

Inflammation and Remodeling in Infantile, Juvenile, and Adult Allergic Sensitized Mice

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Summary. Background: Airway structural changes occur early in childhood asthma, but it is unknown whether the development of airway alterations in children is similar to that of adults. We compared inflammation and remodeling parameters in allergic sensitized infantile, juvenile, and adult mice. Methods: Infantile mice (18D) were sensitized with three intraperitoneal injections (i.p.) of ovalbumin (OVA) at days 5 and 7 and challenged with OVA at days 14–16. The 18D1 group received an additional challenge at days 9–11. The juvenile mice (40D) received challenges at days 22–24 and 36–38. Adult mice (100D) were sensitized at days 60–62 and received three inhalations at days 77–79 and 96–98. Animals were submitted to whole body plethysmography. Airway eosinophils, CD3+ T-lymphocytes, IL-5+ cells, mucus content, collagen and reticular fibers density, and smooth muscle thickness were quantified. Results: All sensitized animals presented with airway hyperresponsiveness, without differences in eosinophil cell density. The density of CD3+ T-cells was higher in the 100D and 18D1 groups than in the 18D and 40D groups. Infantile sensitized groups demonstrated increased interleukin-5 expression in the airways. Infantile mice demonstrated more mucus in the bronchiolar epithelium than the 40D and 100D mice. The 18D animals demonstrated less collagen than the 18D1 group. Juvenile and adult mice had increased airway smooth muscle thickness when compared to age-matched controls, but no differences were observed in the infantile groups. Conclusion: We have shown that infantile mice develop inflammatory and structural alterations in the airways that are partially different from those developed in older animals. **Pediatr Pulmonol.** © 2011 Wiley-Liss, Inc.

Key words: asthma; mice BALB/c; pulmonary inflammation; airway remodeling.

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INTRODUCTION

Asthma is a chronic inflammatory disorder of the lungs that involves complex interactions between mast cells, eosinophils, T-lymphocytes, epithelial cells, and cellular mediators. The inflammation and structural changes in the airways that occur in asthma are believed to contribute to the development of airway obstruction and increased bronchial hyperresponsiveness to a variety of stimuli.^{1,2}

The prevalence of childhood asthma is high worldwide. Data from the International Study of Asthma and Allergies in Childhood (ISAAC) phase three study indicate that the worldwide prevalence of current wheeze is 11.5% in 6- to 7-year-old children and 14.1% in adolescents, displaying wide geographical variations. The prevalence of severe asthma is 4.9% and 6.9% in younger children and adolescents, respectively.³

Phenotypically, pediatric and adult asthma seem to differ. In adult-onset asthma, symptoms tend to be persistent and more severe, and many patients are not atopic. In children, asthma has a more episodic character and is

usually related to atopy.⁴ Clinical symptoms frequently remit during adolescence. However, it has been shown that

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childhood asthma may relapse in later adulthood, and the more severe the symptoms are in childhood, the greater the chances of relapse.⁵ In addition, even in asthmatic adolescents without symptoms, there are signs of persistent inflammation and lung function abnormalities such as hyperinflation and increased residual volume.^{6–8}

These findings are in accordance with pathological findings in biopsies of asthmatic children that show early findings of structural abnormalities, such as the thickening of the reticular basement membrane (RBM) and increased airway smooth muscle (ASM) mass.^{9,10} These alterations seem to be absent in wheezing infants¹¹ but may be present already in children at the age of 3.¹² In children with severe asthma, the amount of ASM mass, but not the RBM thickening, correlates with signs of bronchial obstruction.¹⁰

Recently, mouse models of infantile/juvenile allergic sensitization have been described.^{13–16} These studies using sensitized young mice were able to show features of airway allergic inflammation similar to the ones observed in adult mice. In addition, some authors demonstrated that the young animals develop signs of airway remodeling in parallel with inflammation.¹³

We hypothesized that inflammation and structural alterations would be different in adult sensitized mice when compared to mice sensitized at younger ages. Such differences could be related to the differences of the immune responses between young and adult animals and to the phenotypic differences observed in pediatric versus adult asthma. Few studies have compared the development and

magnitude of airway allergic inflammation and remodeling in mice of different ages.^{14,15}

Therefore, our aim in this study was to compare the features of airway hyperresponsiveness (AHR), allergic inflammation, and remodeling in allergen-sensitized animals of different ages: infantile, juvenile, and adult mice.

MATERIALS AND METHODS

This study was approved by the institutional review board.

Animals

All animals received humane care in compliance with the “Principles of Laboratory Animal Care” published by the National Institutes of Health (NIH publication 86-23, revised 1985). Animals were fed ad libitum with water and commercial pelleted food for small rodents from Nuvital (Nuvilab CR-1, Colombo, PR, Brazil).

Sensitization and Challenge Protocols

Infantile and Juvenile Mice

Female BALB/c mice and their 3-day-old pups were obtained from the animal facility core of the Sao Paulo University Medical School. Each mother and her litter were housed separately. At 5 days of age, pups were immunized intraperitoneally (i.p.) with 10 µg of ovalbumin (OVA) (Advanced Nutrition, Rio de Janeiro, Brazil) in 1 mg of alum (Al(OH)₃) in 0.1 ml saline (0.9% NaCl), and they received an OVA boost at day 7. Animals were then divided into three groups: one group was challenged with aerosolized 3% OVA solution using an ultrasonic nebulizer (Pulmoclear II Luxo—Soniclear, São Paulo, Brazil) for 10 min a day for 3 consecutive days from day 14 to 16. These animals were euthanized on day 18. The second group received challenges on days 9–11 and days 14–16 and was euthanized on day 18. A third group received challenges on days 21–23 and days 36–38 and was euthanized on day 40.

Adult Mice

Sixty-day-old BALB/c mice (day 60) were immunized i.p. with 10 µg OVA in 1 mg of alum in 0.1 ml saline, and they received a boost on day 62. Animals were then challenged with aerosolized 3% OVA solution using an ultrasonic nebulizer for 10 min a day for 3 consecutive days from days 76 to 78 and days 96 to 98. These animals were euthanized on day 100.

The control groups received i.p. immunization with alum alone and challenges with vehicle (saline) under the same protocols for infantile, juvenile, and adult mice. The number of animals in each group was six to eight. The study design is depicted in Figure 1.

ABBREVIATIONS:

ASM	Airway smooth muscle
Al(OH) ₃	Alum
BAS	Baseline
Br	Bronchiole
DAB	Diaminobenzidine
ED50	Effective dose
H&E	Hematoxylin and eosin
IgE	Immunoglobulin E
IgG1	Immunoglobulin G1
IL-5	Interleukin 5
IQR	Interquartile range
Th2	T helper 2
i.p.	Intraperitoneal injections
ISAAC	International Study of Asthma and Allergies in Childhood
i.v.	Injected intravenously
Mch	Methacholine
OVA	Ovalbumin
PAS/AB	PAS/Alcian blue
PBS	Phosphate-buffered saline
PCA	Passive cutaneous anaphylaxis
<i>P_{enh}</i>	Enhanced pause
RBM	Reticular basement membrane
SAL	Saline
V	Vessel

Measurement of Airway Hyperresponsiveness

Pulmonary responsiveness of mice to increasing concentrations of aerosolized methacholine (Mch) was measured through a whole-body plethysmography system (BUXCO, Winchester, UK). Briefly, each mouse was placed in a chamber, and the box pressure-time wave was continuously measured via a transducer connected to a computer data-acquisition system. The main indicator of air bronchoconstriction, enhanced pause (P_{enh}), which shows a correlation with airway resistance measured according to standard evaluation methods in BALB/c mice,¹⁷ was calculated from the box pressure-time wave form.¹⁸ After measurement of the baseline P_{enh} , either aerosolized saline or Mch in increasing concentrations (6, 12, 25, 50 mg/ml) was nebulized through an inlet of the chamber for 3 min. P_{enh} values for each dose were collected for 5 min and averaged. Results were expressed as P_{enh} values, $P_{\text{enh Max}}$ values and effective dose (ED50).

Passive Cutaneous Anaphylaxis (PCA) to IgE and OVA-Specific IgG₁

All mice were bled by ophthalmic plexus with a graduated heparinized Pasteur pipette 1 day before euthanize. The blood, 0.2 ml, was immediately diluted in 0.4 ml of saline (1:5 plasma dilution). After centrifugation the plasma samples were kept at -20°C until to use for PCA reaction.

