

Montelukast reverses airway remodeling in actively sensitized young mice

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Abstract

Asthma is characterized by airway hyperresponsiveness (AHR) and inflammation leading to airway remodeling (AR). In children, AR may occur very early prior to the age of 6 years. Treatments to prevent or reverse AR are unknown.

Aim: We sought to determine (i) whether short allergenic sensitization at a young age in a mouse model may induce enhanced AR and inflammation compared to adults; (ii) the effect of Montelukast on such AR.

Methods: Immature and adult Balb/c mice were sensitized and challenged with ovalbumin. AHR and AR were measured using cultured precision-cut lung slices and inflammation by bronchoalveolar lavage. Experiments were repeated after administration of Montelukast.

Results: OVA-challenged mice developed AHR to methacholine regardless of age of first exposure to OVA. Young mice developed greater thickened basement membrane, increased smooth muscle mass, and increased area of bronchovascular fibrosis compared with adult mice. Cellular infiltrates in BAL differed depending upon animal age at first exposure with higher eosinophilia measured in younger animals. Montelukast decreased ASM mass, BAL cellularity.

Conclusion: We provide thus evidence for a greater degree of AR after allergenic sensitization and challenge in younger mice versus adults. This study provides proof of concept that airway remodeling can be prevented and reversed in this case by anti-asthmatic drug Montelukast in this model.

KEYWORDS

asthma & early wheeze, respiratory and airway muscle

1 | INTRODUCTION

Asthma affects around 300 million people throughout the world. It is a complex disease of the airways involving, smooth muscle cells and non-contractile components such as epithelium, submucosa glands, and inflammatory cells. Asthma is characterized by airway hyper

responsiveness (AHR) and inflammation (eosinophils, mast cells, lymphocytes) leading to airway remodeling (AR) that, in severe cases, can generate permanent airflow obstruction.¹

AR in asthma was first described in cases of fatal asthma and is associated with clinical outcomes such as more severe airflow obstruction and AHR.² In childhood asthma, AR may be an early

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phenomenon occurring prior to the age of 6 years.^{3,4} Asthma may have its origins in the airways themselves involving defective structural and functional behavior of, for instance, the epithelium in relation to environmental insults (viruses, repeated allergen exposure).^{5,6} With respect to childhood, in human bronchial smooth muscle cells (ASMC), we have previously demonstrated increased contractile response, ASMC proliferation, and inflammation in young compared to adult ASMC (34). This was accompanied by increased leukaemia inhibitory factor secretion (a member of the IL-6 family) and intracellular calcium response.⁷ Epidemiological studies in asthmatic children have shown that lung function in early childhood influences lung function in adults⁸ suggesting that the remodeling process may be irreversible.

Since lung biopsies are difficult to obtain in children, there is a need for the development of appropriate young animal models of asthma to better understand the disease process. Usually, asthma models are induced in adult animals by intra-peritoneal ovalbumin sensitization followed by nasal or nebulized allergenic challenges.⁹ Acute adult asthma models are generated within a time frame of 3–4 weeks and are characterized by inflammation and AHR^{9,10} whereas chronic asthma models with AR require up to 10–11 weeks.^{9,10} Immature mice models have also been proposed in the past¹¹: thrice-weekly intranasal house dust mite (HDM) in neonatal Balb/c mice induced significantly increased AHR and increased total and eosinophilic lung inflammation from Week 2 onwards, airway remodeling from Week 3–4.

The Leukotriene Receptor Antagonists (LTRA) Montelukast reduces airway eosinophilic infiltration and goblet cell metaplasia, as well as the established increase in ASM mass and subepithelial collagen deposition in some *in vitro*^{12,13} and murine^{14,15} experiments. However, it remains unclear as to when is the best time to initiate such treatment to modify remodeling, which components to target, and how best to monitor interventions on remodeling.¹⁶ In the present study, we chose to target ASM in particular since in human studies, asthma severity is greatly determined by ASM surface area.¹⁷

The aim of the present study was thus study the effect the anti-asthmatic drug Montelukast in a similar model of asthma in young mice, based on relatively short-term active ovalbumin sensitization, in order to determine whether AR can be prevented or cured. We compared AR (histology and immuno-histochemistry), bronchial reactivity (lung-slice technique¹⁸) and airway inflammation (bronchoalveolar lavage BAL) in young mice versus adult mice. Using this model, we assessed the ability of Montelukast to prevent or reverse airway inflammation, AHR, and AR in immature mice.

2 | METHODS

2.1 | Animals

Female Balb/c mice, 4 weeks-old for the young group and 8 weeks-old for the adult group, were obtained from IFFA CREDO, FRANCE. The respective weights of 4-week old young and 8-week old adult mice were (mean $\pm$ SD) 14.0 $\pm$ 0.5 g (n = 21) and 21.0 $\pm$ 0.7 g (n = 22). All procedures employed in this study were approved by the Animal Care

and Use Committee of our institution. The sensitization protocol appeared to be well tolerated since, overall, only five mice did not survive (four in the ovalbumin group [two young and two adult mice] and one in the control group (a young mouse). Balb/c mice acquire sexual maturity between the age of 6 and 8 weeks and growth maturity between 8 and 12 weeks.¹¹

2.2 | Active ovalbumin sensitization

Mice were sensitized by intraperitoneal injections of ovalbumin on day (D) 0 and D14 and challenged by intranasal ovalbumin (#A5503) (Sigma-Aldrich, Saint-Quentin-Fallavier, France) injections in anesthetized mice on D14, D28, D29, and D30.

