

Neurogenic inflammation in allergen-challenged obese mice: a missing link in the obesity-asthma association?

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ABSTRACT

Aim: A number of studies have shown an association between obesity and asthma. Controversy remains on the mechanisms supporting this association. In this study we aimed to assess neurogenic inflammation in a model of diet-induced obesity and allergen-challenged mice. **Methods:** High fat diet-induced (HFD) obese Balb/c mice were sensitized and challenged with ovalbumin (OVA). Glucose, insulin, OVA-specific IgE and substance P (SP), and the main tachykinin involved in neurogenic inflammation, were quantified in sera. Cell counts were performed in bronchoalveolar lavage fluid (BALF). The extent of peribronchial infiltrates was estimated on lung tissue sections and inflammation was score based on inflammatory cell counts surrounding the bronchi. **Results:** Obesity per se and allergen-sensitization per se increased serum SP ($P = .027$, $P = .004$, respectively). Further increased was observed in obese-sensitized mice ($P = .007$). Obese-sensitized mice also showed higher insulin ($P = .0016$), OVA-specific IgE ($P = .016$), peribronchial inflammatory score ($P = .045$), and tendency for higher glycemia. The interaction of obesity and asthma on SP levels was confirmed ($P = .005$, $R^2 = 0.710$). SP was positively correlated with metabolic (glycemia, $r = 0.539$, $P = .007$) and allergic inflammation parameters (BALF eosinophils, $r = 0.445$, $P = 0.033$; BALF mast cells, $r = 0.574$, $P = .004$; peribronchial inflammation score, $r = 0.661$, $P < .001$; and OVA-specific IgE, $r = 0.714$, $P < .001$). **Conclusions:** Our findings provide support to the neurogenic inflammation link between obesity and asthma in mice. These two conditions independently increased SP and the presence of both pathologies further increased this level. Neurogenic inflammation may be a previously unrecognized mechanism beyond the obese-asthma phenotype. Further studies are need to confirm this role of SP in human obesity-asthma association.

KEYWORDS Asthma, neurogenic inflammation, obesity, specific IgE, substance P

INTRODUCTION

Prospective, cross-sectional and meta-analysis of cohort studies show obesity as a risk factor for asthma [1]. A substantial volume of data from cross-sectional studies indicates that obesity is associated with 2–3 fold increase in risk of a range of self-reported asthma outcomes in adults and 1–2 fold increased

risk in children [2]. Additionally to the increase risk, obese patients also exhibit a distinct asthma phenotype [3]. Actually, increased asthma severity, decreased response to control medication and presence of metabolic/immune imbalances related to the obese state are main features of this obese-asthma phenotype [4]. Therefore, evidence from epidemiological studies points to an association between obesity and asthma which may be partially influenced by common molecular/mechanistic pathways [5].

Low-grade inflammation and mechanical changes in lungs are hypothesized as possible explanations for the obesity-asthma association [6]. There is some evidence that systemic inflammation is increased in obese asthmatics [7], which may contribute to the

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phenotype. Obesity has direct impact on respiratory function due to the mechanical effects of excess abdominal and thoracic fat. Thus, obese patients show a mildly restrictive pattern of lung function associated with reduced vital capacity (FRC) and total lung capacity [2]. As BMI increases, FRC decreases. However, this effect of BMI on lung function is slight and argues against a mechanical hypothesis for the obese-asthma association [8]. As the mechanistic basis of this association remains unclear, research must be redirected towards new hypothesis.

The tachykinin substance P (SP) can be presumed as being an unstudied molecular pathway linking obesity and asthma. SP is an 11 amino acid peptide and a member of the tachykinin family of peptides [9]. Its primary high affinity receptor is a member of the G protein-coupled neurokinin receptor family–NK1-R [9]. SP-NK1-R interactions mediate neurogenic inflammation in the lungs [10] and inflammatory responses in the gut [11]. Within the airways, SP was shown to affect airway smooth muscle cells contraction, induce mucus secretion from submucosal glands, mediate vasodilation and increase vascular permeability [12]. It also acts as potent chemoattractant for neutrophils, monocytes, eosinophils, natural killer cells, and lymphocytes in vitro [13]. In the adipose tissue, SP was shown to affect fat tissue mass through effects on preadipocyte replication and apoptosis [14]. Besides, SP was also suggested as an appetite and weight gain promoter and a relevant player in glucose metabolism [15–17]. Also, antagonism for its preferential receptor–NK1, led to decreased appetite, reduced weight gain and lower levels of insulin and leptin in mice models of diet-induced obesity [15]. Similar results were observed with knockout mice for NK1 [16]. Increased concentrations of SP were also found in sputum [18], serum, and bronchoalveolar lavage of allergic asthmatic patients and increased NK1 receptor mRNA expression was demonstrated within human airways [19]. In a mice model of allergic asthma, increased SP concentrations in the bronchial lumen and increased number of SP-positive pulmonary nerves were found [20]. Moreover, allergen-induced bronchial hyper-responsiveness was blocked in sensitized mice treated with a NK1 receptor antagonist [20]. The release of neuropeptides, as SP neurokinin A and neurokinin B, by peripheral terminals of sensory neurons, and their action on target cells in the periphery producing inflammation is known as neurogenic inflammation [21]. SP is the main tachykinin involved in neurogenic inflammation. Studies aiming to assess neurogenic inflammation in murine models of asthma include studies of capsaicin depletion, inhibition of neuroendopeptidase (which cleaves substance P), antagonism