Plasma levels of anti-OVA IgE and anaphylactic IgG₁ were measured by PCA as previously described.^{19,20} Briefly, the backs of naive rats were shaved, and several 0.1 ml intradermal injections of plasma samples dilutions

submitted to the different protocols of immunization were made. After a latent period of 24 hr for IgE, these animals were injected intravenously (i.v.) with 1 ml of a 0.5% solution of Evans blue in saline (0.9% NaCl) containing 1 mg of antigen. Thirty minutes after antigen injection, the animals were euthanized, and the diameters of the blue spots on the inner surface of the flayed skin were measured.

To detect IgG₁ antibody, the anti-sera were heated for 1 hr at 56°C to destroy the PCA activity due to IgE. Briefly, the backs of the naive mice were shaved and several 0.05 ml intradermal injections of plasma samples dilutions submitted to the different protocols of immunization were made. After a latent period of 2 hr, the animals were injected i.v. with 0.5 ml of a 0.5% solution of Evans blue in saline (0.9% NaCl) containing 1 mg of antigen. Thirty minutes after antigen injection, the animals were euthanized, and the diameters of the blue spots on the inner surface of the flayed skin were measured.

The PCA titer was expressed as the reciprocal of the highest dilution that produced a lesion of more than 5 mm in diameter in duplicate tests. The detection threshold was established at 1:5 dilutions to avoid positive reaction due to non-specific degranulation of mast cells.

Tissue Preparation

Animals were euthanized with sodium pentobarbital (50 mg/kg body weight, i.p.). Lungs were fixed by intratracheal instillation of formalin (4% formaldehyde in 2% buffer) for 24 hr and were then paraffin embedded. Transversally cut lung tissue specimens were cut into 5- μm thick sections and prepared for immunohistochemical and histochemical analysis.

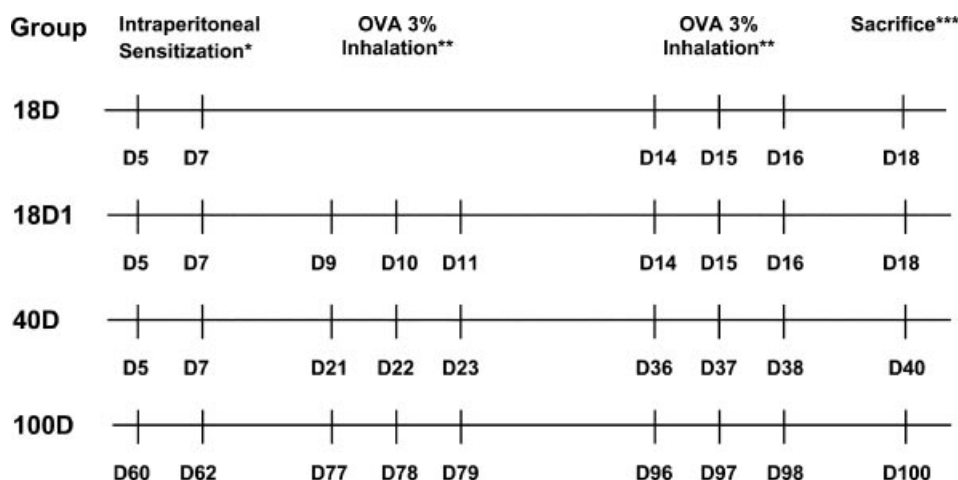


Fig 1. Study design. *OVA 10 μg in 1 mg of alum in saline 0.1 ml (0.9% NaCl). **Challenge 3% OVA or saline for 10 min/day, on 3 consecutive days in some experiments. ***Sacrifice 48 hr after the last OVA challenge. D = Day of life.

Histochemistry

Sections were stained with hematoxylin and eosin (H&E), PAS-Alcian blue, Masson's trichrome, Sirius red (collagen), Resorcin-Fuchsin with oxidation (elastic/reticular fibers) and Luna staining (eosinophils).²¹

Immunohistochemistry

Immunohistochemistry was performed using the polyclonal rat antibody to CD3 (1:300, Serotec, Inc., Raleigh, NC) and using the polyclonal rabbit antibodies to interleukin 5 (IL-5) (1:800, Santa Cruz Biotechnology, Santa Cruz, CA). Sections were deparaffinized, and a 0.3% hydrogen peroxide solution was applied for 35 min to inhibit endogenous peroxidase activity. Antigen retrieval was performed with citrate solution for 45 min. Sections were incubated with the primary antibody overnight at 4°C. The biotin-streptavidin peroxidase method (VECTASTAIN, Irvine, CA) was used as the secondary antibody and 3,3-diaminobenzidine (DAB) (Sigma Chemical Co, St. Louis, MO) was used as the chromogen. The sections were counterstained with Harris hematoxylin. For negative controls, the primary antibody was replaced with phosphate-buffered saline (PBS).

Morphometry

Bronchovascular inflammation was semiquantitatively analyzed using H&E stained slides using a score system in 1–3, as previously described:²² 1+ Occasional areas cuffed by inflammatory cells. 2+ Most areas surrounded by a thin layer (1–5 cells thick) of inflammatory cells. 3+ Most areas surrounded by a thick layer (>5 cells thick) of inflammatory cells.

We further quantified the bronchial density of eosinophils and CD3+ T-cells in the airways using a point-counting technique. Using a 168-point grid with a known area (11,449 μm^2 at a 1,000 $\times$ magnification) attached to the microscope ocular; we counted the number of points hitting airway wall. Counting was performed in 25 fields of airway walls (mean of 5 airways) of each animal, and results were expressed as cells/ μm^2 . The ASM thickness and epithelial mucus content were measured by counting the number of points that hit on smooth muscle and on mucus (Masson staining and PAS staining, respectively) divided by the number of intercepts that hit on the basement membrane (number of points/intercept).²³

Airway collagen content, elastic/reticular fibers and the expression of IL-5+ cells were obtained by image analysis using the software Image-Pro[®] Plus 4.5 for Windows[®] (Media Cybernetics, Silver Spring, MD) on a compatible microcomputer connected to a digital camera and coupled to a light microscope (Leica DMR, Leica Microsystems GmbH, Wetzlar, Germany). We analyzed five airways per

animal at a magnification of 400 $\times$. The area of positive staining in the peribronchiolar region was determined by color threshold. Results were expressed as percentages [positive area (μm^2) divided by total airway area (μm^2)]. We only analyzed medium to large sized airways cut in cross sections.²⁴

Statistical Analysis

Parametric and non-parametrical data are expressed as means $\pm$ SD or median/interquartile range (IQR), respectively. The statistical significance between groups was assessed by a *t*-test or Mann–Whitney *U*-test. For comparisons in more than two groups, one-way analysis of variance (parametric data) or the Kruskal–Wallis (non-parametric data) test followed by the Bonferroni post hoc test were used; significance level was adjusted to 5% ($P < 0.05$). The Statistical Package for the Social Sciences (SPSS) version 15.0 for Windows was used for the analysis.

RESULTS

OVA-treated groups showed signs of allergic inflammation with monocytes and eosinophils and increased mucus content in the airways (Fig. 2).

Airway Responsiveness

OVA exposure induced an increase in lung responsiveness, causing significant changes in $P_{\text{enh Max}}$ in the infantile group with one or two challenges ($P = 0.023$) and in juvenile mice ($P = 0.046$). The analysis of the ED50 values showed significantly decreased values in the juvenile ($P = 0.05$) and adult ($P = 0.014$) groups. In the infantile group with one or two challenges, there was a trend towards increased P_{enh} values at the Mch doses of 50 mg/ml ($P = 0.06$). In the juvenile group, there were significant differences at the Mch doses of 12, 25, and 50 mg/ml ($P = 0.017$). In the adult group, differences were observed at the Mch doses of 6 and 12 mg/ml ($P = 0.036$) (Fig. 3).

Effects of OVA Sensitization at Different Ages on Anaphylactic Antibodies IgE and IgG1

All sensitized animals presented positive titers for IgE and IgG1. The PCA titers were higher in the juvenile and adult mice than in the infantile sensitized mice ($P < 0.001$) (Fig. 4).

