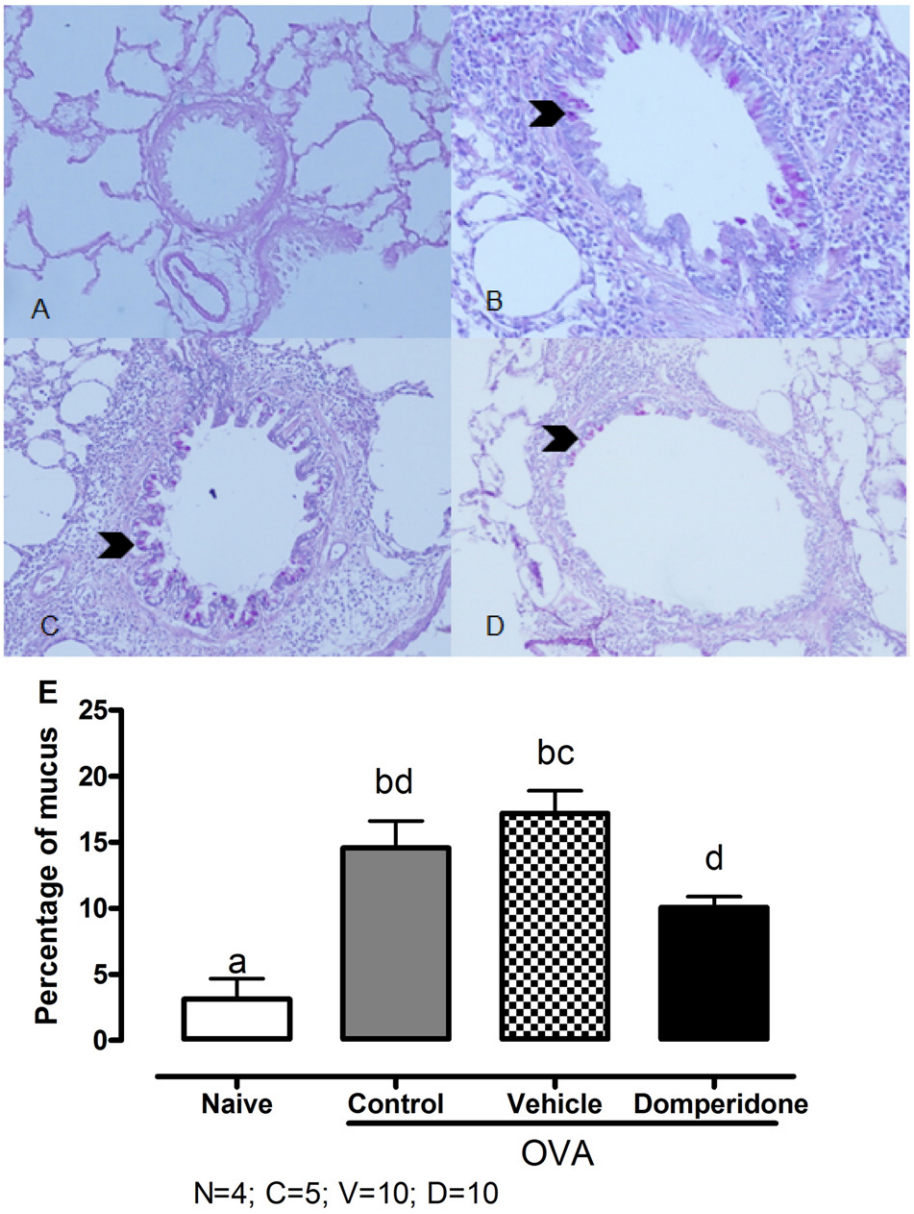

Fig. 6. Domperidone inhibits OVA-induced increase in peribronchiolar mucus deposition. Representative images of mucus staining with PAS in each treatment group. The arrows indicate positive PAS mucus staining (A, naïve; B, control; C, vehicle; D, domperidone). Effects of domperidone treatment before OVA inhalation on the percentage of mucus in the inflammatory response of the lungs (E). N, naive group; C, control group; V, vehicle group; D, domperidone group. Different letters above the columns indicate significant differences. The data are expressed as mean ± SE. $p < 0.05$ (ANOVA and Newman–Keuls multiple-comparison test).

IL-6 is synthesized by Th2 cells and antigen-expressing cells and involved in the inflammatory response [4]. Increases in IL-6 levels stimulate the secretion of ACTH, and IL-6 plays a role in activating the hypothalamic–pituitary–adrenal axis [39]. The concentrations of IL-6 and IL-10 in rat lung explants in the D group in the present study were higher than in the N, V, and C groups challenged with OVA. IL-6 levels were significantly different between the hyperprolactinemic group treated with domperidone and the other three groups (N, V, and C). T lymphocytes activation mediated by prolactin pathways, prolactin receptors (PRL-R) and JAK2/Stat5, lead to the production and release of a range of cytokines including IL-1, IL-4, IL-5, IL-6, IL-10 and IFN-g, which stimulated activated B cells to proliferate and differentiate. These findings support the notion that prolactin favors tolerance by modulating the biological activity of several cell types and tissues, as well as many aspects of the immune response [47]. Prolactin concentration is an important modulatory factor of the inflammatory response [30,38]. Prolactin has an important physiological role in maintaining of survival and function of the immune system in stress conditions, wherein both prolactin and glucocorticoids are elevated but with opposed functions [30].