of tachykinin receptors, and inhibition of neuropeptides release [21]. Animal models of asthma are a tool that allows studies to be conducted in the setting of an intact immune and respiratory system, overcoming some limitations of human studies. Therefore, animal models have been vital in understanding the mechanisms that are involved in the development and progression of asthma, as well as in the study of neurogenic inflammation [22].

We hypothesized that obesity and asthma may have an interaction effect on serum levels of SP. So, to test our hypothesis, we used a model of diet-induced obesity and allergen-challenged mice, and determined serum levels of SP, allergic inflammatory and metabolic parameters.

MATERIAL AND METHODS

Animals and Study Design

Balb/c mice aged 6–8 weeks old were used in this study. The mice were purchased from Charles–River (USA) and kept in a controlled environment (Temperature: 22°C–24°C; Humidity: 55%; Air renovations/hour: 15; and Light cycle (hour): 12/12) and free access to food and water. Mice were divided into the following groups matched by weight ($n = 6$ –8 per group): lean (23.5 ± 1.7 g), obese (23.5 ± 0.6 g), OVA-Lean (23.7 ± 0.8 g), and OVA-Obese (23.3 ± 0.5 g). All in vivo manipulations were handled by trained technicians and in agreement with the Portuguese Act 1005/92 (number 3, iii) and European Community guidelines (86/609/EEC) for the use of experimental animals.

Diet-induced Obesity (DIO)

Mice were fed a high fat diet (HFD) (45 kcal% fat, 4.73 kcal/gm, Research Diets #D12451) for 17 weeks. Control mice received normal diet for the same period. Weight was weekly assessed. Final weight per group was: lean 25.2 ± 0.98 g; obese 30.0 ± 1.3 g; OVA-lean 25.3 ± 0.52 g; and OVA-obese 30.5 ± 1.05 g. The Glucose levels were measured using standard glucose test strips with an Onetouch® Ultra® monitoring system (LifeScan, Inc., USA) prior to sacrifice.

Allergen Sensitization and Challenge

As previously described [23], animals were systemically sensitized by means of intraperitoneal injections of 10 μ g ovalbumin (OVA, #LS003048, Worthington, NJ, USA) adsorbed to 1.5 mg of

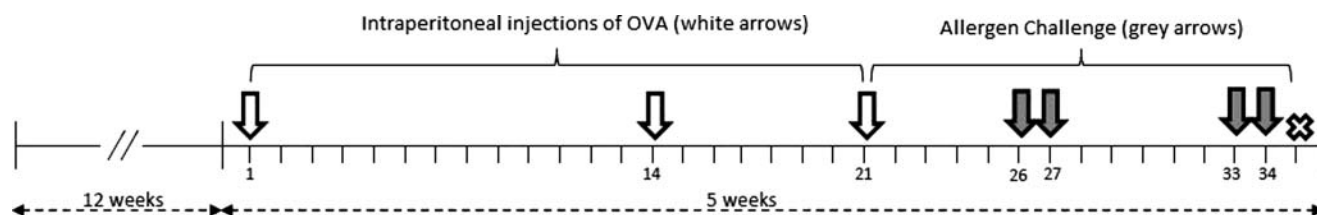


FIGURE 1. Allergen-sensitization and challenge protocol. After 12 weeks mice were sensitized with OVA or PBS intra-peritoneal injections on days 1, 14, and 21 of allergen-sensitization and challenge protocol. On days 26, 27, 33, and 34 of this protocol, mice were challenged with OVA aerosols. On day 34 mice were sacrificed. From the overall experience of 17 weeks as well the allergen-sensitization and challenge protocol lasted for 5 weeks, and diet-induced obesity lasted for the entire protocol. Arrow at the bottom represent the time-table of protocol.

aluminum hydroxide $\text{Al}(\text{OH})_3$ (AlumImject, Pierce, Rockford, IL, USA) as adjuvant on days 1, 14, and 21. Animals received two consecutive local aerosol challenges of 1% OVA (in phosphate-buffered saline, PBS) for a period of 20 min on days 26 and 27 ($2 \times$ challenge). Challenge was repeated on days 33 and 34, prior to mice sacrifice. Nonsensitized animals were treated with intraperitoneal PBS and aerosol OVA challenge. Allergen sensitization and challenge protocol is presented in Figure 1.