Inflammation

Semiquantitative analysis showed that bronchovascular inflammation was higher in all OVA groups compared to

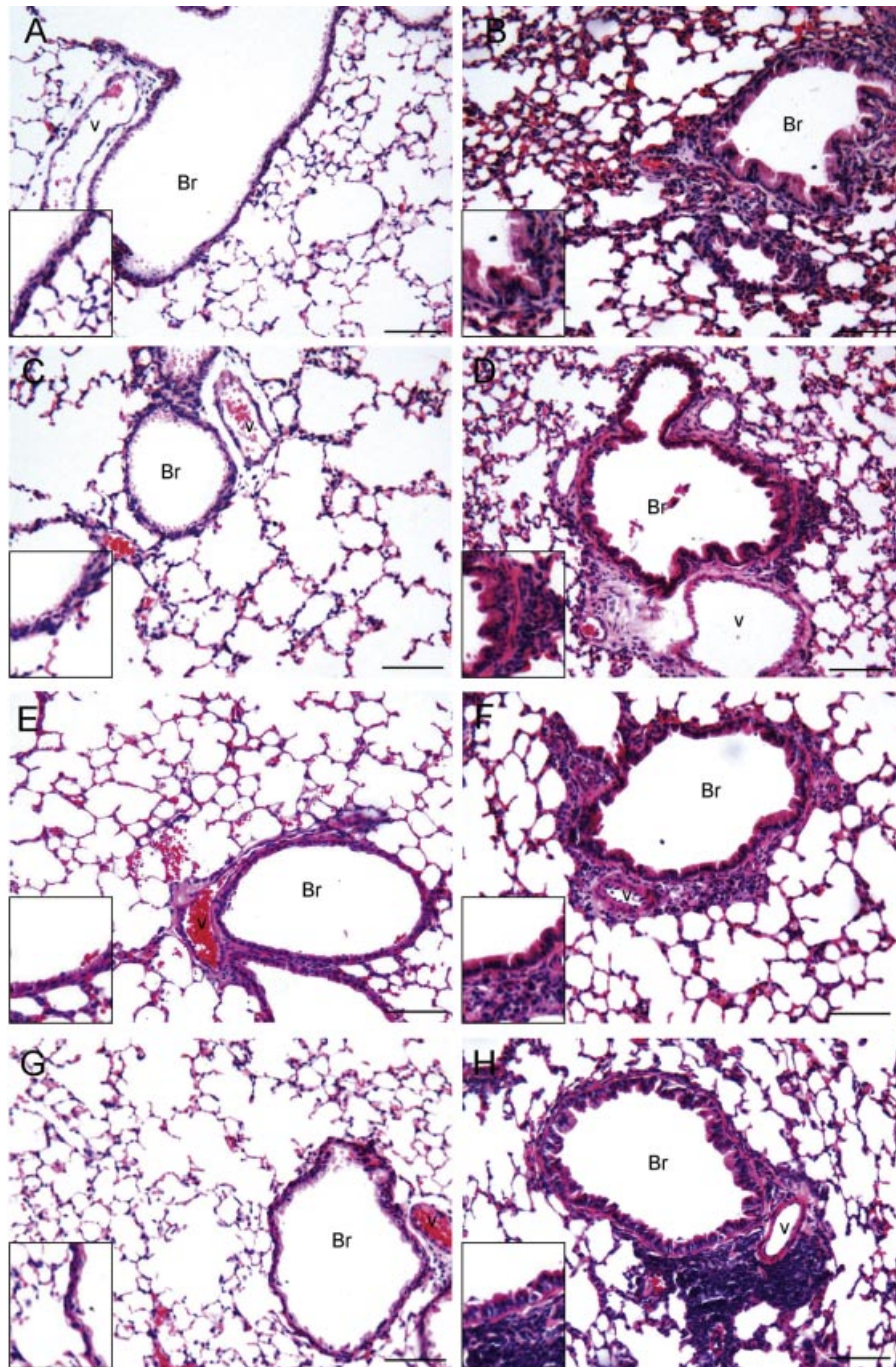


Fig 2. Histological findings in controls and OVA sensitized mice. The microphotographs show H&E stained sections of mice of different ages. The left panel (A,C,E,G) shows sections of control animals and the right panel shows sections of OVA-sensitized mice (B,D,F,H). Sensitized infantile mice with one challenge (B), infantile mice with two challenges (D), juvenile mice (E) and adult mice (H) show the presence of inflammation in the peribronchiolar areas. The insets show details of the airway wall cellularity in the different study groups. Scale bar = 60 μ m. Br, bronchiole; V, vessel.

control groups ($P < 0.001$). There were no differences among OVA or control groups. Data are presented in Table 1. The mean $\pm$ SD perimeter of the analyzed airways was $763 \pm 39 \mu$ m in the infantile groups, $926 \pm$

30μ m in the juvenile groups and $1,345 \pm 96 \mu$ m in the adult groups.

There were no significant differences in eosinophil and CD3+ T cell densities among controls (eosinophils

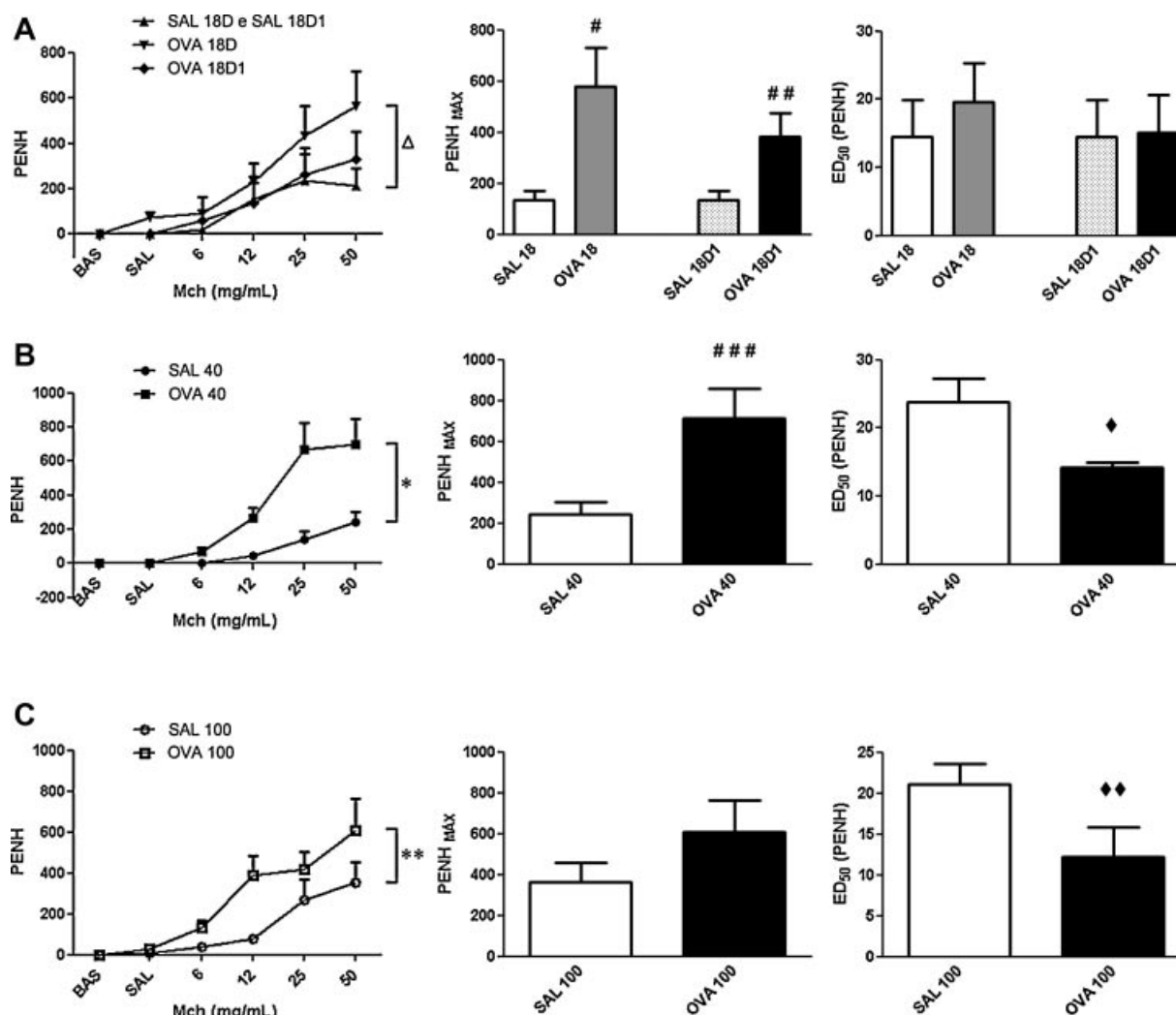


Fig 3. Airway hyperresponsiveness measured through the whole-body plethysmography system in mice at different ages in bronchial responsiveness. **A:** Infantile with one or two challenges; **(B)** juvenile mice; **(C)** adult mice. Values expressed as enhanced pause (P_{enh}), P_{enh} Max and effective dose (ED_{50}). BAS: baseline, SAL: control animals, and OVA: sensitized animals; 6–50: metacholine (mg/ml) in increasing concentrations. $P_{enh} - \Delta P = 0.002$ (saline dose); $*P = 0.017$ and $**P = 0.036$; P_{enh} Max— $\#P = 0.013$, $##P = 0.023$ and $###P = 0.046$; ED_{50} — $P = 0.050$ and $P = 0.014$.

