

ORIGINAL ARTICLE

Chronic intranasal administration of *Aspergillus fumigatus* spores leads to aggravation of airway inflammation and remodelling in asthmatic rats

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ABSTRACT

Background and objective: Epidemiological evidence indicates a close link between exposure to fungi and deterioration of asthma. However, the role of fungi as an exogenous precipitant for initiation and progression of asthma has been incompletely explored. In this study, the effects of *Aspergillus fumigatus* exposure on airway inflammation and remodelling in a rat model of chronic asthma were investigated.

Methods: The rat model of chronic asthma was established by systemic sensitization and repeated challenge with ovalbumin (OVA). The asthmatic rats were exposed to chronic intranasal inhalation of *A. fumigatus* spores. Changes in airway inflammation, remodelling and BHR were measured after exposure to the fungus.

Results: Chronic inhalation of *A. fumigatus* spores elevated the production of T helper 2 (Th2) cytokines, increased the concentration of total serum IgE, and resulted in the recruitment of eosinophils and lymphocyte infiltration into the airways of asthmatic rats. Goblet cell hyperplasia, mucus hyperproduction and subepithelial collagen deposition were also induced by inhalation of the fungus. The remodelling changes induced by inhalation of the fungus paralleled the changes in BHR in this rat model of asthma.

Conclusions: Chronic exposure to *A. fumigatus* aggravated Th2 airway inflammation, promoted airway remodelling and increased BHR in OVA-sensitized and -challenged rats.

Key words: airway remodelling, *Aspergillus fumigatus*, asthma, bronchial hyperresponsiveness, inflammation.

INTRODUCTION

Fungal spores are universal atmospheric components and are also recognized as an important cause of

SUMMARY AT A GLANCE

The effects of chronic exposure to *Aspergillus fumigatus* on airway inflammation and remodelling were investigated in a rat model of chronic asthma. Chronic exposure to the fungus increased T helper 2 airway inflammation, airway remodelling and BHR in ovalbumin-sensitized and challenged rats. Avoidance of *A. fumigatus* exposure may prevent the progression of asthma.

respiratory allergies. The most common airborne fungi include *Cladosporium herbarum*, *Alternaria alternata* and *Aspergillus fumigatus*, although a multitude of other species are readily found.¹ Accumulated epidemiological evidence shows a close association between fungal exposure and asthma deterioration. Asthma deaths, hospital admissions, respiratory symptoms and PEF can be adversely affected by high concentrations of fungal spores in outdoor air.²⁻⁵ The risk of an asthmatic patient dying increases 2.16-fold following exposure to >1000 spores/m³ compared with exposure to <1000 spores/m³.² In a Canadian study, there was a positive association between spore counts, but not pollen counts, and emergency hospital visits for asthma.⁶

Aspergillus fumigatus is one of the most common fungi found in both indoor and outdoor environments. Engelhart and colleagues detected mean indoor and outdoor *A. fumigatus* counts of 8.1 and 9.4 cfu/m³, respectively.⁷ *A. fumigatus* spores are 2–3 µm in diameter and their surface is highly hydrophobic, allowing them to remain suspended in the air for long periods of time.^{8,9} Some estimates suggest that roughly 2000 spores lodge in the terminal airways every day.⁹ One key difference between *A. fumigatus* and other allergens, such as house dust mite, cat dander or grass pollen, is that the fungus can germinate and colonize the respiratory tract.¹ For most healthy individuals, inhalation of the spores of this ubiquitous airborne fungus is innocuous, because the innate immune system effectively eliminates the inhaled spores, preventing invasive infection or allergic airway inflammation.^{9,10} In 1–2% of patients with

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chronic asthma, intrapulmonary colonization with *A. fumigatus* elicits a chronic hypersensitivity disorder referred to as allergic bronchopulmonary aspergillosis (ABPA).^{11,12} However, the role of this fungus as an exogenous precipitant for the initiation and progression of asthma, in patients without clinically diagnosed ABPA, has been incompletely explored.

Allergic inflammation results in a changed milieu in the airways, with considerable infiltration of inflammatory cells, copious secretion of T helper 2 (Th2)-type cytokines, injury and detachment of the epithelium, and exposure of the basal membrane. *In vitro* studies mimicking the asthmatic airway microenvironment showed that changes in the airway epithelium facilitated the adhesion of *A. fumigatus* spores to the mucosal surface.^{13,14} In an allergic environment, Th2-type cytokines and allergens have the potential to sustain airway inflammation and promote remodelling, through a cooperative effect on cytokine and growth factor release by the bronchial epithelium.¹⁵ A study by Beisswenger and colleagues showed that after *Pseudomonas aeruginosa* infection, ovalbumin (OVA)-sensitized and -challenged mice had significantly more viable bacteria in their lungs compared with non-sensitized controls, indicating that allergic airway inflammation suppressed the innate antimicrobial host defence in the asthmatic animals.¹⁶ These findings suggest that asthmatic patients may respond differently to *A. fumigatus* exposure compared with healthy individuals.

We hypothesized that allergic airway inflammation and damage to epithelial cells may alter the response to chronic *A. fumigatus* exposure, resulting in colonization of this fungus in the airways, thus aggravating airway inflammation and promoting airway remodelling and BHR in asthmatic subjects. To address this, the present study investigated whether chronic inhalation of *A. fumigatus* spores led to colonization of the fungus in the airways, and aggravation of airway inflammation, remodelling and BHR in OVA-sensitized and -challenged rats.

METHODS

Animals

Male Wistar rats weighing 220–250 g were purchased from Shanghai SLAC Laboratory Animal Co. Ltd (Shanghai, China). The rats were housed under specific pathogen-free conditions and maintained on a 12-h light–dark cycle, with food and water *ad libitum*. All experiments were approved by the Shanghai Jiaotong University Animal Investigational Committee and were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the Ministry of Health, China.

Rats were randomly divided into seven experimental groups (eight in each group): (i) OVA-sensitized asthma rats (sensitized and challenged with OVA); (ii–iv) OVA-sensitized asthma + *A. fumigatus* spores rats (sensitized and challenged with OVA, and 1 week after cessation of OVA exposure rats were given intranasal inhalations of *A. fumigatus* spores for 1, 3 or 5 weeks);

(v) OVA-sensitized and saline-challenged rats (sensitized with OVA and challenged with saline); (vi) OVA-sensitized and saline-challenged + *A. fumigatus* spores rats (sensitized with OVA and challenged with saline, and 1 week after cessation of saline challenge rats were given intranasal inhalations of *A. fumigatus* spores for 5 weeks); and (vii) OVA-sensitized asthma + saline rats (sensitized and challenged with OVA, and 1 week after cessation of OVA exposure rats were given intranasal inhalations of saline for 5 weeks). Animals were killed 24 h after the last challenge or intranasal inhalation.