Bronchoalveolar Lavage (BAL) and Blood Collection

After 17 weeks, mice were sacrificed with an intraperitoneal anesthetic overdose with sodium pentobarbital 6 g/mL. Mice were tracheotomized and BAL was performed by instillation of $5 \times 200 \mu\text{L}$ of sterile saline solution. BAL fluid (BALF) was immediately processed. Total cell count was performed with a Neubauer chamber, and results expressed as cell per milliliter [24]. For differential cell count, two smear slides were prepared using $40 \mu\text{L}$ of BALF samples, stained with Wright and Giemsa, and 500 cells per slide were counted.

Blood was collected by heart puncture and sera immediately separated and frozen at -80°C for further analysis.

Histopathological Analysis of Pulmonary Inflammation

After BALF and blood collection, the thorax was opened. Lungs were fixed with 10% paraformaldehyde and embedded in paraffin. Sections of $4\text{--}5 \mu\text{m}$ from all lobes were stained with hematoxylin and eosin. The extent of peribronchial infiltrates was estimated by an inflammation score: slides were coded and, blinded to the investigator, the peribronchial inflammation was graded using a reproducible scoring

system previously described [25]. Briefly, a 0–3 score was used: 0 when no inflammation was detectable, 1 for occasional cuffing with inflammatory cells, 2 when most bronchi were surrounded by a thin layer (1–5 cells) of inflammatory cells, and 3 when most bronchi were surrounded by a thick layer (more than 5 cells) of inflammatory cells. Five randomly selected tissue sections per mouse were scored and inflammation scores were expressed as a mean value per animal.

Serum OVA-Specific IgE Measurement

Serum OVA-specific IgE was measured using a commercial available ELISA kit (#3010, Chondrex Inc., Redmond, USA). Accordingly to manufacturer's instructions, an anti-IgE monoclonal antibody was used to capture IgE in serum samples followed by biotinylated-OVA to determine OVA-specific IgE captured by anti-IgE antibody coated on plate surfaces. The capture antibody used (rat anti-mouse IgE antibody, Clone 77-1) does not cross-react with any mouse immunoglobulin subclasses (IgG1, IgG2a, IgG2b, IgG3, and IgM). The kit contained a mixture of various types of OVA-specific antibodies that was able to accurately measure OVA-specific IgE levels in samples with less than 500 ng/mL IgE.

Serum Insulin Measurements

Serum insulin levels were measured by a Milliplex[®] map kit (#MADPK-71K, Millipore Corporation, USA) based on the Luminex[®] xMAP technology and accordingly to the manufacturer instructions. Briefly, Luminex[®] uses proprietary techniques to internally color-code microspheres with two fluorescent dyes. Through precise concentrations of these dyes, 100 distinctly colored bead sets can be created, each of which is coated with a specific capture antibody. After an analyte from a test sample is captured by the bead, a biotinylated

detection antibody is introduced. After incubation with streptavidin-PE (reporter molecule) conjugate to complete the reaction on the surface of each microsphere, the microspheres are allowed to pass rapidly through a laser which excites the internal dyes marking the microsphere set. A second laser excites PE, the fluorescent dye on the reporter molecule. Finally, high-speed digital signal processors identify each individual microsphere and quantify the result of its bioassay based on fluorescent reporter signals. The minimum detectable concentration for insulin was 24.9 pg/mL. The intra- and interassay variations were <4.5% and <10.3%, respectively.

Serum SP Measurement

SP was measured in serum by a competitive enzyme-immunoassay kit suitable for mice samples (#583751, Cayman Chemical Company, USA) accordingly to the manufacturer instructions. The assay was based on the competition between SP and a SP acetylcholinesterase conjugate (SPACHe tracer) for a limited number of substance-P specific rabbit antiserum binding sites. Concentration of SP was inversely proportional to the amount of SP tracer conjugated to the rabbit antiserum. Ellman's Reagent was used to wash the plate and remove any unbound reagents. A distinct yellow color identifies the product of this enzymatic reaction that absorbed strongly at 412 nm. The intensity of this color, determined spectrophotometrically was proportional to the amount of SP tracer bounded to the well. This assay provides accurate measurements of SP with a working range of 3.9 to 500 pg/mL, typically with an IC_{50} (50% B/B₀) of 32 pg/mL, and IC_{80} (80% B/B₀) of 8.2 pg/mL.