$P = 0.097$, CD3+ T cells $P = 0.339$). In all OVA groups, there was a significant increase in the density of eosinophils ($P = 0.002$) and CD3+ T cells ($P = 0.005$) when compared to controls irrespective of age. When the OVA-treated animals were compared, there were no differences regarding eosinophil cell density ($P = 0.052$) (Figs. 5 and 6).

The density of CD3+ T cells was higher in the adult mice and in the infantile group with two challenges when compared to the infantile group with one challenge and the juvenile mice ($P < 0.001$) (Figs. 5 and 7).

IL-5+ Cells

Among controls, IL-5+ cells expression was lower in the infantile group with one challenge in relation to the

juvenile and adult mice ($P < 0.001$). When the OVA groups were compared to their respective controls, the infantile groups demonstrated increased IL-5+ cells expression in the airways ($P = 0.017$), but no differences were observed in the juvenile and adult mice. No differences among OVA groups were observed ($P = 0.14$) (Figs. 5 and 8).

Goblet Cell Hyperplasia

Signs of increased mucus production/accumulation were present in all OVA-treated groups, which differed significantly from controls ($P = 0.004$). There was no difference among controls ($P = 0.704$). Infantile mice with one or two challenges presented with higher PAS

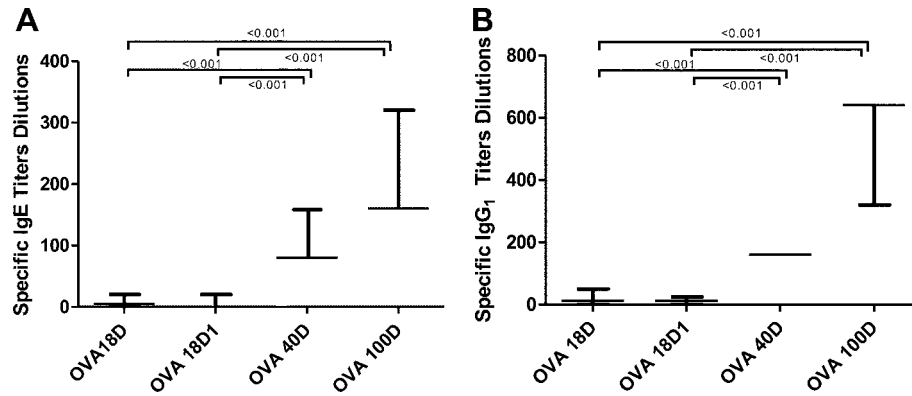


Fig 4. Specific IgE antibodies titers dilution (A) and specific IgG1 antibodies titers dilution (B) in OVA-sensitized animals. Control animals presented undetectable titers. Titers were higher in juvenile and adult groups than the infantile sensitized mice.

TABLE 1—Semiquantitative Scores on the Bronchovascular Inflammation

Study group	Control group [median (range)]	Sensitized group [median (range)]	P-values
18D	1 (1–1)	2 (2–3)	<0.001
18D1	1 (1–1)	2 (2–3)	<0.001
40D	1 (1–1)	2 (2–3)	<0.001
100D	1 (1–2)	3 (2–3)	<0.001

The inflammation score was similar in all sensitized groups. Study groups: 18D—infantile mice with one challenge; 18D1—infantile mice with two challenges; 40D—juvenile mice and 100D—adult mice.

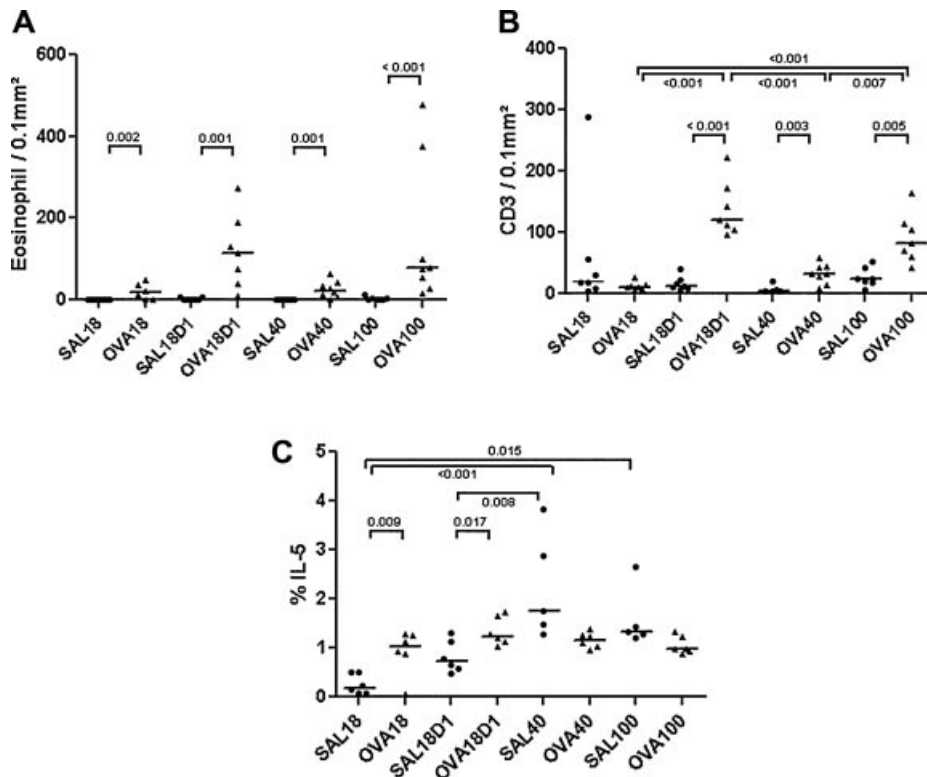


Fig 5. Quantification of eosinophils, CD3+ T cells and IL-5+ cells in the different study groups. **A:** Eosinophil cell density in the airway of the different study groups, results were expressed as cells/area (μm^2). **B:** Lymphocyte density in the airway of the different study groups, results were expressed as cells/area (μm^2). **C:** Positively stained area of IL-5+ cells in the airways wall. Results were expressed as %.