Sensitization and antigen challenge protocol

Animals were sensitized and challenged as previously described.^{17,18} On day 1, rats were sensitized by intraperitoneal injection of 1 mg of OVA (Grade III; Sigma-Aldrich, St Louis, MO, USA), together with 100 mg of Al(OH)₃ in 0.5 mL of saline. Simultaneously, 1 mL of *Bordetella pertussis* vaccine, containing 6×10^9 heat-killed organisms, was administered intraperitoneally as an adjuvant. A booster sensitization using the same procedure was performed on day 8. From day 15 to day 57, the rats were exposed to aerosolized OVA (1% in saline) or saline alone, for 30 min thrice weekly. The aerosol was generated with a nebulizer (PARIBOY N037; PARI, Starnberg, Germany) and was drawn into the exposure chamber containing the awake animals.

Preparation and administration of *A. fumigatus* spores

A strain of *A. fumigatus* isolated from a patient with invasive aspergillosis was used in this study. The spore suspension was prepared as previously described, with slight modifications.¹⁹ The organism was grown on Sabouraud dextrose agar plates (Biomérieux, Marcy Etoile, France) for 7–10 days at 37°C. The surface of each plate was washed with 100 mL of sterile 0.1% Tween-80 (Sigma-Aldrich) in saline. The resulting suspension of spores was filtered through sterile gauze to remove clumps and hyphal debris, and then washed once and resuspended in 4 mL of 0.1% Tween-80 in saline. The concentration of spores in the suspension was counted in a haemocytometer. The suspension was then diluted to the desired concentration, and the concentration of spores was again measured before administration. Bacterial contamination was excluded by the absence of colony-forming units after plating on an agar plate. Endotoxin was measured by the Limulus Amebocyte Lysate Assay (Westang Co. Ltd, Shanghai, China) and shown to be <1 EU/mL. Virulence was confirmed by the invasive pulmonary infection caused by inoculation of the spores into cyclophosphamide-treated rats.

The validity and reliability of the techniques used for administering *A. fumigatus* spores to rats have been established in previous studies.^{20,21} One week after the last challenge (day 64), rats were intranasally

administered with *A. fumigatus* spores or saline twice a week, on Monday and Friday. Animals were anaesthetized with isofluran (Qiupai Pharmaceutical Co. Ltd, Hebei, China). When the rats were unresponsive but breathing comfortably, they were placed in a vertical position and 100 μ L of spore suspension (1×10^4 spores) or saline was instilled directly into the nostrils. The animals were kept in the vertical position for 10 min, and then allowed to recover in a supine position.

Measurement of airway reactivity

Airway reactivity was measured as previously described.¹⁸ Rats were anaesthetized by intraperitoneal injection of 50 mg/kg pentobarbital (Sigma-Aldrich), and were placed on a heating pad (37°C). The trachea was cannulated with a Teflon cannula, and then connected to an animal ventilator (JiangXi TeLi Anaesthesia & Respiration Co. Ltd, Jiangxi, China). Spontaneous respiratory movements were interrupted by injection of pancuronium bromide (Huapai Chemical Industry Corp., Jiangsu, China), at 0.2 mg/kg bodyweight. The rats were ventilated with oxygen-enriched air at 60 strokes/min and a stroke volume of 2–2.5 mL. A polyethylene catheter was inserted into the external jugular vein for administration of i.v. drugs and fluids. The animals' arterial blood gases were measured after 10 min of ventilation, and the tidal volume was adjusted to keep PaO₂ and PaCO₂ within the normal ranges. The transpulmonary pressure was measured with a differential pressure transducer (Smup-B bio-signal processing system, Medical Equipment Research Center of Shanghai Medical College, Shanghai, China), one end of which was attached to an air-filled catheter inserted into the pleural cavity and the other end to a catheter connected to a side-port of the outlet of the tracheal cannula. Airflow at the outlet of the intratracheal cannula was measured with a pneumotachograph (Hans Rudolph, Kansas City, MO, USA). The tidal volume was obtained by integration of the flow. Airway resistance (R_{aw}) was continuously calculated from tidal volume, air flow and transpulmonary pressure (PRS800, Mumed, London, UK). After the real-time data stabilized, baseline R_{aw} was measured for a period of 10 min. Acetylcholine chloride (300 μ g/kg) was administered through the external jugular vein, and acetylcholine-induced bronchoconstriction was measured as the percentage increase in R_{aw} , by comparing the peak of the response with baseline R_{aw} .

BAL

Lungs were lavaged through the tracheal cannula with 6×5 mL of PBS (pH 7.2). Lavage fluid was recovered by gentle aspiration with a syringe. The BAL fluid was centrifuged, the supernatant was stored at –80°C for measurement of cytokines and the cell pellet was resuspended in 1 mL of PBS. Total cell counts were determined in a haemocytometer. Differential cell

counts were obtained by examining at least 500 cells on Wright–Giemsa-stained cytospin slides.

Morphometric analysis of lung tissue

After BAL, the lungs were fully inflated by intratracheal perfusion with 4% paraformaldehyde. Lungs were then dissected and placed in fresh paraformaldehyde for 24 h. Routine histological techniques were used to paraffin-embed the tissue, and 5- μ m sections of lung tissue were stained with HE, periodic acid Schiff (PAS) and Masson trichrome to visualize peribronchial inflammation, hyperplasia of goblet cells and subepithelial deposition of collagen. Gomori methanamine silver (GMS) stain was used to identify *A. fumigatus* spores or hyphae that were present in the rat lungs.

Digital image analysis was performed on histological sections, using Image-Pro Plus software version 5.0 (Media Cybernetics, Silver Springs, MD, USA), as previously described.^{22–24} Nuclear profiles in HE-stained sections were counted in the lamina propria. The extent of inflammatory cell infiltration was expressed as the mean number of cells per 100 μ m of epithelial basement membrane in the lamina propria. The full length of the epithelial surface was examined using a $\times 100$ oil immersion lens to enumerate intraepithelial eosinophils. The data were expressed as number of cells per millimetre of epithelial basement membrane. The inner perimeter of the airway epithelium and the length of the section not covered with intact ciliated cells or goblet cells were measured. The ratio of the length of detached epithelium to the inner perimeter was used to assess the extent of epithelial detachment. Subepithelial collagen deposition in the airways was determined by digital colour analysis of Masson's trichrome-stained histological sections. The area of collagen was calculated as the non-cellular portion 20 μ m beneath the basement membrane that was stained blue. The extent of collagen deposition was assessed as the percentage of blue-stained area relative to the total area of the region. The PAS-stained area within the basal border and the inner border of airway epithelium were measured in PAS-stained sections. The extent of goblet cell hyperplasia was assessed as the percentage of PAS-stained area relative to the total epithelial area. For each lung compartment, image data from a total of five sections, each taken from a separate paraffin block, were averaged. In each set of three sections, measurements were taken from all available bronchioles and small bronchi, ranging in diameter from 200 to 2000 μ m (calculated on basement membrane perimeter), which were cut in transverse section and free of branching.