Statistical Analysis

Data are presented as mean $\pm$ SEM, unless otherwise stated. All tests were performed using Graphpad Prism version 5.00 (Graphpad Software, San Diego, CA, USA) and PASW Statistics 18.0 (SPSS Inc., Chicago, Illinois, USA). Student's *t*-test was used to analyze weight gain, peribronchial inflammation scores, serum OVA-specific IgE, serum SP, and glycemia and serum insulin. Pearson correlation was used to perform the correlation study of these parameters. Due to the skewed distribution, cell counts in BALF comparisons were made after logarithmic transformation, and one-way ANOVA followed by Tukey's test were used. Linear regression approach to ANCOVA was used to evaluate the interaction between obesity and asthma on serum SP levels. A *P* value <.05 was considered significant.

RESULTS

Effect of Obesity and Allergen Sensitization on Glycemia and Insulinemia

We first examined whether HFD and allergen-sensitization affected metabolism by evaluating mice body weight, blood glucose and insulin in sera. As expected, mice fed a HFD gained more weight than those fed a normal diet (Figure 2A: lean 8.98% $\pm$ 2.70% and obese 27.89% $\pm$ 3.16%, *P* = .007; OVA-lean 7.09% $\pm$ 0.96% and OVA-obese 28.59% $\pm$ 1.5%, *P* < .0001). The cumulative energy intake was also increased in mice fed a HFD (lean 3049 $\pm$ 69.3 kcal and obese 5305 $\pm$ 78.8 kcal, *P* = .0043; OVA-lean 3086 $\pm$ 74.4 and OVA-obese 4978 $\pm$ 40.9 kcal, *P* = .0003).

No significant differences in percentage of body weight gain were observed between sensitized and nonsensitized obese mice (Figure 2A: obese 27.89% $\pm$ 3.16% and OVA-obese 28.59% $\pm$ 1.5%) and between sensitized and nonsensitized lean mice (Figure 2A: lean 8.98% $\pm$ 2.70% and OVA-lean 7.09% $\pm$ 0.96%). Absolute weight gain followed the same tendency (weight gain: lean 2.0 $\pm$ 0.58 g and obese 6.50 $\pm$ 0.67 g, *P* = .0045; OVA-lean 1.67 $\pm$ 0.21 g and OVA-obese 7.17 $\pm$ 0.48 g, *P* = .0003), as well as cumulative energy intake (lean 3049 $\pm$ 69.3 kcal and OVA-lean 3086 $\pm$ 74.4; obese 5305 $\pm$ 78.8 kcal, and OVA-obese 4978 $\pm$ 40.9 kcal). This reflects that allergen sensitization per se does not change the total percentage of body weight nor the absolute weight gain.

OVA-obese mice showed a tendency for higher levels of glycemia (Figure 2B: OVA-obese 140.8 $\pm$ 8.9 mg/dL and OVA-lean 123.2 $\pm$ 3.5 mg/dL) and significant higher levels of insulinemia than OVA-lean (Figure 2 C: OVA-obese 1.21 $\pm$ 0.25 ng/mL and OVA-lean 0.51 $\pm$ 0.07 ng/mL, *P* = .0016). These differences were also found between nonsensitized obese and lean mice (Figure 2B: glycemia, obese 133.5 $\pm$ 4.9 mg/dL and lean 115.8 $\pm$ 2.4 mg/dL, *P* = .004; Figure 2C: insulinemia, obese 1.69 $\pm$ 0.10 ng/mL; and lean 0.78 $\pm$ 0.05 ng/mL, *P* < .001). However, no differences were found between OVA-obese and nonsensitized obese mice regarding these serum parameters. This may indicate that, while obesity has a direct effect on glycemia and insulinemia, sensitization per se does not affect these parameters.

Effect of Obesity on BALF Cellularity, Lung Histology, Airway Inflammation and Allergic Sensitization

We next determined if obesity affected lung inflammation. In contrast to nonsensitized mice where