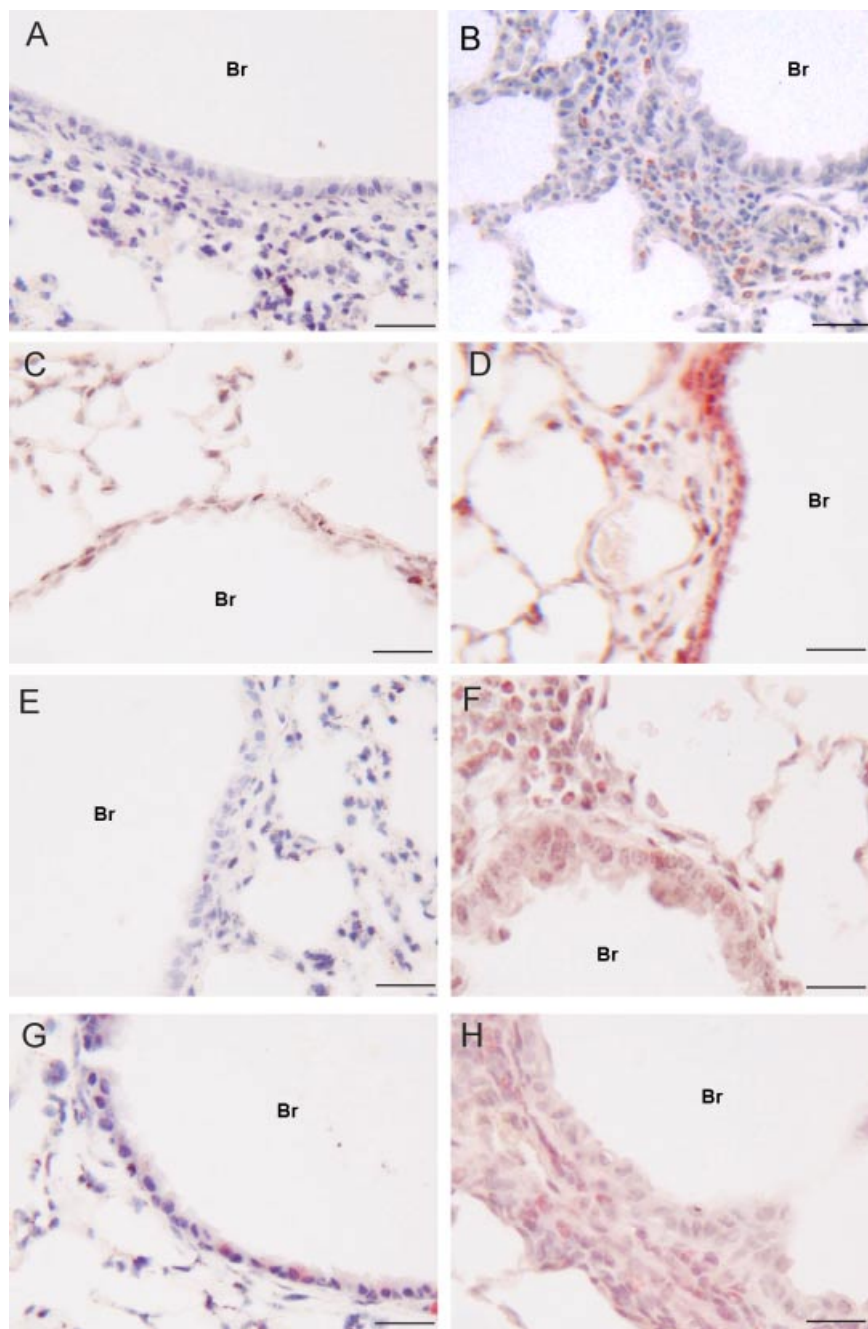


Fig 6. Eosinophilic airway inflammation in the different study groups. Luna staining showing eosinophils in the bronchial walls (Br) of the OVA-sensitized animals. Eosinophilia was similar in all sensitized groups. The left panels (A,C,E,G) show sections of control animals and the right panels show sections of OVA-sensitized mice (B,D,F,H). Scale bar = 60 μ m.

positivity in the bronchiolar epithelium than the juvenile animals ($P = 0.007$) (Figs. 9 and 10).

Collagen and Elastic/Reticular Fraction Areas

Among controls, there was an increased collagen fraction area in relation to the total airway area in adult mice

when compared to infantile mice ($P < 0.001$), but there were no differences among the other groups. All OVA-sensitized groups demonstrated a higher collagen area fraction than their respective control group ($P = 0.037$) with the exception of the juvenile mice ($P = 0.336$). Among the OVA mice, infantile mice with one challenge demonstrated a lower collagen area fraction than the group

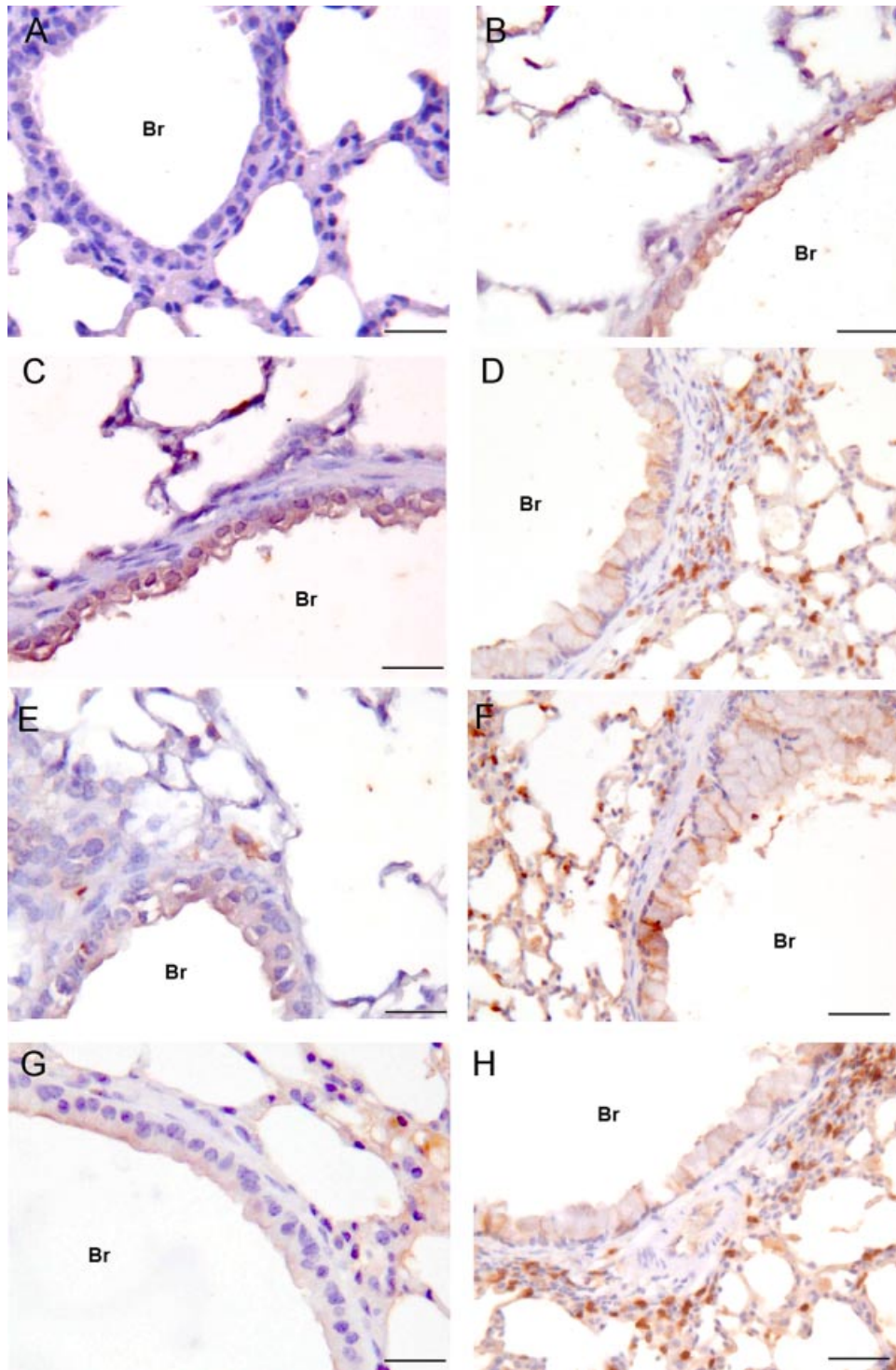


Fig 7. Lymphocytic CD3+ T cell airway inflammation in the different study groups. Immunohistochemistry with CD3 antibody showing positive CD3+ T-lymphocytes in the bronchial walls (Br) of OVA-sensitized animals. Lymphocytic inflammation was prominent in the infantile mice with two challenges (D) and in the adult OVA-sensitized animals (H). The left panels (A,C,E,G) show sections of control animals and the right panels show sections of OVA-sensitized mice (B,D,F,H). Scale bar = 60 μ m.