Total IgE determination

Serum total IgE was measured using an ELISA kit, according to the manufacturer's instructions (USCN-LIFE Co., Wuhan, China). The limit of detection was 0.2 ng/mL.

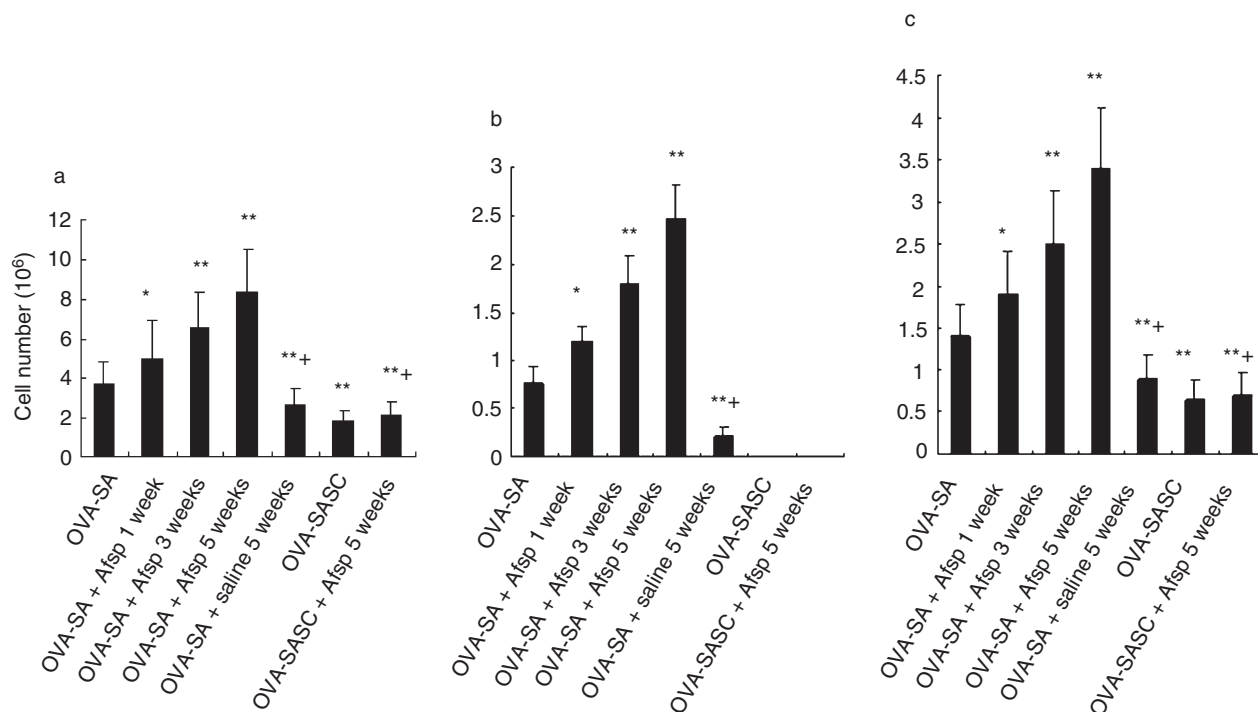


Figure 1 Cell counts in BAL fluid in the different groups. Total cells (a), eosinophils (b) and lymphocytes (c) in BAL fluid were significantly increased in OVA-sensitized asthmatic rats after intranasal inhalation of *Aspergillus fumigatus* spores for 1–5 weeks. Data are expressed as mean $\pm$ SEM of eight individual samples. * $P < 0.05$, ** $P < 0.01$ versus the OVA-SA group; + $P < 0.01$ versus the OVA-SA + Afsp 5 weeks group. OVA-SA, ovalbumin-sensitized asthma; OVA-SASC, ovalbumin-sensitized and saline-challenged; Afsp, *A. fumigatus* spores.

Cytokine measurements

The levels of IL-13, IL-5, interferon- γ (IFN- γ) and transforming growth factor- β (TGF- β) in supernatants of BAL fluid were quantified by ELISA (detection limits 1.5, 7, 2 and 4.6 pg/mL, respectively), using Quantikine kits according to the instructions provided by the manufacturer (R&D Systems Inc., Chicago, IL, USA).

Statistical analysis

Data are presented as mean $\pm$ SEM. Statistical analyses were performed using SAS version 6.12 software (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was used to compare mean values for the different groups, followed by Student–Newman–Keuls test for multiple comparisons. A P -value < 0.05 was accepted as statistically significant.

RESULTS

Inflammatory cells in BAL fluid

The numbers of total cells, eosinophils and lymphocytes in BAL fluid of OVA-sensitized asthmatic rats were significantly elevated after inhalation of *A. fumi-*

gatus spores for 1–5 weeks. Conversely, the numbers of inflammatory cells were decreased after inhalation of saline for 5 weeks in OVA-sensitized asthmatic rats. There were no obvious changes in the numbers of inflammatory cells in OVA-sensitized and saline-challenged rats, after inhalation of fungal spores for 5 weeks (Fig. 1).

Airway reactivity

The percentage increase in R_{aw} in OVA-sensitized asthmatic rats was significantly greater after inhalation of *A. fumigatus* spores for 3–5 weeks, and was sustained after saline inhalation for 5 weeks. There was no obvious increase in R_{aw} in OVA-sensitized and saline-challenged rats, after inhalation of fungal spores for 5 weeks (Fig. 2).