However, corticosterone levels in the hyperprolactinemic group were low and different from the N and C groups. These results are consistent with the physiological antagonism of ACTH and corticosteroids by PRL in stress situations [34]. As such the administration of prolactin after the hemorrhage improves the function of the macrophages and splenocytes, and reduces both levels plasma corticosterone and mortality [31]. Prolactin reverses the effects of corticosteroids and increases the Th1 type of cellular responses [13]. Thus, Th1 responses in the asthma model might be anti-inflammatory. This is consistent with the observations in this study. These results are consistent with the findings of other authors who reported that PRL promotes the secretion of Th1 cytokines in vivo and in vitro [6,13,30]. In mice, high levels of PRL

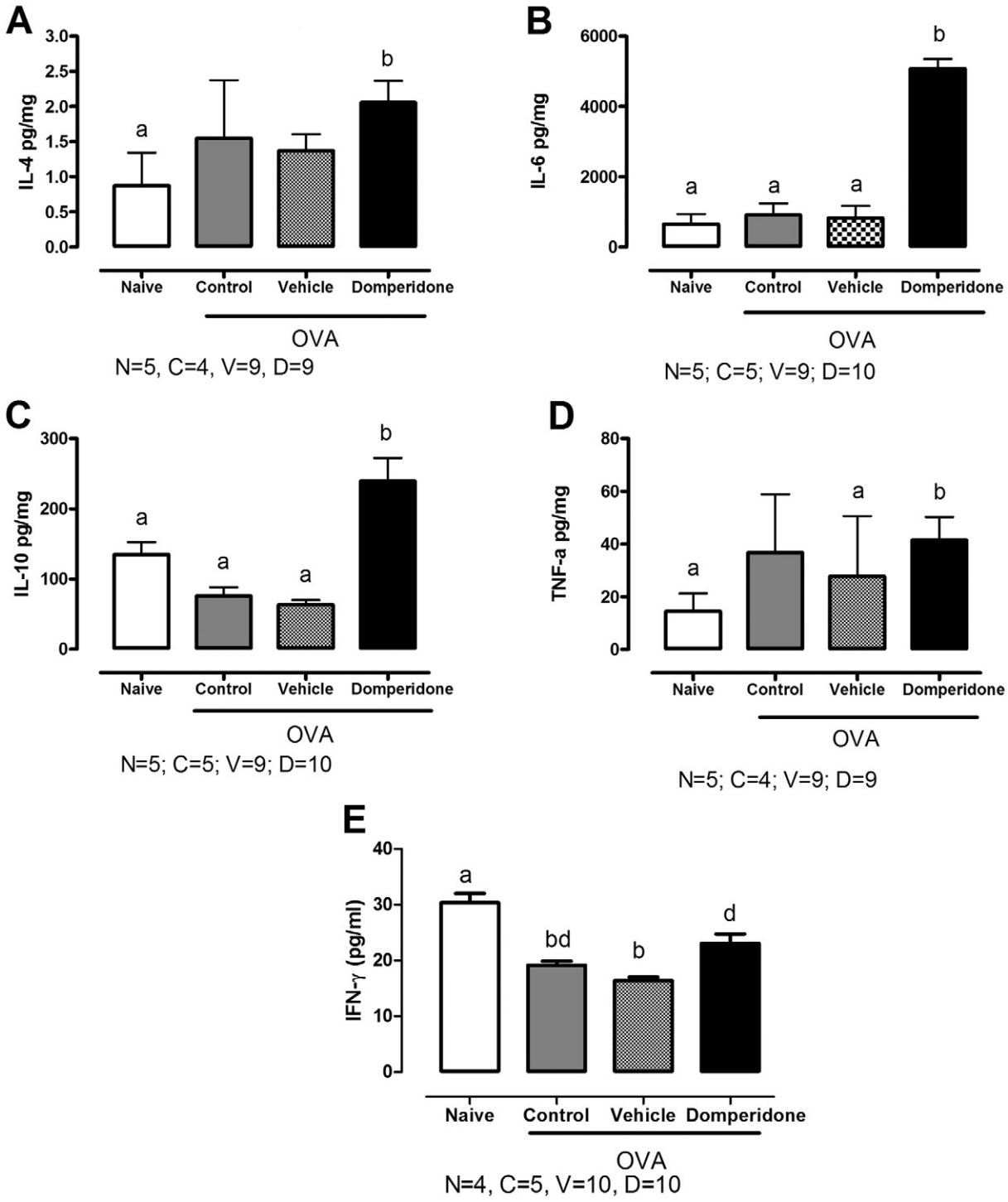

Fig. 7. Domperidone increases IL-6 and IL-10 levels. Effects of domperidone treatment before OVA inhalation on the expression of inflammatory cytokines in allergic rats. IL-4, IL-6, IL-10, and TNFα were measured in supernatants of lung explants and IFNg was measured in BAL. (A) IL-4. (B) IL-6. (C) IL-10. (D) TNF-α. (E) IFNg. N, naive group; C, control group; V, vehicle group; D, domperidone group. Different letters above the columns indicate significant differences. The data are expressed as mean ± SE. IL-4: $p < 0.05$ (Kruskal–Wallis test, followed by Mann–Whitney test). IL-6: $p < 0.01$ (ANOVA and Newman–Keuls multiple-comparison test). IL-10: $p < 0.0001$ (ANOVA and Newman–Keuls multiple-comparison test). TNF-α: $p < 0.05$ (Kruskal-Wallis test, followed by Mann–Whitney test). IFNg: $p < 0.0001$ (Kruskal–Wallis test, followed by Dunn's Multiple Comparison post test).

increase Th1 cell activity and antigen-specific T lymphocyte function [13] Th1 cells secrete IL-2 and IFNg, whose function is to inhibit bronchial asthma [3].

Prolactin is also able to modulate the response of Th1 cells to exogenous IL-2 and the response of B and T cells to competence factors (e.g., mitogens). This modulatory action can be either positive or negative, depending on the concentration of the hormone. However, the stimulatory effect of PRL was decreased at high concentrations (200 ng/ml), and comparable amounts of IL-2 are also known to effectively increase NK cell proliferation [27]. Prolactin is a versatile molecule and this depends on both its structural polymorphism and the wide distribution of its membrane receptors [30].

IL-10 prevents eosinophil deposition and activation, induced by the allergen in animal asthma models. It also regulates the cellular immune response [37]. The D group exhibited an increase in the levels of IL-10, a cytokine that is produced by Th2 cells, B cells, and macrophages. IL-10

acts on antigen-expressing cells by inhibiting their function [4]. Hyperprolactinemia in this group activated a control system by IL-10, thereby limiting pro-inflammatory reactivity [34]. When IL-10 is increased in allergic diseases, it suppresses the activity of mast cells and neutrophils. IL-10 has also been associated with the reduced synthesis of IgE in allergic responses that involve mast cells [19]. High prolactin doses (1000 ng/ml) in murine macrophages induced increase of IL-10 synthesis [16,38] with significant suppression of cytokine production in the same cell line, correlated to pSTAT3 expression [38].