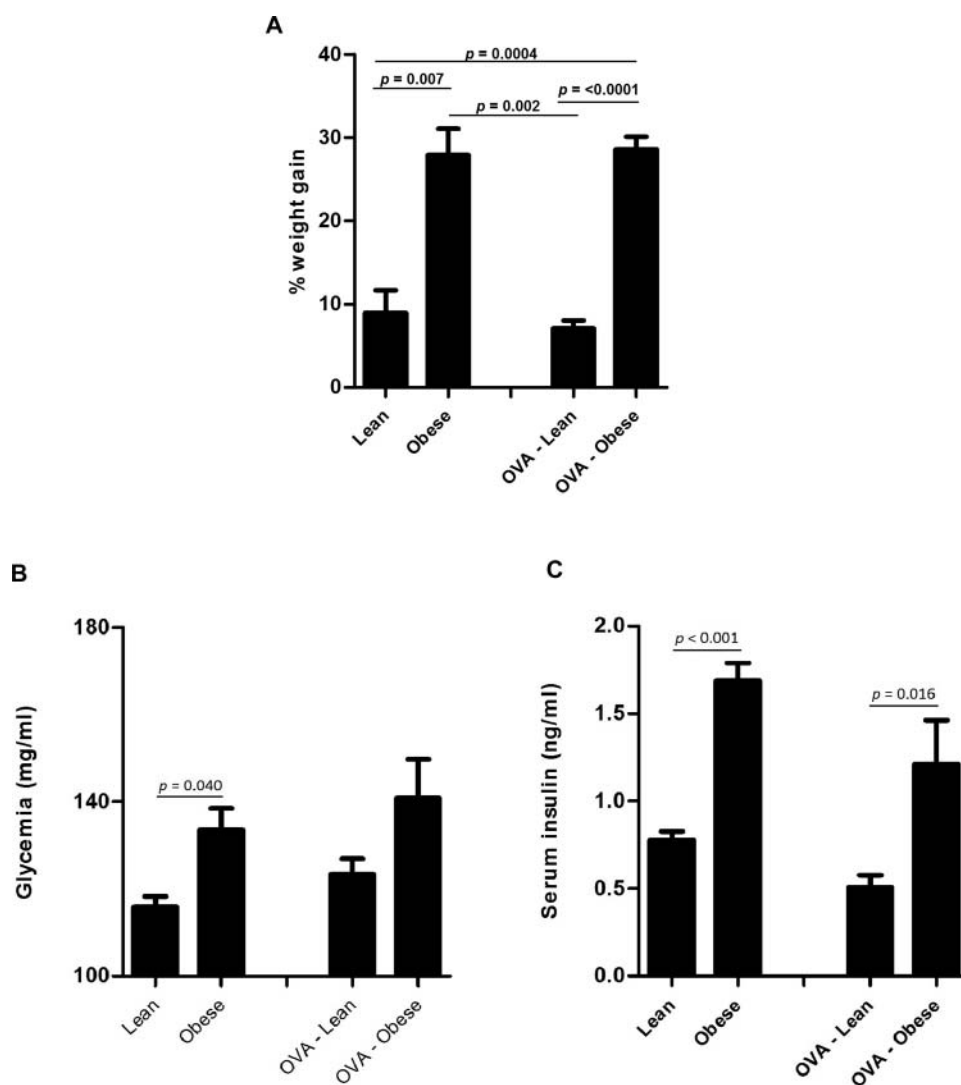


FIGURE 2. Effect of allergen sensitization on body weight, glycemia, and insulinemia. A significant increase in weight gain was found in obese mice independently of allergen sensitization (A). OVA-obese mice showed a tendency for higher levels of glycemia (A) and significant higher levels of insulinemia than OVA-lean (C). These differences were also found between non-sensitized obese and lean mice (B and C). OVA=ovalbumin sensitization.

only a few macrophages were observed in the bronchoalveolar lavage fluid (BALF), sensitization with OVA showed a significant eosinophilia, and lymphocytosis in BALF (Figure 3A). This was also demonstrated by differential cell counts in BALF (Table 1). In fact, OVA-obese mice showed higher eosinophil and lymphocyte counts in BALF than obese mice (eosinophils: OVA-obese $4.293 \pm 2.006 \times 10^5/\text{mL}$ and obese zero; lymphocytes: OVA-obese $2.215 \pm 0.916 \times 10^5/\text{mL}$, and obese 0.012 ± 0.004 , $P = .001$). OVA-lean also showed higher eosinophil and lymphocyte counts in BALF than lean mice (eosinophils: OVA-lean $3.530 \pm 1.007 \times 10^5/\text{mL}$ and lean zero; lymphocytes: OVA-lean $1.976 \pm 0.451 \times$

$10^5/\text{mL}$; and obese 0.014 ± 0.004 , $P = .001$). However, when OVA-obese and OVA-lean mice were compared for total cell counts and eosinophils in BALF no significant differences were found (Table 1).