Anesthesia for intranasal injections was performed by the intraperitoneal administration of xylazine 2g/100 mL (Sigma-Aldrich) (2 μ L for 16 g body weight + 1 μ L/g above 16 g) and ketamine (Panpharma, Fougères, France) 50 μ g/mL (2 μ L/g body weight). Ovalbumin doses in the adult group were 100 and 500 μ g. This is a relatively high dose due to the absence of aluminium hydroxide¹⁹ for the intraperitoneal and intranasal approaches, respectively. In young mice, the doses were adapted, in proportion to the weight per se of the animal. Intranasal administration was performed in the supine position, the upper part of the body inclined 45°.

The study design is shown in Figure 1.

2.3 | Assessment of *in vitro* cholinergic bronchial contractility (lung slices): Response to carbachol

We have established, for young animals, a previously described method,¹⁸ based on the capacity of 2% agarose to sufficiently solidify a live lung in order to obtain lung sections for the study of bronchial contractility. Briefly, 2% liquid agarose (VII-A, Low Gelling Temperature, Sigma-Aldrich) was prepared at 60°C in a PBS 1x solution then cooled to 37°C. Mice were sacrificed on D42 by cervical dislocation. Their lungs were then inflated with warm (37°C) 2% liquid agarose through a tracheal catheter. Following the solidification of the agarose by cooling the whole lung to 4°C, the lungs were excised and cut into serial sections 200 μ m thick using a vibratome. The slices were placed in DMEM medium supplemented with antibiotics and antimycotics (100 000 μ g/L), FCS (foetal calf serum) (10% or 100 mL/L) at 37°C and 5% CO₂ for up to 3 days.

To determine bronchial contractile response, lung slices were placed in culture dishes, immersed in Krebs Hepes solution with 2 mM Ca²⁺ and maintained in position using a strip of nylon mesh. Contraction was induced by the cholinergic agonist carbachol (10^{−6} M to 10^{−3} M). Only lung slices including viable airways (active ciliated epithelium), which were free of agarose, were selected. Airway lumen areas were measured prior to and after adding carbachol and normalized with respect to airway wall thickness. All image analyses were performed using Quancoul software (Quant Image 1995–7, France).²⁰ Regarding the reproducibility of the measurements our preliminary data indicated that the coefficient of variation was 2.8% for the measurement of bronchial lumen area and 5% for bronchial wall thickness.

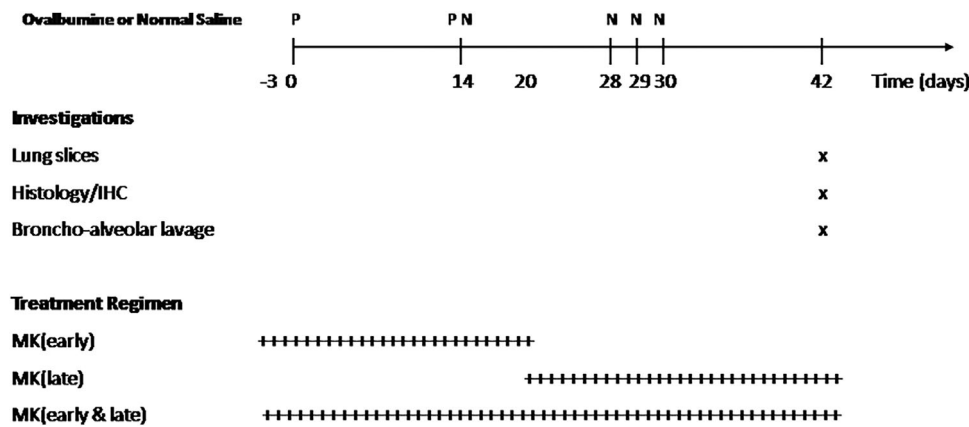


FIGURE 1 Study design. Mice received intraperitoneal injections of either ovalbumin solution or vehicle on Days 0 and 14. Aerosol challenges with either ovalbumin or vehicle were performed three times per week starting on Day 14 until Day 30. Montelukast (1 mg/kg) or vehicle was administered from Days−3 to 20, Days 20–42, or Days−3 to 42. Animals were sacrificed and studied on Day 42. IHC, Immunohistochemistry; MK, montelukast; N, intranasal; NS, normal saline; P, Intraperitoneal