Serum concentration of total IgE

As depicted in Figure 3, the serum IgE concentration in OVA-sensitized asthmatic rats was significantly upregulated after inhalation of *A. fumigatus* spores for 1–5 weeks, and downregulated after saline inhalation for 5 weeks. There was no significant increase in total IgE concentration in OVA-sensitized and

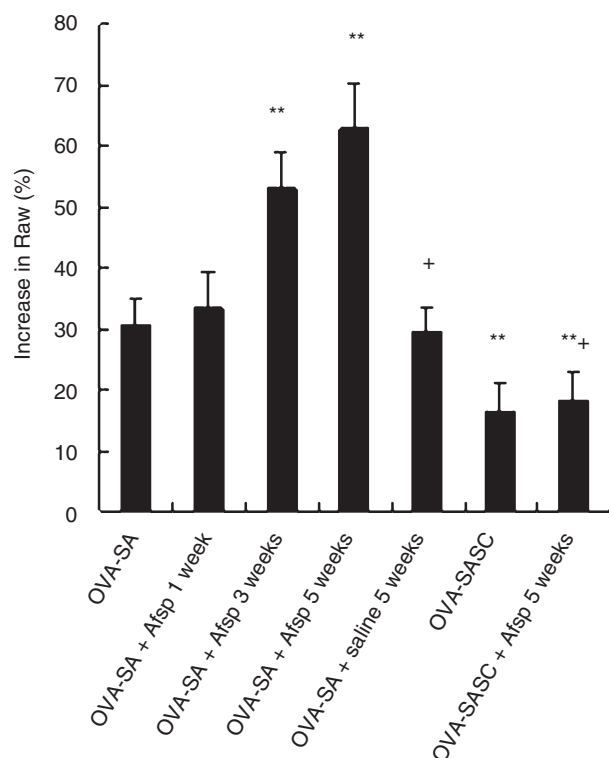


Figure 2 Changes in airway reactivity in the different groups. The percentage increase in airway resistance (R_{aw}) was significantly greater in OVA-sensitized asthmatic rats after intranasal inhalation of *Aspergillus fumigatus* spores for 3–5 weeks. Data are expressed as mean $\pm$ SEM of eight individual samples. ** $P < 0.01$ versus the OVA-SA group; + $P < 0.01$ versus the OVA-SA + Afsp 5 weeks group. OVA-SA, ovalbumin-sensitized asthma; OVA-SASC, ovalbumin-sensitized and saline-challenged; Afsp, *A. fumigatus* spores.

saline-challenged rats, after inhalation of fungal spores for 5 weeks (Fig. 3).

Concentration of cytokines in BAL fluid

The concentrations of IL-13, IL-5 and TGF- β , but not IFN- γ , in BAL fluid of OVA-sensitized asthmatic rats were upregulated after inhalation of *A. fumigatus* spores for 1–5 weeks, and downregulated after saline inhalation for 5 weeks. There were no significant changes in the concentrations of IL-5, IL-13, TGF- β or IFN- γ in OVA-sensitized and saline-challenged rats, after inhalation of fungal spores for 5 weeks (Fig. 4).

Morphometric analysis

The extent of inflammatory cell, as well as eosinophil infiltration, epithelial detachment, goblet cell hyperplasia and subepithelial collagen deposition in the airways of OVA-sensitized asthmatic rats, was significantly increased after inhalation of *A. fumigatus*

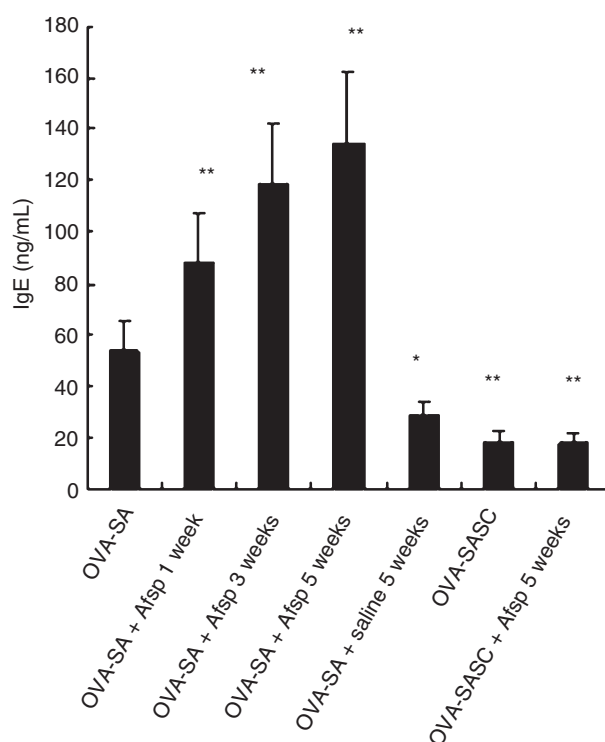


Figure 3 The changes in serum IgE concentration in the different groups. The concentration of IgE was significantly elevated in OVA-sensitized asthmatic rats after intranasal inhalation of *Aspergillus fumigatus* spores for 1–5 weeks. Data are expressed as mean $\pm$ SEM of eight individual samples. * $P < 0.05$, ** $P < 0.01$ versus the OVA-SA group. OVA-SA, ovalbumin-sensitized asthma; OVA-SASC, ovalbumin-sensitized and saline-challenged; Afsp, *A. fumigatus* spores.

spores for 1–5 weeks. Although airway inflammation, as well as epithelial detachment, was significantly reversed, airway remodelling was unchanged in OVA-sensitized rats after saline inhalation for 5 weeks. There were no airway inflammation and remodelling changes in OVA-sensitized and saline-challenged rats after inhalation of fungal spores for 5 weeks (Figs 5–7).

Observation of *A. fumigatus* colonization

Gomori methanamine silver stain was used to identify *A. fumigatus* spores or hyphae present in the rat lungs. No hyphae were found in the airways or lung parenchyma in OVA-sensitized asthmatic and OVA-sensitized and saline-challenged rats, after inhalation of *A. fumigatus* spores for 5 weeks. However, entire or degraded fungal spores persisted within lung mononuclear cells in the asthmatic rats (Fig. 8). The absence of colonization of *A. fumigatus* in the rat lungs was also confirmed by lung chitin assay (data not shown).