Histological evidence of inflammatory cells was found surrounding bronchia of sensitized mice (Figure 3B). In addition, these peribronchial inflammatory infiltrates seemed more prominent in OVA-obese than in OVA-lean mice (Figure 3B). This histological evidence was confirmed by counting inflammatory cells surrounding bronchia and calculating a peribronchial inflammatory score. Interestingly, OVA-obese mice showed higher peribronchial inflammatory score (Figure 3C: OVA-obese $2.33 \pm$

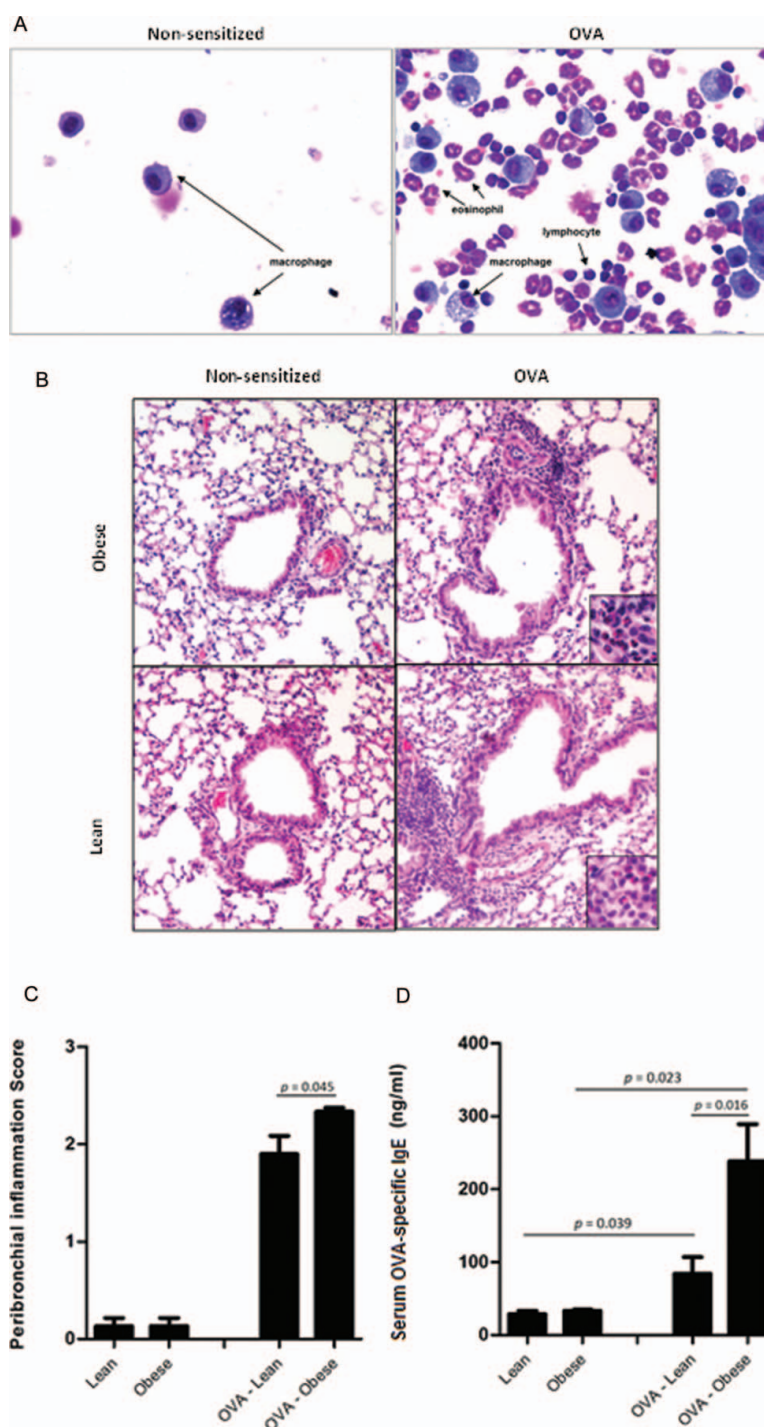


FIGURE 3. Effect of allergen sensitization and obesity on BALF cellularity, histology, peribronchial inflammatory score, and serum OVA-specific IgE. In contrast to nonsensitized mice, where only a few macrophages were observed in BALF, sensitization with OVA showed a significant eosinophilia and lymphocytosis in BALF (A, Wright and Giemsa, 40 $\times$). Histological evidence of inflammatory cells was found surrounding bronchia of sensitized mice and these peribronchial inflammatory infiltrates seemed more prominent in OVA-obese than in OVA-lean mice (B, Hematoxylin and Eosin, 20 $\times$ -magnifiers on right inferior side, 100 $\times$). This histological evidence was confirmed by a higher peribronchial inflammatory score in OVA-obese than in OVA-lean mice (C). The effect of obesity on allergic inflammation was demonstrated by higher serum anti-OVA IgE levels in OVA-obese than in OVA-lean mice (D).