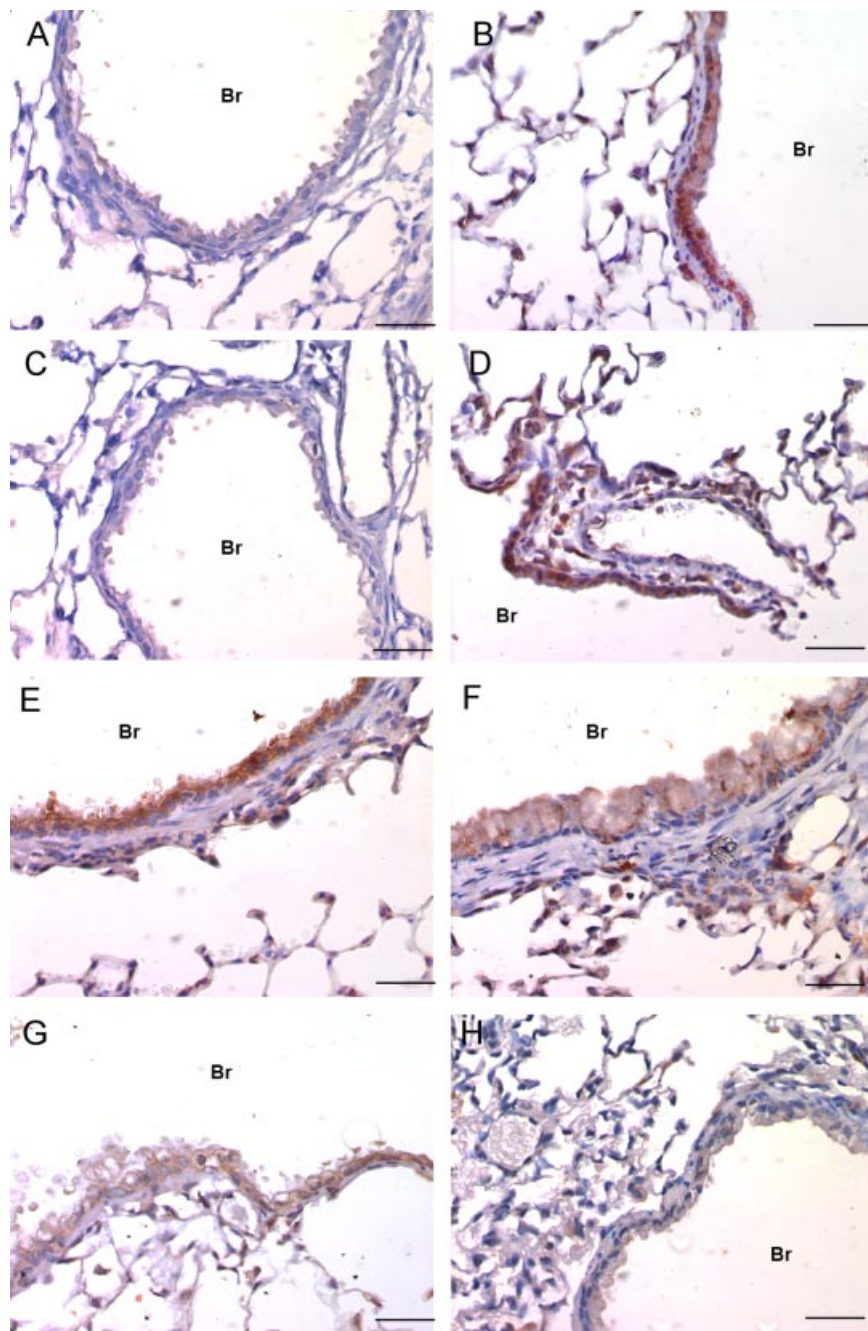


Fig 8. IL-5+ cells in the airways of the different study groups. Immunohistochemistry with IL-5 antibody showing positive in inflammatory cells in the bronchial walls (Br) and in the epithelium. The infantile OVA a sensitized animal (B and D) presented higher expression of IL-5+ cells in the Br their respective controls (A and C). The left panels (A,C,E,G) show sections of control animals and the right panels show sections of OVA-sensitized mice (B,D,F,H). Scale bar = 60 μ m.

of infantile mice that received two challenges ($P < 0.001$) without further differences among the groups (Figs. 9 and 11).

There were no significant differences in elastic/reticular fibers among all groups. The area fraction was $1.34 \pm 0.68\%$ in the infantile controls with one saline challenge, $1.24 \pm 0.44\%$ in the infantile controls with one OVA

challenge, $1.39 \pm 0.41\%$ in the infantile controls with two saline challenges, $1.52 \pm 0.50\%$ in the infantile controls with two OVA challenges, $1.74 \pm 0.47\%$ in the juvenile controls, $1.19 \pm 0.45\%$ in the sensitized juvenile group, $1.59 \pm 0.47\%$ in the adults controls, and $1.10 \pm 0.48\%$ in the sensitized adult group ($P = 0.37$).

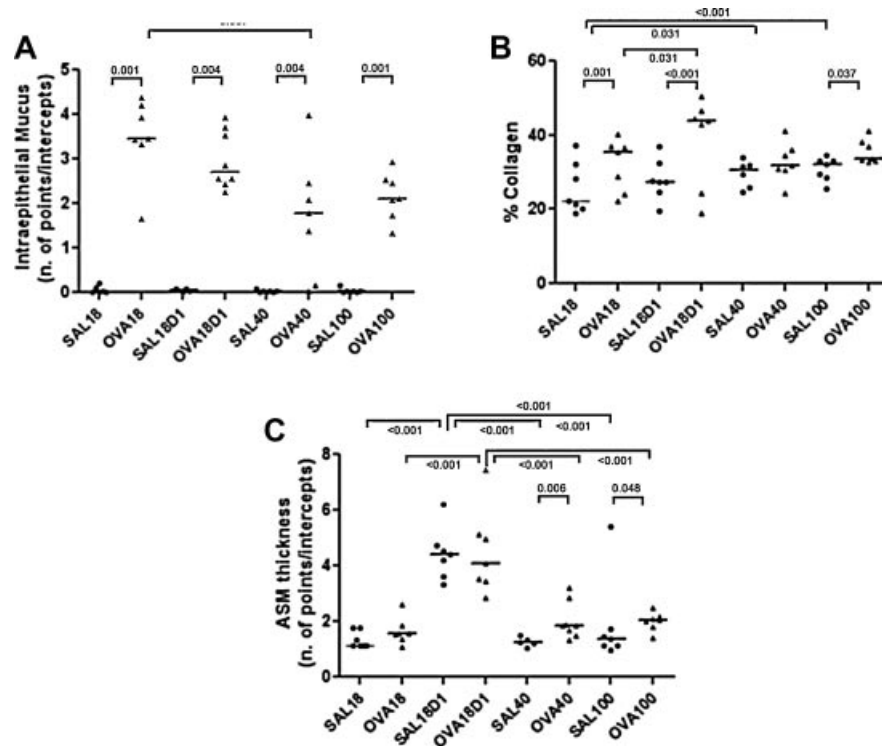


Fig 9. Quantification of airway epithelial mucus, collagen and airway smooth muscle in the different study groups. **A:** Epithelial mucus content in the airway of the different study groups, results were expressed as number of points/intercept. **B:** Positively stained area of collagen content in the airways wall. Results were expressed as %. **C:** Positively stained area of airway smooth muscle in the airway walls. Results were expressed as number of points/intercept.

Airway Smooth Muscle (ASM) Thickness

Among controls, the group of infantile mice that received two saline inhalations had greater ASM thickness than the other control animals ($P = 0.006$). Infantile mice sensitized with two challenges had a thicker ASM than other groups ($P < 0.001$); however, when OVA-sensitized animals were compared to their respective controls, infantile mice did not demonstrate significant differences ($P = 0.848$). Juvenile and adult mice had increased ASM thickness when compared to their age-related controls ($P = 0.048$) (Figs. 9 and 12).

DISCUSSION

In this study, we compared inflammatory and remodeling parameters in infantile, juvenile, and adult mice submitted to similar protocols of allergic sensitization. Our data show that sensitized infantile mice develop inflammatory and structural alterations in the airways, which differed from juvenile and adult mice in the airway compartments that are altered. Furthermore, the magnitude of some inflammatory and remodeling parameters in infantile mice seemed to be related to the frequency of allergen exposure.