In addition, high levels of pro-inflammatory Th2 cytokines as IL-5, [51] IL6 [52] and a low profile of Th1 cytokines as IFN-g [37] and anti-inflammatory cytokine IL10 [45] have been observed in asthmatic patients. It needs to be recognized that activated vitamin D (calcitriol) modulates the immune response through inhibition or enhancement on multiple levels of cell function, such as production of both pro- and anti-inflammatory cytokines, and inhibition of B cell differentiation, proliferation, and antibody secretion. Activated vitamin D enhances the development of interleukin-IL-10- and reduces the number of IL-6- and IL-17-secreting cells [15].

Vitamin D seems to act similarly to prolactin. Both vitamin D and prolactin increase IL-10. Thus part of the effects reported in this article may be due to vitamin D actions in addition to those of prolactin. Domperidone has limitations as a model for hypeprolactinemia. Since this drug blocks peripheral dopamine receptors and some immune cells have dopamine receptors, this very action may have influenced the response of those immune cells [44,46]. In vitro studies have addressed this issue [9–11]. Yet, future studies will address the question whether prolactin actions described here occur using one or more of the different prolactin signaling pathways. Neuron-specific STAT5 knockout mouse may be a useful tool to address this issue [7].

In the present study, the histochemical results showed purple collagen staining, thus allowing its quantification. Several regulatory cytokines that are produced by T cells and eosinophils have profibrotic effects, such as TGF-β, IL-10, and IL-17. The concentrations of these cytokines may increase in the airways of allergic humans. They promote fibrosis by decreasing the activity of T and B cells [40]. All of the treatment groups (C, V, and D) exhibited higher collagen staining compared with naive animals. TNF-α may also play a more integrated role in airway remodeling and the repair response that occurs as a result of the inflammatory response and is associated with airway epithelial injury through the modulation of epidermal growth factor receptors [41].

Airway epithelial cells also secrete mucus when stimulated with TNF-α [41]. The D group had higher levels of TNF-α than the V group. Therefore, the D group exhibited the highest levels of mucus compared with the N group. These results are consistent with those described previously [29]. These authors reported that the production of TNF-α (measured by the MTT assay/cytotoxicity assay) by neutrophils was markedly increased in mice treated with PRL and in mice that received ectopic pituitary grafts compared with controls. Nonetheless, the D group exhibited a decrease in mucus production compared with the V group.

5. Conclusion

In summary, the present results suggest that an anti-asthmatic effect resulted from hyperprolactinemia induced after ovalbumin treatment. Hyperprolactinemia induced before antigenic challenge decreased allergic lung inflammation. The role played by endogenous PRL as a relevant immunomodulator of asthma appears likely. This study shows a prospective beneficial side effect of domperidone for asthmatic patients.

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