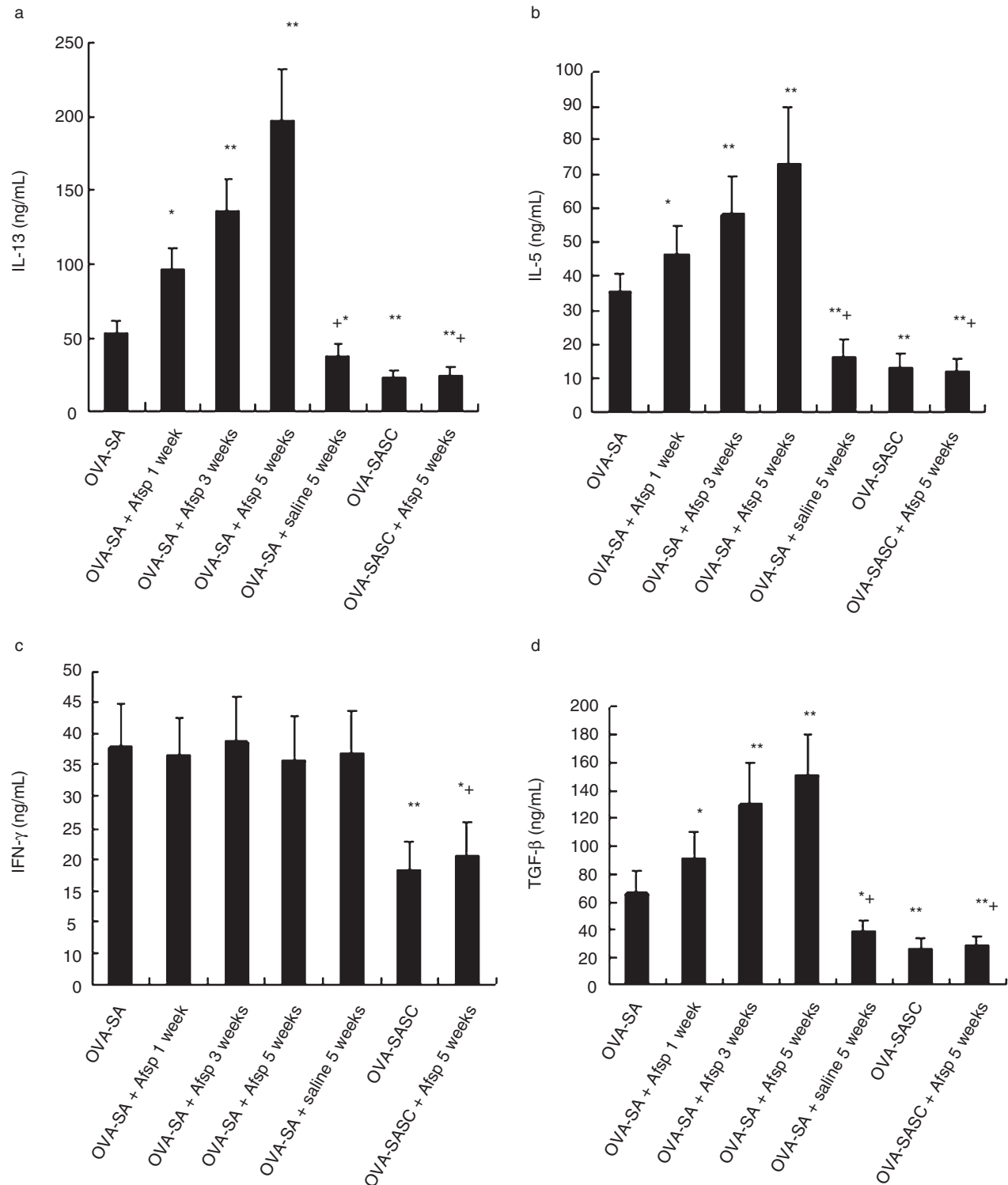


Figure 4 Changes in cytokines in BAL fluid in the different groups. The concentrations of IL-13 (a), IL-5 (b) and transforming growth factor- β (TGF- β) (d) in BAL fluid were significantly upregulated, whereas the concentration of interferon- γ (IFN- γ) (c) was unchanged in OVA-sensitized asthmatic rats after intranasal inhalation of *Aspergillus fumigatus* spores for 1–5 weeks. Data are expressed as mean $\pm$ SEM of six to eight individual samples. * $P < 0.05$, ** $P < 0.01$ versus the OVA-SA group; + $P < 0.01$ versus the OVA-SA + Afsp 5 weeks group. OVA-SA, ovalbumin-sensitized asthma; OVA-SASC, ovalbumin-sensitized and saline-challenged; Afsp, *A. fumigatus* spores.