2.4 | Histology and immunohistochemistry

Mice were sacrificed by cervical dislocation on D42, that is, 12 days after the last challenge. This is in contrast to most models in which mice are sacrificed 24–28 h after the final challenge, since we sought to study residual remodeling. The right lung was dissected and immediately fixed with 10% formalin. After successive ethanol bath dehydration, lung tissues were embedded in paraffin, cut into 3 μ m sections, mounted onto slides, and stained with HES (Hematoxylin Eosin Safran) to quantify reticular basement membrane thickness, according to previously described methods.^{14,15} Similarly, Masson's trichrome was used to quantify airway collagen deposition, and α -actin to quantify bronchial smooth muscle. Of note, for immunohistochemical processing, 3 μ m sections were incubated 45 min with mouse monoclonal anti alpha smooth muscle actin antibody (monoclonal anti- α SM actin, mouse IgG2a, clone 1A4; Sigma; 1/10 000). After rinsing in PBS, biotinylated rabbit anti-mouse secondary antibody followed by the streptavidin-biotin-peroxidase complex was applied according to the kit Dako REAL, Peroxidase/DAB+ procedure (Sigma A 2547). After rinsing, the sections were counterstained with Mayer's hematoxylin and mounted. Reticular basement membrane (RBM) thickness was expressed as the mean of 12 measurements on three different bronchi. Smooth muscle, collagen deposition area and total wall area were determined automatically using Quancoul software (Quant Image 1995–7, France) after color calibration and manual selection of the area of interest. The boundaries of the total wall were determined by the epithelium and the submucosa, large blood vessels excluded. For each parameter and for each mouse, three different non-cartilaginous bronchi were selected: the first, a large proximal airway was cut according to a longitudinal axis, the second, a medium-size one was cut in a axial axis (mean perimeter measured at the level of the RBM: 702 μ m) and the third, of a smaller size (mean perimeter: 443 μ m) was also cut in a axial axis. The ASM area or collagen deposition was normalized according to the overall area of the bronchial wall. Of note, the coefficient of variation was 10.9% for RBM thickness measurements, 3.2% for the ASM, and 4% for bronchovascular collagen deposition.

2.5 | Bronchoalveolar lavage (BAL)²¹

Mice were sacrificed 12 days after completion of sensitization (D42) by a lethal intraperitoneal injection using 500 μ L of urethane (120 mg/mL). Briefly, the trachea was cannulated using a 22-gauge soft catheter, and distal lung cells were obtained by BAL. BAL was performed with two 1 mL aliquots of phosphate-buffered saline (PBS), which were pooled. Total leukocyte numbers in the BAL fluid were counted in a Neubauer chamber. Following cyto-centrifugation (Shandon Cutospin 4, Thermo Scientific) and staining with Diff-Quik (Baxter Healthcare, Miami, FL), at least 400 cells per slide were differentiated by a blinded investigator using standard haematological criteria.

2.6 | Treatment with Montelukast

Only immature animals were given Montelukast. The final Montelukast solution was administrated at a 1 mg/kg dose, intra peritoneally, as per Figure 1. Montelukast was purchased from Cayman Chemical (Catalog N°10008318). As recommended, a stock solution was produced by dissolving Montelukast in an organic solvent (ethanol) purged with an inert gas (helium). The stock solution was stored at −20°C. A solution of Montelukast was reconstituted each day prior to administration by adding to the stock solution a 1:9 solution of ethanol:PBS.

2.7 | Data analysis

Data are presented as mean $\pm$ SEM. Animals were attributed to four groups according to their age (young [IMM] or adult [MAT]) and to the sensitization (ovalbumin [OVA] or normal saline [NS]) protocol and referred to as IMM-NS ($n = 11$), MAT-NS ($n = 12$), IMM-OVA ($n = 10$) and MAT-OVA ($n = 10$). One-way ANOVA followed by unpaired two-tailed t -test were performed for the analysis. When data were not normally distributed, log transformation was applied. The Newman-Keuls correction was used to correct for multiple comparisons. P -values <0.05 were considered significant.

3 | RESULTS

3.1 | Validation of the animal model

3.1.1 | Bronchial responsiveness

In vitro responsiveness of lung slices was only assessed in immature mice, since preliminary immunochemistry results showed greater airway remodeling in immature animals. We normalized the percentage of airway lumen area reduction according to airway wall thickness, since immuno-histochemical data showed that the smooth muscle (α -actin) content varied according to the size of the airways: 23% of the total bronchial wall area in the largest bronchi, 16% in medium-sized bronchi and 10% in the smallest bronchi. Carbachol-induced bronchial constriction was significantly greater at all concentrations studied (10^{-6} M ($P = 0.007$) to 10^{-3} M ($P = 0.01$)) in IMM-OVA than in IMM-NS mice (Figures 2).