TABLE 1 Differential Cell Counts in BALF Collected After Mice Sacrifice.

Cellularity in BALF	Animals			
	Obese	Lean	OVA-Obese	OVA-Lean
PMN ($10^5/\text{mL}$)	0.005 ± 0.038	0.010 ± 0.007	–	0.001 ± 0.001^a
Eosinophils ($10^5/\text{mL}$)	–	–	4.293 ± 2.006	3.530 ± 1.007
Lymphocytes ($10^5/\text{mL}$)	0.012 ± 0.004	0.014 ± 0.004	$2.215 \pm 0.916^{a,b}$	$1.976 \pm 0.451^{a,b}$
Monocytes ($10^5/\text{mL}$)	0.683 ± 0.150	1.484 ± 0.449	1.564 ± 0.428	0.968 ± 0.204
Macrophages ($10^5/\text{mL}$)	0.004 ± 0.001	0.011 ± 0.039	0.026 ± 0.007^b	0.040 ± 0.016^b
Mast cells ($10^5/\text{mL}$)	–	–	0.002 ± 0.002	–
Total cells ($10^5/\text{mL}$)	0.700 ± 0.1528	1.520 ± 0.450	$8.100 \pm 3.282^{a,b}$	$6.567 \pm 1.463^{a,b}$

PMN, polymorphonuclear neutrophils. Values are expressed as mean $\pm$ SEM. *P* values were calculated by Mann–Whitney *U* test.

^aSignificantly different from Lean mice ($P < .05$); ^bSignificantly different from Obese mice ($P < .05$)

0.04 and OVA-lean 1.9 ± 0.18 , $P = .045$). The effect of obesity on allergic inflammation was paralleled by higher serum anti-OVA IgE levels in OVA-obese than in OVA-lean (Figure 3D: OVA-obese 238.2 ± 50.9 ng/mL and OVA-lean 83.9 ± 22.8 ng/mL, $P = .016$) indicating that obesity potentiated the allergic inflammation.

Effect of Obesity and Allergen Sensitization on Serum SP

We next studied whether obesity and allergic inflammation affected serum levels of SP.

As expected, OVA sensitization led to an increasing in serum SP levels in lean mice (Figure 4:

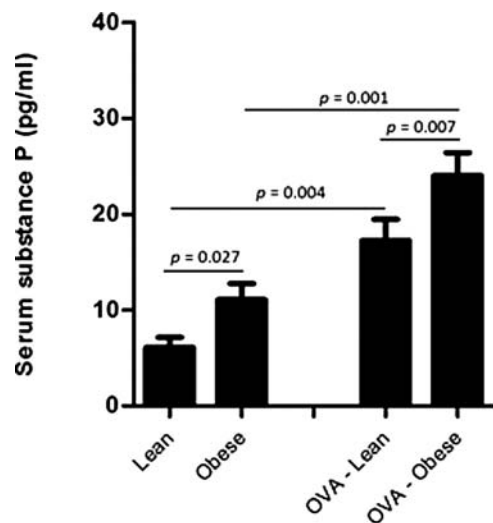


FIGURE 4. Effect of obesity and allergen sensitization on serum levels of SP. OVA sensitization led to an increasing in serum SP levels in lean mice. Obesity per se led to an increasing in serum levels of SP. Also, obesity and allergic sensitization together led to a further increased in serum SP, OVA–ovalbumin sensitization.

OVA-lean 17.23 ± 2.21 ng/mL and lean 6.102 ± 1.06 ng/mL, $P = .004$). In addition, obesity per se led to an increasing in serum levels of SP. In fact, these levels were significantly increased in obese relatively to lean mice (Figure 4: obese 11.07 ± 1.70 ng/mL and lean 6.102 ± 1.06 ng/mL, $P = .027$) implying a role of this neuropeptide in adipose metabolism.

Most interestingly, obesity and allergic sensitization together led to a further increased in serum SP (Figure 4: OVA-obese 24.02 ± 2.41 ng/mL and obese 11.07 ± 1.70 ng/mL, $P = .001$; OVA-obese 24.02 ± 2.41 ng/mL; and OVA-lean 17.23 ± 2.21 ng/mL, $P = .007$). In fact, the presence of an interaction between obesity and asthma was confirmed for SP serum levels ($P = .005$, $R^2 = 0.710$), revealing that the joint-effect of obesity and asthma in SP levels is higher than the sum of both effects. Together, these results may suggest a role of this neuropeptide in obese-asthma condition.