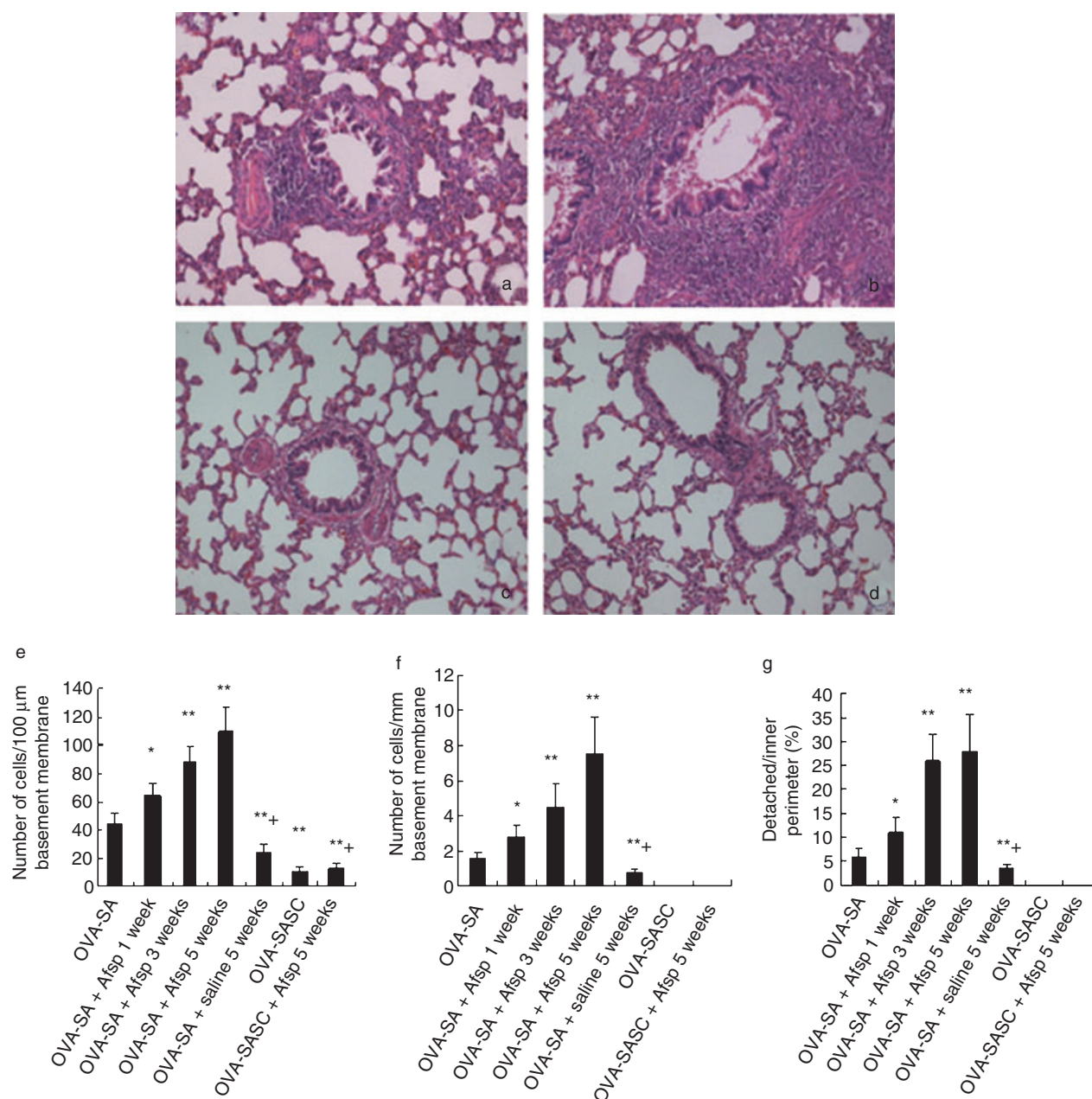


Figure 5 Airway inflammatory cell infiltration in the different groups. HE-stained lung sections from OVA-SA group (a), OVA-SA + Afsp 5 weeks group (b), OVA-SASC group (c) and OVA-SASC + Afsp 5 weeks group (d), showing inflammation around small airways. Original magnification, $\times 200$. Morphometric analysis showed that inflammatory cell infiltration (e), eosinophil infiltration (f) and epithelial detachment (g) were significantly increased in OVA-sensitized asthmatic rats after intranasal inhalation of *Aspergillus fumigatus* spores for 1–5 weeks. Data are expressed as mean $\pm$ SEM of five individual samples. * $P < 0.05$, ** $P < 0.01$ versus the OVA-SA group; + $P < 0.01$ versus the OVA-SA + Afsp 5 weeks group. OVA-SA, ovalbumin-sensitized asthma; OVA-SASC, ovalbumin-sensitized and saline-challenged; Afsp, *A. fumigatus* spores.

DISCUSSION

In the present study, OVA-sensitized asthmatic rats were exposed to prolonged intranasal inhalation of *A. fumigatus* spores, in order to explore the effects of chronic *A. fumigatus* exposure on asthma. The main finding from the study is that chronic *A. fumigatus*

exposure aggravates airway inflammation and remodelling, as well as BHR, in a dose-dependent fashion in OVA-sensitized asthmatic rats. This is the first study to focus on the influence of chronic *A. fumigatus* exposure on non-ABPA asthmatic animals that mimics asthma. The findings from the study enhance our understanding of the deleterious effects

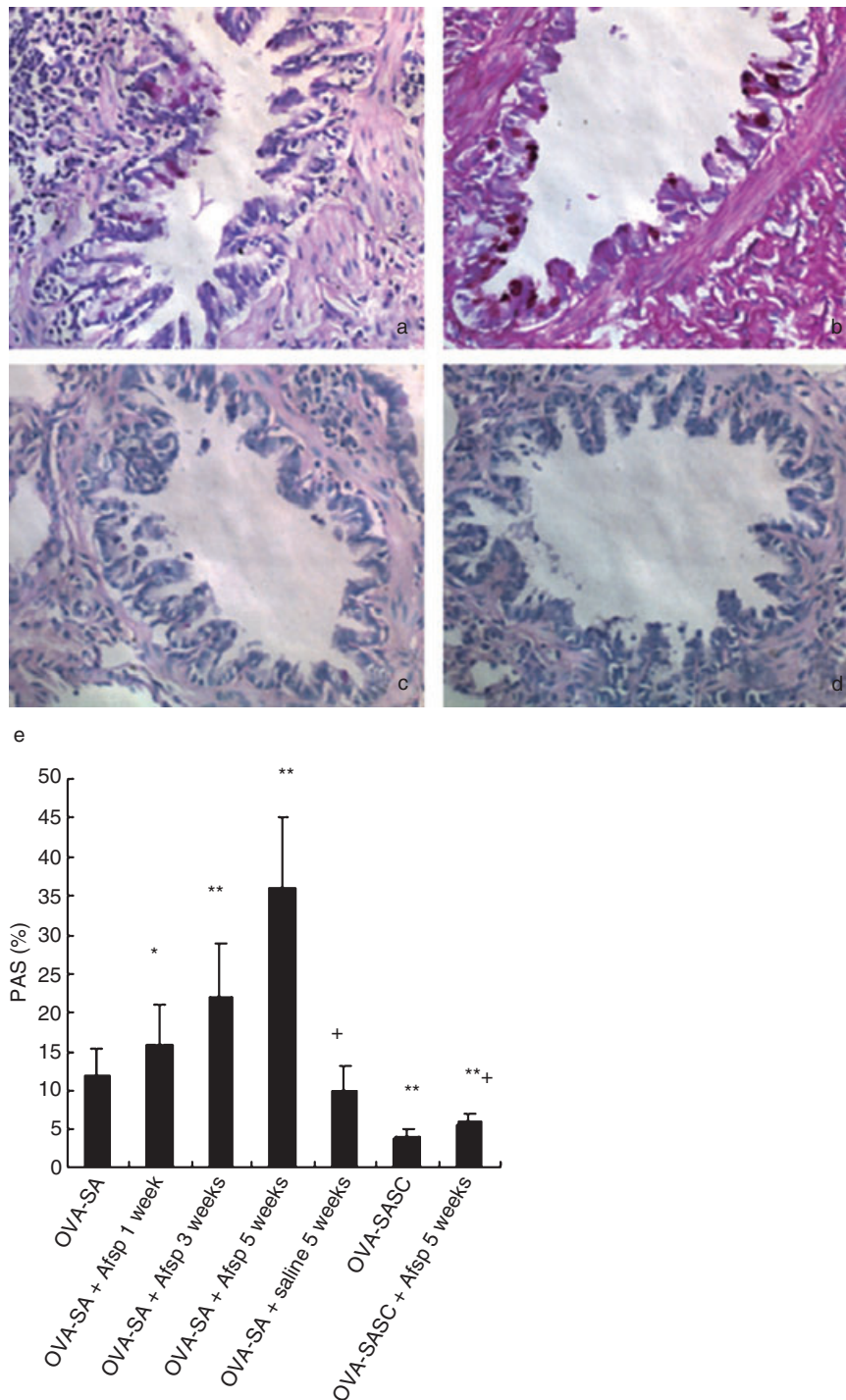


Figure 6 Goblet cell hyperplasia in the different groups. Periodic acid Schiff (PAS)-stained lung sections from OVA-SA group (a), OVA-SA + Afsp 5 weeks group (b), OVA-SASC group (c) and OVA-SASC + Afsp 5 weeks group (d), showing goblet cell hyperplasia (dark magenta staining) in the rat airways. Original magnification, $\times 400$. Morphometric analysis showed that goblet cell hyperplasia (e) was significantly increased in OVA-sensitized asthmatic rats after intranasal inhalation of *Aspergillus fumigatus* spores for 1–5 weeks. Data are expressed as mean $\pm$ SEM of five individual samples. * $P < 0.05$, ** $P < 0.01$ versus the OVA-SA group; + $P < 0.01$ versus the OVA-SA + Afsp 5 weeks group. OVA-SA, ovalbumin-sensitized asthma; OVA-SASC, ovalbumin-sensitized and saline-challenged; Afsp, *A. fumigatus* spores.

of chronic *A. fumigatus* exposure on airway inflammation and remodelling in non-ABPA asthmatic patients.