Much attention has been drawn to the development of inflammation and airway remodeling in asthmatic children. The safe use of bronchoscopy to obtain bronchial biopsies has shed light on several phenomena occurring in childhood asthma. These studies have shown that inflammation and remodeling occur already in persistent wheezers at the pre-school age.¹² In children with severe asthma, thickening of the RBM may occur irrespective of inflammation; importantly, these airway structural changes seem to be of similar magnitude to those occurring in adult asthma.^{9,25}

Few studies have used models of neonatal/infantile allergic sensitization in mice. The available studies have shown that it is possible to develop such models using both systemic and local sensitization.^{13–15} We used the model developed by Hamada et al.,¹⁶ and, in accordance with their findings, functional and inflammatory features were observed in the younger mice. Since previous studies have shown that airway eosinophilia and the degree of airway hyperresponsiveness were both present and correlated at 48 hr after allergen challenge, we obtained our data at this time point of the study design.^{26,27}

The development of asthma seems to be related to re-exposure to aeroallergens in sensitized children.²⁸ In this study, we analyzed sensitized infantile mice submitted

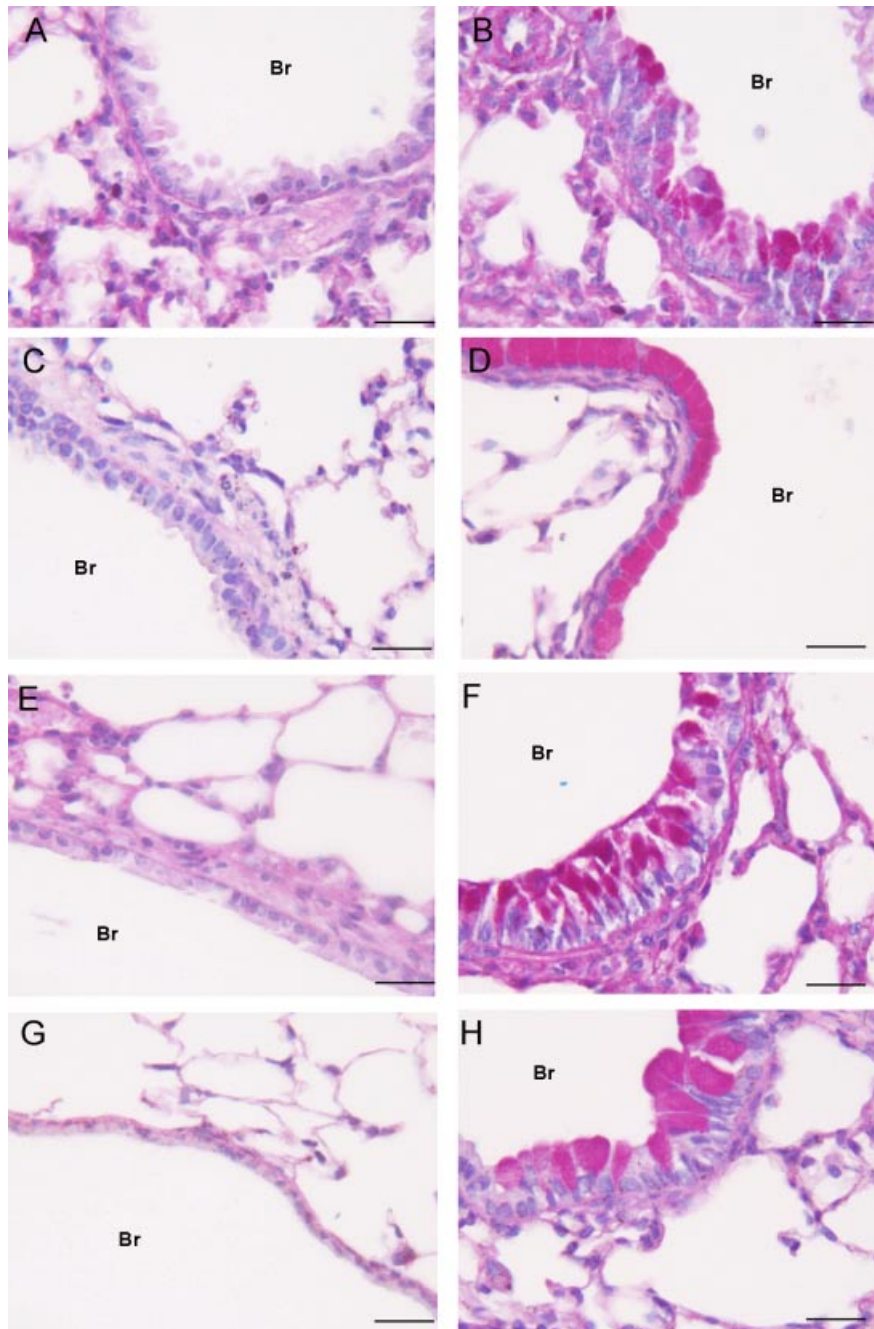


Fig 10. Mucus content in the different study groups. PAS staining (in pink) revealing marked goblet cell hyperplasia in all OVA-sensitized animals. The infantile groups had more goblet cell metaplasia (A and D) than the juvenile and adult mice (F and H). There is no mucus in the control groups. The left panels (A,C,E,G) show sections of control animals and the right panels show sections of OVA-sensitized mice (B,D,F,H). Scale bar = 60 μ m. Br, bronchiole.

to one or two challenges early in life. Our data show that infantile mice sensitized with two challenges demonstrated a more prominent lymphocytic inflammation than infantile mice with one challenge and displayed higher collagen content in the airways. These data suggest that the development of inflammation and remodeling

might be linked to more frequent exposures early in life.

We acknowledge that measures of AHR obtained via the whole-body plethysmography system probably does not accurately reflect airway resistance when compared to other more invasive methods.^{18,29} However, given the

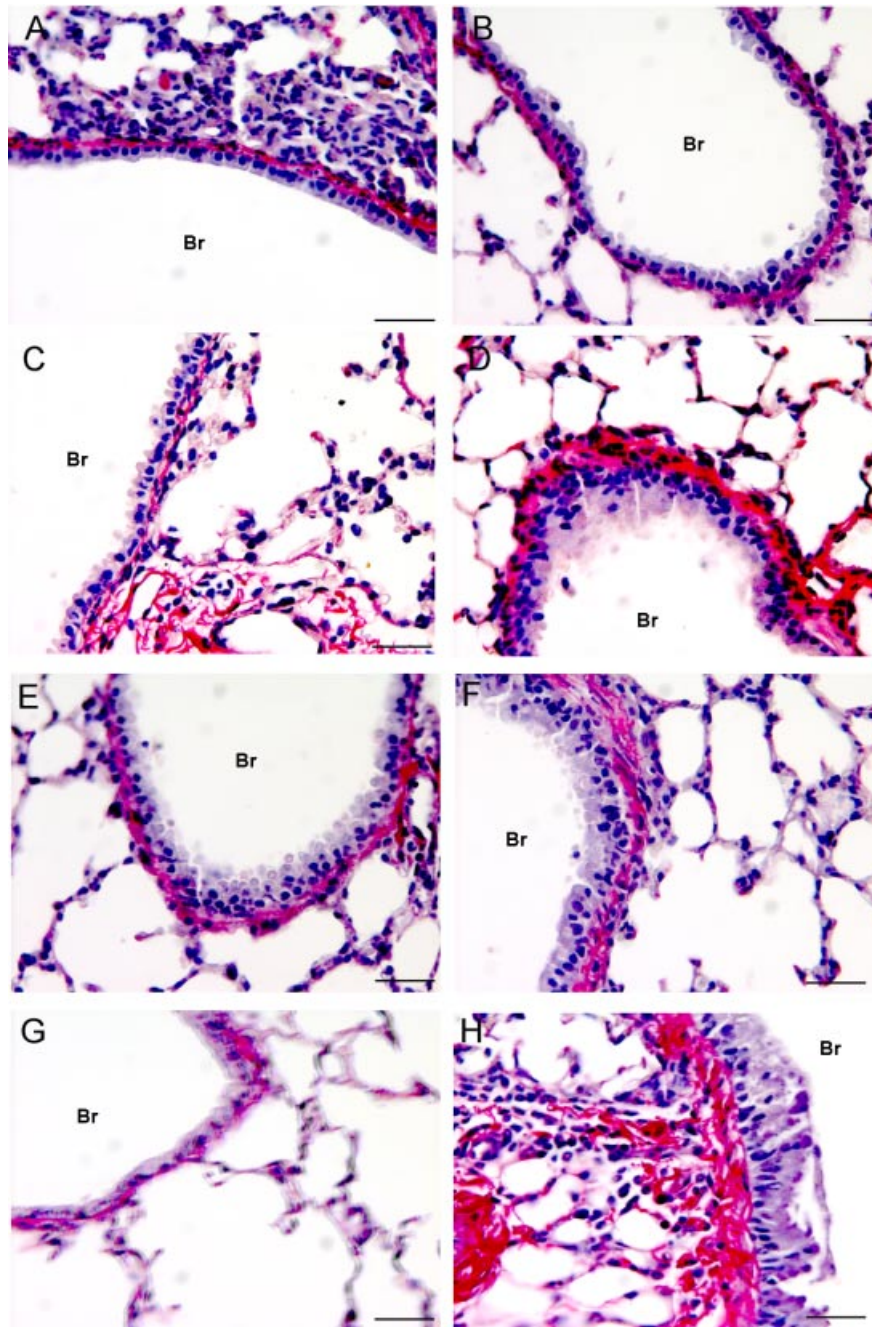


Fig 11. Collagen content in the different study groups. Picrosirius staining collagen fibers in red. Bronchial deposition of collagen was observed in all OVA groups when compared to the respective controls, except in the juvenile group (F). Infantile animals with two challenges (D) present more airway collagen than infantile mice with one challenge (B). The left panels (A,C,E,G) show sections of control animals and the right panels show sections of OVA-sensitized mice (B,D,F,H). Scale bar = 60 μ m. Br, bronchiole.