3.1.2 | Airway and vascular remodeling

In adult animals, there was no significant increase in RBM thickness ($0.42 \pm 0.25 \mu\text{m}$ [MAT-NS] and $0.42 \pm 0.28 \mu\text{m}$ [MAT-OVA]), in ASM area

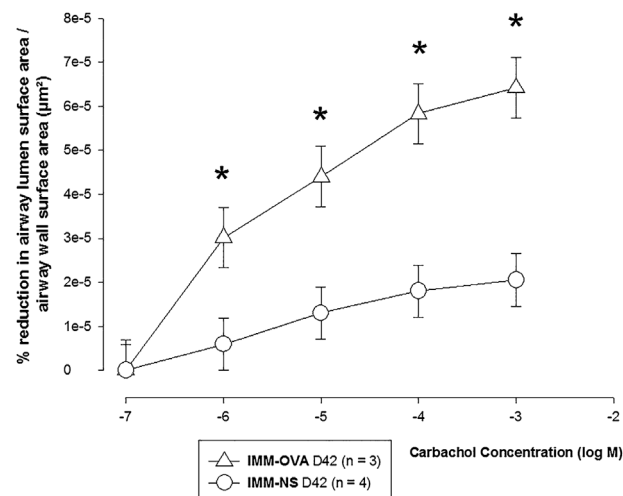


FIGURE 2 Precision cut lung slice carbachol induces contraction in young mice. Airway hyperresponsiveness was found at all concentrations of carbachol (10^{-6} M to 10^{-3} M). D: day. OVA, ovalbumin; NS, normal saline; IMM, Young mice; Mean $\pm$ SEM. * $P < 0.05$ (IMM-OVA vs IMM-NS [on D42])

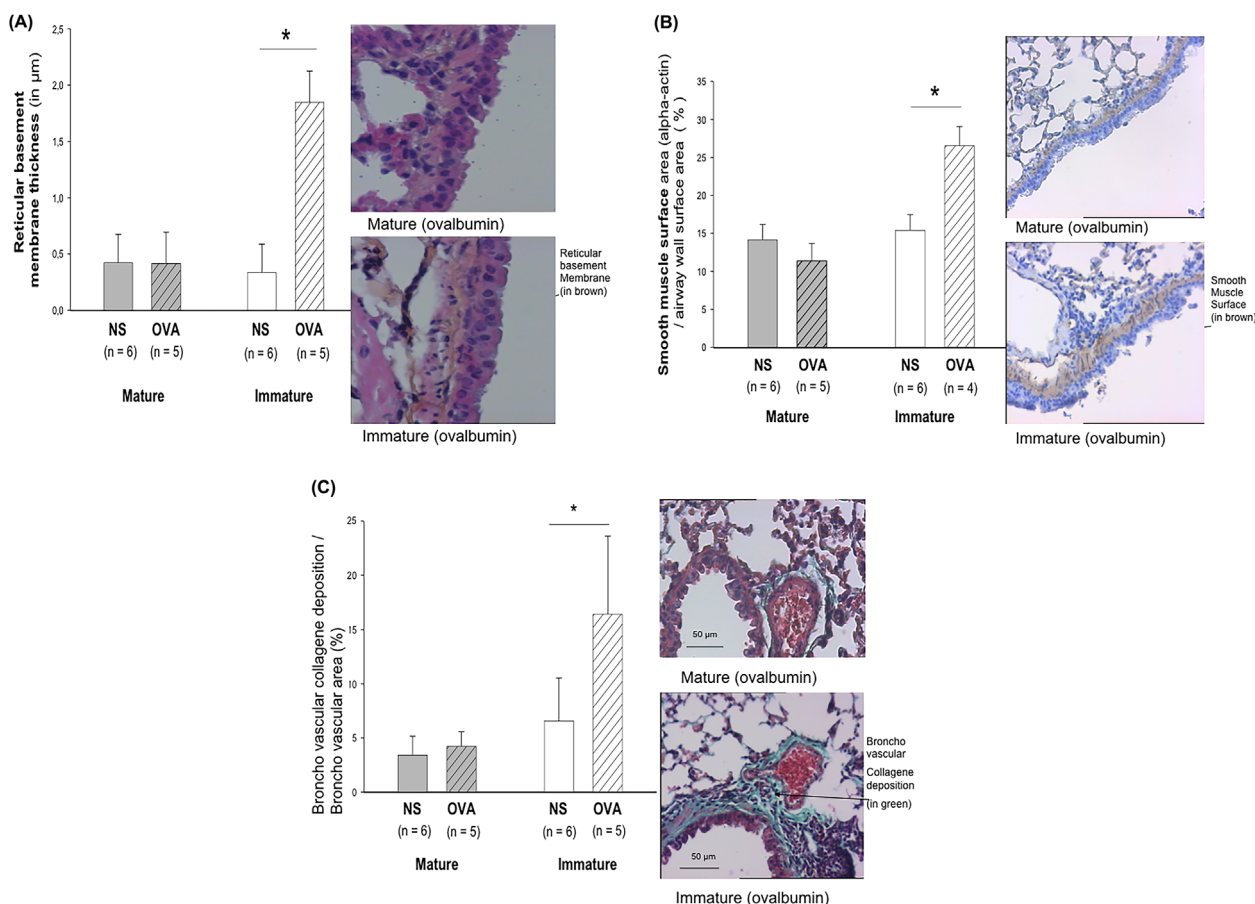


FIGURE 3 Effect of ovalbumin. A, Effect of ovalbumin according to age on reticular basement membrane thickness. B, Effect of ovalbumin according to age on smooth muscle area. C, Effect of ovalbumin on bronchovascular collagen deposition area. IMM, Young mice; OVA, ovalbumin; NS, normal saline. IMM-OVA versus IMM-NS (on D42). Mean $\pm$ SEM * $P < 0.05$