Ovalbumin-sensitized asthmatic rats were exposed to low concentrations of *A. fumigatus* spores for a long time, to mimic the actual exposure of asthma patients. The concentration of *A. fumigatus* spores (1×10^4 spores) used in this study was less than that used in the development of murine models of ABPA and invasive pulmonary aspergillosis.^{25,19} The concen-

tration used was calculated based on the concentration of *A. fumigatus* spores in the environment, the tidal volume and respiratory rate of the rat, and the species coefficient between rats and humans, and was determined after preliminary experiments.

Although the asthmatic rats were not sensitized to *A. fumigatus* before spore inhalation, chronic inhalation of *A. fumigatus* spores resulted in the recruitment of more eosinophils, lymphocyte infiltration in the airways, elevated levels of inflammatory cytokines in

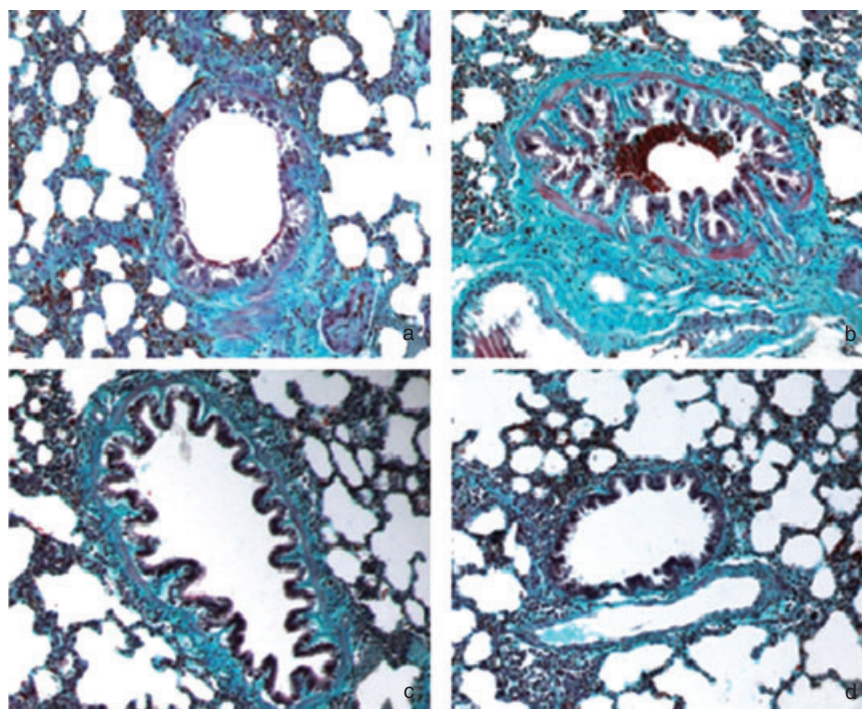
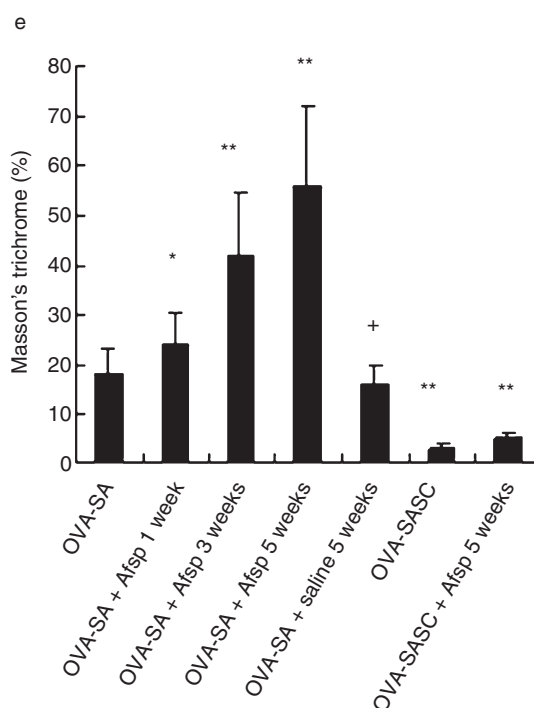


Figure 7 Subepithelial collagen deposition in the different groups. Masson trichrome-stained lung sections from OVA-SA group (a), OVA-SA + Afsp 5 weeks group (b), OVA-SASC group (c) and OVA-SASC + Afsp 5 weeks group (d), showing subepithelial collagen deposition (light blue staining) in the rat airways. Original magnification, $\times 200$. Morphometric analysis showed that subepithelial collagen deposition (e) was significantly increased in OVA-sensitized asthmatic rats after intranasal inhalation of *Aspergillus fumigatus* spores for 1–5 weeks. Data are expressed as mean $\pm$ SEM of five individual samples. * $P < 0.05$, ** $P < 0.01$ versus the OVA-SA group; + $P < 0.01$ versus the OVA-SA + Afsp 5 weeks group. OVA-SA, ovalbumin-sensitized asthma; OVA-SASC, ovalbumin-sensitized and saline-challenged; Afsp, *A. fumigatus* spores.