size of the animals in the infantile groups, this was the only method available to obtain functional data. It is interesting to note that in the smaller mice, the development of AHR was associated with inflammation (eosinophilia) and goblet cell hyperplasia and not with ASM hyperplasia, as observed in the juvenile and adult groups.³⁰

Juvenile and adult saline mice demonstrated higher expression of IL-5+ cells than infantile mice in the airways. However, infantile sensitized mice demonstrated higher levels of IL-5+ cells expression when compared to their respective controls, whereas no differences were observed between juveniles and adults when compared

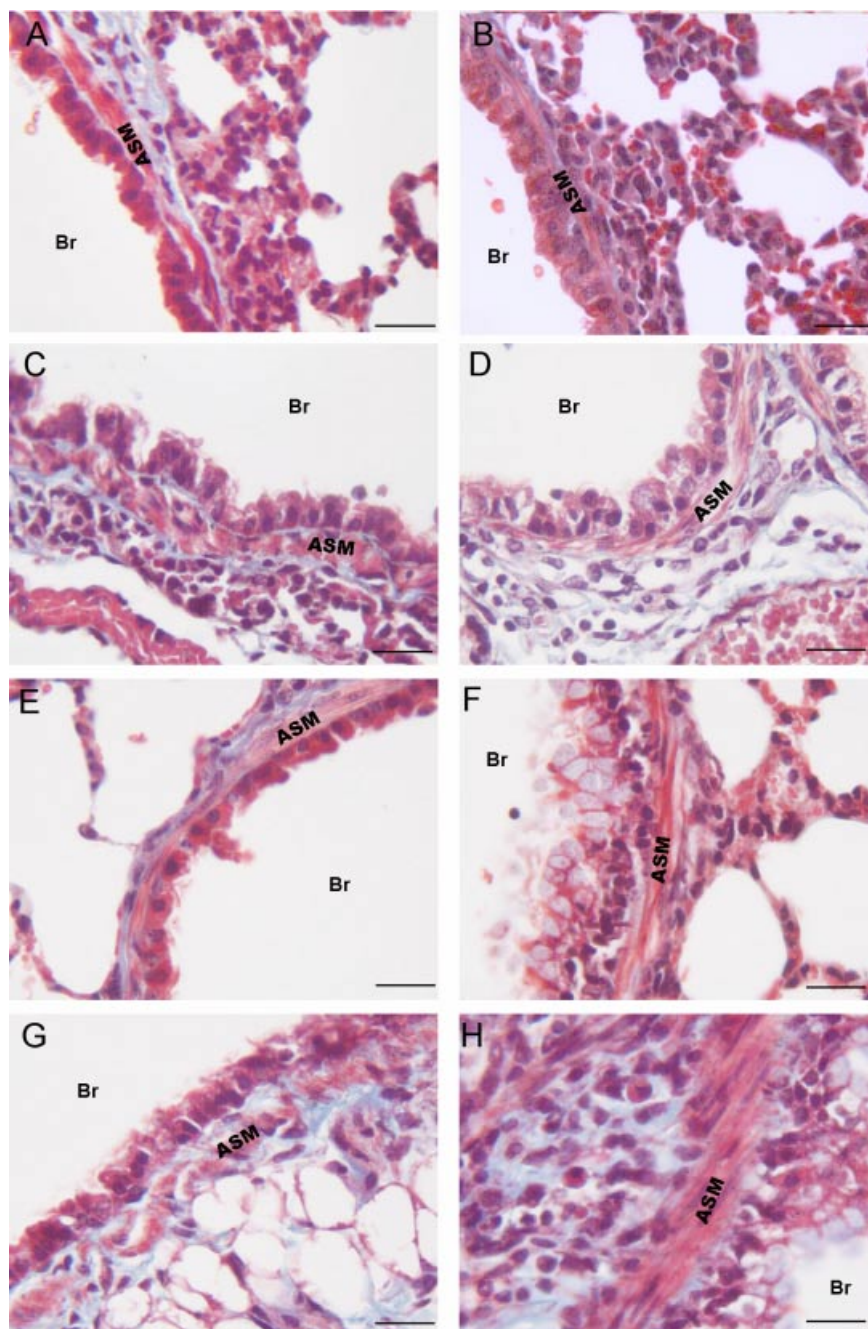


Fig 12. Airway smooth muscle mass in the different study groups. Masson staining shows airway smooth muscle (ASM) fibers in red and collagen fibers in blue. Juvenile (F) and adult (H) mice presented more ASM than their respective controls (E and G); no difference was observed between infantile groups. The left panels (A,C,E,G) show sections of control animals and the right panels show sections of OVA-sensitized mice (B,D,F,H). Scale bar = 60 μ m. Br, bronchiole.

to their age-matched controls. Our results support the previous findings of a polarized Th2 immune response to allergens at younger ages,^{31,32} despite lower levels of IgE.³³

Our results suggest that the development of the airway structural alterations may differ according to the age of

sensitization. With a similar number of challenges, infantile mice developed intense goblet cell hyperplasia, but there was no increase in ASM mass when compared to their respective controls. Mucus hypersecretion is a symptom of several lung diseases in infants, primarily in bronchiolitis. Our study supports the idea that the regulation of

mucus hypersecretion might be a possible therapeutic target in young asthmatic children.³⁴

Our data also show that both the age of sensitization and the period of allergen exposure after sensitization are important to the development of structural alterations. Juvenile challenged mice presented with different structural alterations compared to infantile mice in some parameters, such as increased collagen content and goblet cell hyperplasia, despite having been sensitized at the same age. These changes are likely to be related to the increased maturation of the immune system in the older animals.

Structural alterations in the ASM in asthma are believed to be pivotal to disease pathogenesis and linked to asthma severity.³⁵ To our knowledge, this study is the first to analyze ASM mass in an infantile mouse model. Our data show that the ASM mass only increased in the juvenile and adult mice groups despite similar challenge protocols. In humans, it is unknown when structural changes in the ASM are initiated. It is also unknown whether these changes are intrinsic or allergy related. A limitation of allergic-sensitization models is that all phenomena are allergy driven, which may not be the case in humans, especially in severe, non-atopic asthma.

Previous studies on asthmatic children aged 11 years showed increased ASM mass in relation to controls; the children displayed an ASM proportion in the airways that was similar to the proportion described in adults.¹⁰ Another study on severe asthmatic children (ages ranging from 6 to 14 years) compared children with and without persistent air obstruction. Persistently obstructed asthmatic children showed an increase in the ASM mass and a higher protein expression of muscle contractility markers; however, there was no difference between the levels of eosinophils/CD3+ T-lymphocytes or the thickness of the basement membrane in the two groups.³⁵ These data reinforce the key role of ASM abnormalities in disease severity and the necessity of therapies that target the ASM in asthma. Our results may have important implications, showing that, at a younger age, there may be still time to prevent some structural airway changes such as increased ASM mass.

This study has several limitations. We used an OVA-induced model via i.p. injections instead of sensitization with an aeroallergen that would more naturally mimic childhood asthma. However, with this model, inflammatory and structural abnormalities similar to the house dust model were observed.¹² It would be important to further explore the immunological mechanisms leading to differences in inflammatory and remodeling in young versus adult mice. It is interesting to consider whether adults with late-onset asthma actually have non-diagnosed asthma during childhood that relapsed later in life. The limitations of the allergic sensitization models in mice make a similar study design difficult.

In summary, we have shown that infantile mice develop inflammatory and structural alterations in the airways that are partially different from those developed in older animals. Understanding the phenotypic differences in childhood versus adult asthma is crucial in treating the disease adequately and preventing the escalation of disease severity.

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