($14.1 \pm 2.1\%$ [MAT-NS] and $11.4 \pm 2.3\%$ [MAT-OVA]) or in bronchovascular collagen deposition ($3.4 \pm 1.7\%$ [MAT-NS] and $4.2 \pm 1.3\%$ [MAT-OVA]). In contrast, in young animals, we observed a fourfold increase in RBM thickness ($0.34 \pm 0.25 \mu\text{m}$ [IMM-NS] to $1.85 \pm 0.28 \mu\text{m}$ [IMM-OVA], $P = 0.011$) (Figure 3A), a twofold increase in ASM area ($15.4 \pm 2.1\%$ [IMM-NS] to $26.5 \pm 2.5\%$ [IMM-OVA], $P = 0.007$) (Figure 3B) and a threefold increase in bronchovascular collagen deposition area ($6.5 \pm 3.9\%$ [IMM-NS] to $16.4 \pm 7.2\%$ [IMM-OVA], $P = 0.009$) (Figure 3C).

3.1.3 | Inflammation

In adult animals, ovalbumin sensitization did not increase total cellularity in the BAL fluid ($11.6 \pm 1.6 \times 10^4/\text{mL}$ [MAT-NS] and $15.1 \pm 2.5 \times 10^4/\text{mL}$ [MAT-OVA]) but increased the percentage of lymphocytes ($1.7 \pm 0.5\%$ [MAT-NS] and $8.8 \pm 1.9\%$ [MAT-OVA], $P = 0.0002$) (Figure 4). There was no significant increase in eosinophil count ($0.12 \pm 0.12\%$ vs $1.28 \pm 0.43\%$). In contrast, in young animals, sensitization significantly increased total cellularity (from

$8.8 \pm 0.7 \times 10^4/\text{mL}$ [IMM-NS] to $20.8 \pm 0.8 \times 10^4/\text{mL}$ [IMM-OVA], $P = 0.009$), eosinophil count (from 0% [IMM-NS] to $5.1 \pm 0.8\%$ [IMM-OVA], $P < 0.0001$), and lymphocytes count (from 0% [IMM-NS] to $4.3 \pm 0.8\%$ [IMM-OVA], $P = 0.0002$) (Figure 4).

3.2 | Effect of montelukast in immature animals

3.2.1 | Responsiveness: Lung slices

A lesser reduction in lung slice bronchial lumen area to 10^{-7} M Carbachol was noted following the administration of early + late MK, (IMM-OVA-Vehicle [$1.6 \times 10^{-4} \pm 8.2 \times 10^{-5}/\mu\text{m}^2$ bronchial wall area] vs IMM-OVA-MK [$8.8 \times 10^{-5} \pm 1.6 \times 10^{-5}$], $P < 0.0001$) (Figure 5).

3.2.2 | Remodeling: ASM

In small airways of sensitized animals, there was a marked and significant reduction in ASM area, irrespective of the timing of the administration of