Correlation of SP, Metabolic Parameters and Allergic Inflammation

We next studied the correlation between SP and the metabolic and allergic inflammation parameters evaluated. We found positive correlations between serum SP and serum glycemia ($r = 0.539$, $P = .007$), BALF eosinophils ($r = 0.445$, $P = .033$), BALF mast cells ($r = 0.574$, $P = .004$), peribronchial inflammation score ($r = 0.661$, $P < .001$), and OVA-specific IgE ($r = 0.714$, $P < .001$).

DISCUSSION

This study demonstrates an interaction effect of obesity and allergic asthma on serum levels of SP in a mice model of diet-induced obesity and allergic asthma. Increased levels of SP in obese nonsensitized and in lean-sensitized mice were observed. Also, OVA

sensitization and challenge of obese mice led to a significant increase in serum SP levels. These results suggest a role of this neuropeptide in both lung inflammation and obesity, and may reflect a neurogenic inflammatory response in the obese-asthma phenotype in mice.

In our study, it is possible that presence of both obesity and allergen sensitization may have provided the inflammatory milieu that interacted with neuropeptides release from sensory nerves. In fact, inflammatory mediators present in obesity [26] and in allergic asthma [27] may have led directly to an increase release of SP, or a decrease of SP degradation by the enzyme neuro-endopeptidase. In both the lung and the adipose tissue, binding of SP to NK1-R activates NF- κ B pathway that increases IL-8 and expression of genes that are classically upregulated during inflammatory responses [14, 28]. This could be hypothesized as the molecular mechanism by which SP mediates neurogenic inflammation in the obese-asthma association. Additionally, our study showed that SP is positively correlated with metabolic and allergic inflammation parameters, namely glycemia, BALF eosinophils, BALF mast cells, peribronchial inflammation score, and OVA-specific IgE. Again, these results may indicate that rising of serum SP may be pathophysiological related to specific features of obesity and asthma.

We were also able to show that allergic sensitization did not affect body weight or blood glucose and insulin, revealing that airway inflammation did not interfere with metabolism. Inversely, obesity led to an enhancement of the inflammatory infiltration in the airways. After sensitization, a strong eosinophilia, lymphocytic, and macrophage infiltrate was observed independently of the obesity status. Nevertheless, increased peribronchial inflammatory score was found in obese-sensitized as compared to lean-sensitized mice. This may indicate that diet-induced obesity greater enhance lung inflammation and might be associated with increased airway responsiveness [29].

As proof of active immunization, OVA-specific serum IgE levels were elevated in sensitized mice exposed to OVA compared with those exposed to PBS. Also, higher OVA specific-IgE was observed in OVA-obese mice compared to OVA-lean mice. The OVA-induced increase in IgE is consistent with other reports in mice that indicate that allergen challenge is an important stimulus for IgE production [30, 31]. In obese mice, enhanced airway responsiveness to methacholine and augmented IgE production and/or release following allergen sensitization and challenge has been demonstrated [30, 31]. This could be explained as a result of the impact of obesity on IgE

synthesis, probably by specific effects on B cell function [30].

Obesity probably contributes to asthma through separate mechanisms that are specific of the obese state rather than through conventional Th2-mediated inflammatory pathways [4, 32]. In our study, no difference in BALF cell count from obese-sensitized and lean-sensitized mice was found. This is in agreement with the fact that obesity is dissociated to increased airway eosinophilic inflammation and contributing to a distinct asthma phenotype [3–5, 32, 33]. As this unique obese-asthma phenotype is characterized by increased asthma severity and presence of comorbidities related to the obese state [3], identification of molecular mechanisms involved in its pathophysiology are demanded.

We are aware that neurogenic inflammation is well recognized in murine models and still controversial in humans. However, this controversy mainly arrived from the clinical heterogeneous results obtained in studies evaluating the effect of tachykinin receptors antagonists on human asthma [34]. We are also aware that neurokinin A, acting through its selective NK2-receptor also induces bronchoconstriction. However, SP is the main neuropeptide involved in neurogenic inflammation as it induces bronchoconstriction, increases airway responsiveness, induces mucus secretion, and increases proinflammatory cytokines [12]. Also, we are aware that our OVA-sensitized and challenged model in obese mice, as other murine models, may not reflect all the pathophysiological features of obese-asthma in humans.

Further data from molecular techniques is required to support our findings and to explore inflammatory pathways involved in both obesity and allergic asthma and leading to an increase release of SP. However, the current study highlights a previously unrecognized clue for the mechanistic basis of obese-asthma association.

In conclusion, the present study shows that SP is increased in a mice model of obesity and allergic asthma and that a neurogenic inflammation may be implicated in obesity-asthma association. Further studies elucidating the involvement of SP in humans and its molecular mechanisms are mandatory for the development of therapeutic strategies against this emergent obese-asthma pathological condition.

Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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