BAL fluid, and increased total serum IgE concentration in OVA-sensitized asthmatic rats. The increase in eosinophil infiltration in the airways has been associated with severe exacerbation of asthma.²⁶ Infiltrating T lymphocytes have been shown to be responsible for airway hyperresponsiveness, allergic inflammation and goblet cell hyperplasia in a murine model of acute allergic airway disease.²⁷ Inflammatory cytokines play an important role in the pathogenesis of asthma. IL-13, IL-5 and TGF- β are cytokines of

particular interest because of their potential role in chronic airway changes in asthma. IL-13 upregulates the expression of mucin and induces hyperplasia of goblet cells.²⁸ IL-5 induces the infiltration of eosinophils into the airways.²⁶ The profibrotic growth factor, TGF- β , is closely associated with airway remodelling.²⁹

As a barrier between the external environment and the internal milieu, the airway epithelium is exposed to various stimuli, including allergens. Several

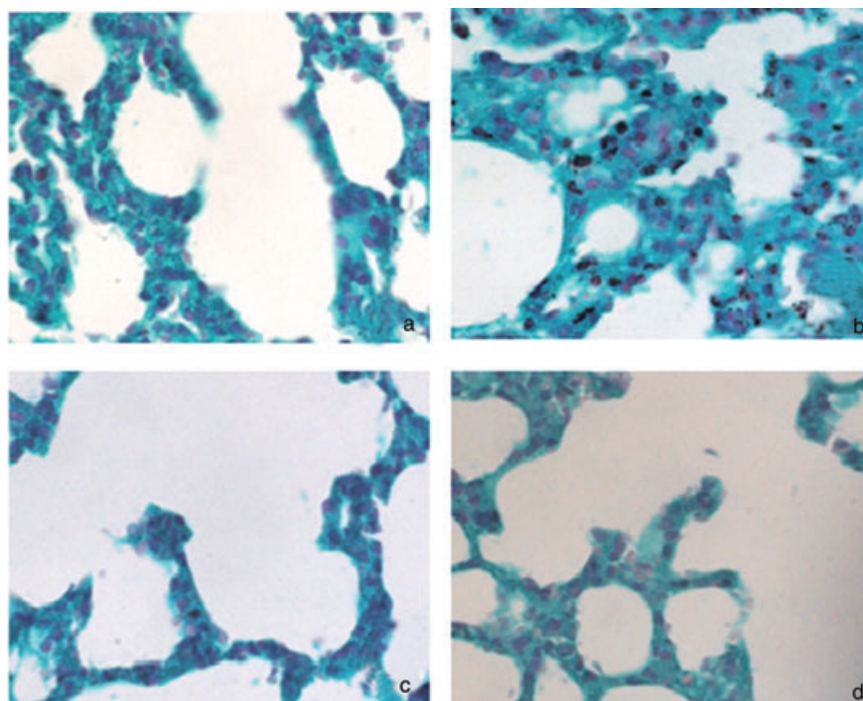


Figure 8 *Aspergillus fumigatus* spores in lungs of rats in the different groups. Gomori methanamine silver-stained sections from OVA-SA group (a), OVA-SA + Afsp 5 weeks group (b), OVA-SASC group (c) and OVA-SASC + Afsp 5 weeks group (d), showing persistence of entire or degraded fungal spores (black material) within lung mononuclear cells in the asthmatic rats. Original magnification, $\times 400$. OVA-SA, ovalbumin-sensitized asthma; OVA-SASC, OVA-sensitized and saline-challenged; Afsp, *A. fumigatus* spores.

pathological changes occur in the airway epithelium in asthma, including epithelial desquamation, goblet cell hyperplasia and mucus hypersecretion, and the extent of epithelial damage correlated with BHR and epithelial permeability in asthma.³⁰ Injured or activated airway epithelial cells are sources of inflammatory cytokines and a range of growth factors involved in the initiation of asthma, as well as its progression, orchestrating both continued inflammation and remodelling.³¹ In a study by Kauffman and coworkers, *A. fumigatus* extracts, which possess protease activity, caused epithelial cell desquamation and release of IL-6 and IL-8.³² In the present study, chronic *A. fumigatus* exposure elicited prominent epithelial detachment in the airways in OVA-sensitized asthmatic rats, and the extent of epithelial injury was paralleled by the extent of airway inflammation and remodelling.

Airway remodelling is a feature of asthma characterized by structural changes, including airway wall thickening due to goblet cell hyperplasia/hypertrophy, collagen deposition beneath the basement membrane, and airway smooth muscle thickening as a result of smooth muscle cell hyperplasia/hypertrophy.³³ In the present study, chronic *A. fumigatus* exposure induced prominent goblet cell hyperplasia and subepithelial collagen deposition in the OVA-sensitized asthmatic rats. It is worth noting that the airway remodelling induced by *A. fumigatus* exposure paralleled the changes in BHR in the asthmatic animals. This supports there being a contribution of airway remodelling to the increase in BHR induced by chronic exposure to *A. fumigatus* in the OVA-sensitized asthmatic rats.

Allergic bronchopulmonary aspergillosis occurs in non-immunocompromised patients and is one of the hypersensitivity disorders induced by *A. fumigatus*. It

has been estimated to occur in 1–2% of chronic asthmatic patients and in up to 10% of patients with cystic fibrosis.³⁴ The inhalation of spores in the environment is followed by growth of hyphae in the airway mucus and stimulation of an immune response involving Th2 CD4+ T cells and IgE and IgG antibodies.¹² In the present study, chronic *A. fumigatus* exposure did not lead to invasive infection or colonization of the fungus in the airways of OVA-sensitized asthmatic rats. However, the lack of *A. fumigatus* colonization in the lungs does not rule out the deleterious effects of chronic *A. fumigatus* exposure on OVA-sensitized asthmatic rats. GMS-stained histological sections showed the persistence of entire or degraded fungal spores within lung mononuclear cells in the asthmatic rats after chronic inhalation of *A. fumigatus* spores. This result is consistent with the findings of Hogaboam and colleagues in a mouse model of ABPA that involved sensitization with *A. fumigatus* extracts and challenge by inhalation of 5.0×10^6 *A. fumigatus* spores.²⁵ The persistence of fungal material in the lungs of allergic animals promotes the chronicity of inflammation and BHR, as elimination of this fungal material reverses these major changes.¹¹ Therefore, the effects of chronic exposure to *A. fumigatus* on airway inflammation and remodelling do not depend on infection or colonization of the fungus.

The OVA-sensitized and saline-challenged rats were also exposed to the same concentration of *A. fumigatus* spores for 5 weeks. There was no airway inflammation, remodelling or BHR in these rats. GMS-stained histological sections of the rats in this group did not show any evidence of hyphae or spores in the lungs. This indicated that the low concentration and prolonged inhalation of *A. fumigatus* spores was safe in non-asthmatic rats, and did not cause allergic

airway inflammation or invasive infection. In the present study, the airway inflammation in OVA-sensitized asthmatic rats showed some degree of reversibility, whereas the remodelling changes, as well as BHR, was sustained after 5 weeks of saline inhalation. This finding is consistent with those from a previous study on a murine model of chronic asthma.³⁵

The present study has shown that chronic exposure to *A. fumigatus* aggravated Th2 airway inflammation, promoted airway remodelling and increased BHR in OVA-sensitized asthmatic rats. The results from this study suggest that avoidance of *A. fumigatus* exposure may be helpful in preventing the progression of asthma.

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