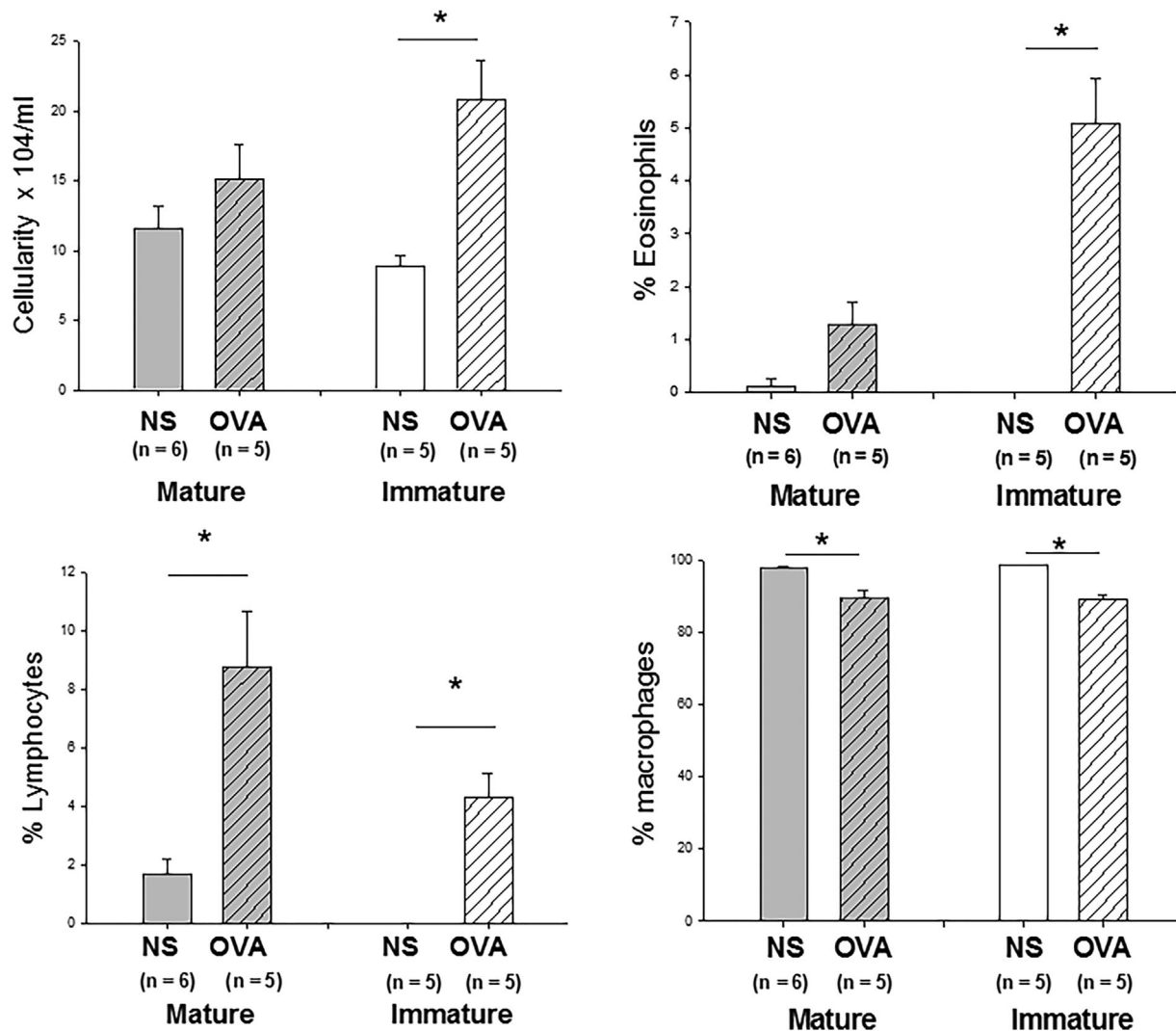


FIGURE 4 Effect of ovalbumin on lung inflammatory cells in bronchoalveolar lavage (D42). IMM, Immature; NS, normal saline; OVA, ovalbumin; Mean $\pm$ SEM. * $P < 0.05$

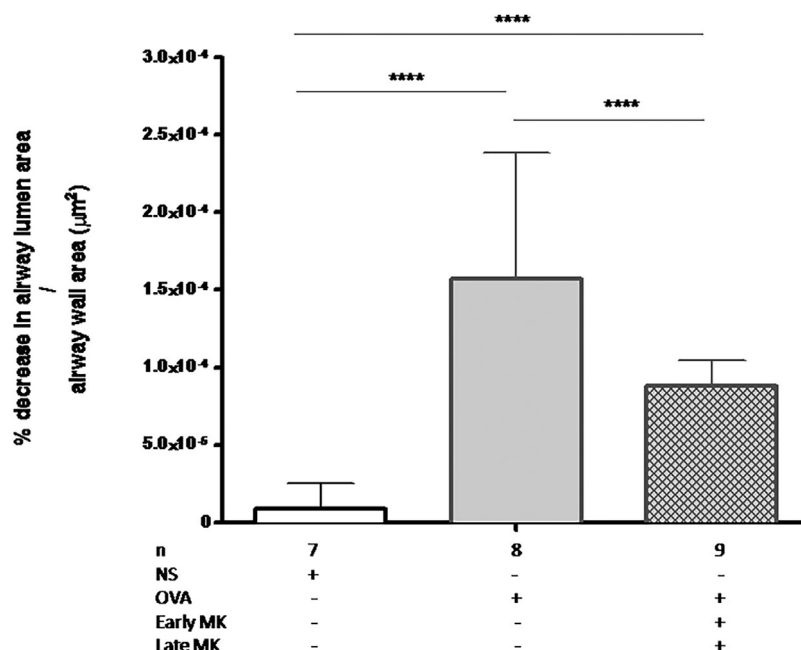


FIGURE 5 Effect of Montelukast in immature mice on bronchial hyperresponsiveness. Airway hyperresponsiveness as determined by the reduction in lung slice bronchial lumen area after the addition of 10^{-7} M Carbachol. MK, Montelukast; NS, normal saline; OVA, ovalbumin. Mean $\pm$ SEM. * $P < 0.05$

MK (IMM-NS-Vehicle = $21.6 \pm 0.8\%$, IMM-OVA-Vehicle = 29.6 ± 3.2 , IMM-OVA-early-MK = 20.9 ± 1.8 , IMM-OVA-late-MK = 20.0 ± 2.2 , IMM-OVA-early-late-MK = 21.3 ± 1.0) ($P < 0.0001$) (Figure 6A).

3.2.3 | Inflammation

The increased total BAL cellularity with ovalbumin confirm inflammation process with IMM-OVA-Vehicle = 19.2 ± 2.3 vs IMM-NS-Vehicle = $6.8 \pm 1.5 \times 10^4$ cells/mL ($P = 0.0393$). There was no significant decrease in total BAL cellularity following MK treatment (IMM-NS-Vehicle = $6.8 \pm 1.5 \times 10^4$ cells/mL, IMM-OVA-Vehicle = 19.2 ± 2.3 , IMM-OVA-early-MK = 13.0 ± 1.8 , IMM-OVA-late-MK = 12.6 ± 2.2 , IMM-OVA-early-late-MK = 15.0 ± 4.0) (Figure 6B).

4 | DISCUSSION

We describe herein a model of asthma in young mice based on a relatively short duration of active ovalbumin sensitization, which, we believe, is well suited to study AR at specific time points in infancy. The classical parameters that characterize AR in asthma were modified in the young sensitized group only, with a fourfold increase in RBM thickness, twofold increase in ASM area and threefold increase in collagen deposition in the small and large airways compared to the adult sensitized group, as well as to the adult and young control groups. Young animals are thus more prone to AR compared to adults. Previously, we described an upper contractility and AHR in immature rat trachea involving an increased secretion of Leukaemia Inhibitory Factor⁷ and a higher airway relaxation in human immature ASM than adult ASM.^{7,22} The leukotriene receptor

antagonist significantly reduced AHR and ASM remodelling, as well as BAL cellularity.

Animal models are very useful for asthma research although no single model mimicks all the features of human asthma. The best animal model of allergic sensitization in asthma appears to be the Canadian model of horses with heaves.²³ Our choice of the more available mouse model was based upon numerous previous studies on asthma and AR within the same species, and the immunological similarities with humans.^{9,24,25} However, this model is not the perfect model of human asthma since mice are not very symptomatic despite Th2 type allergic expression in the airways. The age of the young mouse pups was established at 4 weeks for both technical and theoretical reasons. First, since the sensitization protocol required multiple injections and instillations, it was adapted to this age group by reducing the sensitization phase to 6 weeks, based on previous data by Henderson,¹⁹ compared to, at least, 10–12 weeks in adult mice.¹⁰ Second, it has been shown that earlier (neonatal) ovalbumin sensitization in Balb/c mice may paradoxically induce a protective effect at an adult age regarding bronchial reactivity and inflammation.²⁶ Third, injury in very young animals may result in the cessation of the cycle of repair in the proliferation and squamation phases.²⁵ Fourth, the precision-cut lung slice technique and BAL are difficult to perform in animals whose body weight is less than 10 g.

We assessed bronchial responsiveness in vitro by establishing precision-cut lung slices^{18,27} in young mice. This technique is well suited to study bronchial (or vascular) contractility in small animals, in whom, unlike in adults, isometric contractility studies on whole isolated airways are impossible. Moreover, in lung slices, the lung

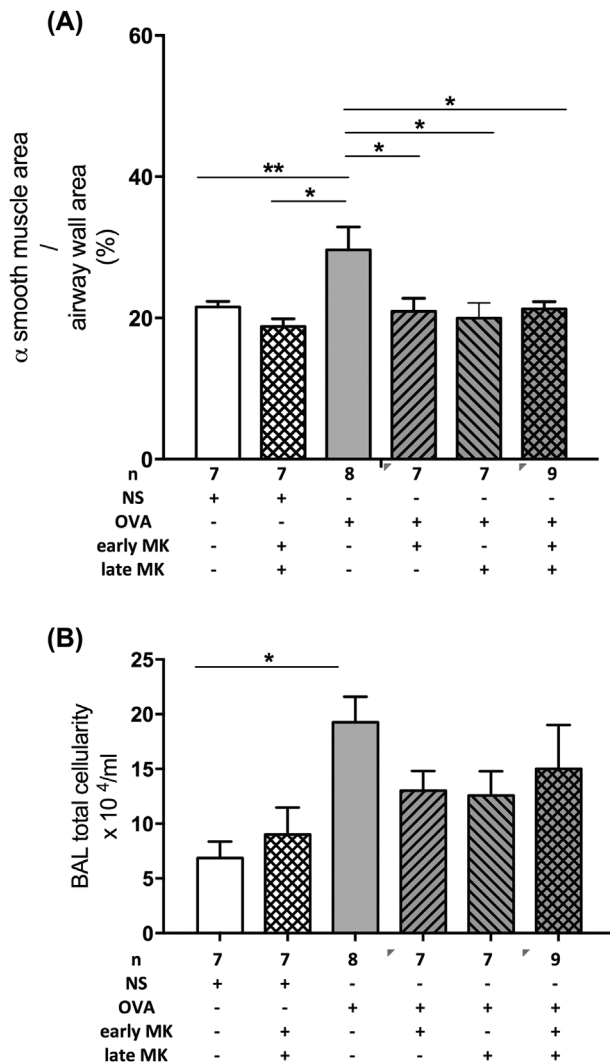


FIGURE 6 Effect of Montelukast in immature mice. A, Effect of Montelukast in Airway smooth muscle area according to sensitization and treatment regimen. B, Effect of Montelukast in Total BAL cellularity according to sensitization and treatment regimen. MK, Montelukast; NS, normal saline; OVA, ovalbumin. Mean $\pm$ SEM. * $P < 0.05$

architecture is better preserved, thus increasing its clinical relevance (eg, including part of elastic recoil). A certain degree of bronchial hyperreactivity may develop during the incubation period²⁸ but it was not a bias since both groups (sensitized and control) were studied at the same time point. Lung slice airway narrowing correlates well with more invasive measurement of airway resistance such as airway resistance, and murin precision-cut lung slices maintain important features of the whole lung.²⁹

We evaluated AR using key parameters, already described in asthma. These included bronchial RBM thickness (1, 2), ASM area and collagen deposition (3). The results of the present study confirm the presence of AR in sensitized young mice by showing a fourfold increase in reticular membrane thickness and a twofold increase in the smooth muscle area compared to adult mice. Saglani et al have shown AR that occurs very early (at week 4) in house dust mite sensitized

immature mice.¹¹ This may have implications regarding the natural history of asthma humans. In school age children, airway disease is established and has been regarded as probably not modifiable.³⁰ The present experimental data however show that these changes may be reversed, at least in part, in the presence of specific agents (ie, Montelukast)¹⁹ and are not permanently fixed, once the allergic airway is established. Noteworthy, there is no eosinophilic inflammation or RBM thickening in wheezing infants at a median age of 1 year but both features are present by the age of 3 years.^{3,30} Along the same line, Tillie-Leblond et al¹⁷ have demonstrated that structural abnormalities are present in children with severe asthma. In particular, the increase in area of ASM and the density of the vascular network are more pronounced in children with a persistent obstructive pattern, whereas RBM thickening is similar. These results are concordant with longitudinal studies, which emphasize the importance of the early onset of bronchial obstruction. Interestingly, we also observed pulmonary vascular collagen deposition in the sensitized group as previously reported in sensitized adult mice. This finding may relate to data obtained in atopic children, suggesting that lung neo-angiogenesis may develop at this age.³¹ Other studies have documented an increase in VEGF expression in association with such angiogenesis.³²

In young sensitized and challenged mice pups, the inflammatory process was more pronounced since total cellularity was significantly increased in the BAL fluid (12 days post-sensitization) with a higher proportion of eosinophils. As an example of the age-dependence of the immune pattern, CD14_159C lymphocytes (receptors for LPS) contribute to the atopic phenotype during mild childhood, which is no longer apparent by early adulthood.³³ Current state of the art in the field indicates that inflammation and airway remodeling are separate, parallel but interrelated processes.³⁴ In the data presented herein examining the inflammatory profiles in the young animals the changes in inflammatory cell counts presented show differences not observed in adult animals. Together with the immunochemistry data, this would suggest that the fit with the current state of the art is more marked in young animals.

In the second part of our study, we chose to focus on the efficacy of montelukast on ASM remodeling. ASM plays a pivotal role in asthma severity.^{17,35-37} ASM area is correlated with lower lung function (FEV1)¹⁷ and is associated with increased exacerbations rates.³⁶ Moreover, targeting ASM, for example with gallopamil improves asthma severity with reduced number of exacerbations.³⁸

There are few data regarding which treatments are most likely to prevent or reverse AR, in particular in children. Long-term inhaled steroids, the mainstay asthma treatment, do not appear to significantly change the natural history of asthma in children.³⁹ In some studies, in adults, prolonged high-dose inhaled steroids have demonstrated limited effects on RBM thickening.¹⁶ However, such findings are inconsistent. In adult asthmatic subjects, ASM mitogenesis and growth have been found to be resistant to glucocorticoids in vitro.⁴⁰ This may be due to lack of cell-specific anti-proliferative transcription factor, C/EBP alpha.⁴⁰ Using ASMC obtained from adults with severe asthma, Trian et al have demonstrated that an increased ASMC proliferation is triggered by extracellular calcium influx, which subsequently activates

mitochondrial biogenesis. A calcium channel blocker treatment in vitro reduced mitochondrial biogenesis involved in ASMC proliferation⁴¹ and attenuated AR, AHR and eosinophilia in a murine asthma model.⁴² In vivo, Gallopamil treatment, a calcium channel blocker reduced AR in adults with severe asthma.³⁸ Anti-leukotriene receptor antagonists, for example, Montelukast, have demonstrated substantial efficacy. In vitro, montelukast abolished the proliferation of asthmatic adult ASMC induced by house dust mite stimulated epithelium.¹³ In mice, established ASM layer thickening and subepithelial fibrosis were reversible following CysLT1-receptor blockade.¹⁴ MK pre-treatment also prevented the increase in ASM mass.^{13,43} Moreover, lower doses of this agent have significant anti-inflammatory effect on allergen-induced lung inflammation and fibrosis but not ASM hypertrophy.¹⁵ In the present study, although a single dose of MK was used, it still appears pharmacologically relevant. Indeed, in previous studies, the effects induced by a large range of doses were similar.^{13,44} The present study provides proof of concept that airway remodeling can be prevented or cured in an immature mouse model.

5 | CONCLUSION

Overall, the present experiments suggest that sensitization at a young age significantly affects the characteristics and magnitude of the airway immune (increased eosinophilia) and structural (enhanced remodeling) cell responses in this murine model. MK prevents and reverses AR in actively sensitized young mice.

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