

ORIGINAL ARTICLE

Effects of chronic exposure to *Aspergillus fumigatus* on epidermal growth factor receptor expression in the airway epithelial cells of asthmatic rats

Fu-Sheng Gao, Tong-Mei Cao, Yan-Yan Gao, Mei-Juan Liu, Yong-Quan Liu, and Ze Wang

Department of Respiratory Medicine, The Affiliated Hospital of Weifang Medical College, Weifang, China

ABSTRACT

Epidemiologic studies suggest that increased concentrations of airborne spores of *Aspergillus fumigatus* closely relate to asthma aggravation. Chronic exposure to *A. fumigatus* aggravates airway inflammation, remodeling, and airway hyperresponsiveness in asthmatic rats. The effects of chronic exposure to *A. fumigatus* on epidermal growth factor receptor (EGFR) expression in the airway epithelial cells of asthmatic rats remain unclear. This study aimed to investigate the effects of chronic exposure to *A. fumigatus* on injury and shedding of airway epithelium, goblet cell metaplasia, and EGFR expression in the airway epithelial cells of asthmatic rats. A rat model of chronic asthma was established using ovalbumin (OVA) sensitization and challenge. Rats with chronic asthma were then exposed to long-term inhalation of spores of *A. fumigatus*, and the dynamic changes in injury and shedding of airway epithelium, goblet cell metaplasia, and EGFR expression were observed and analyzed. Chronic exposure to *A. fumigatus* could aggravate airway epithelial cell damage, upregulate the expression of EGFR and its ligands EGF and TGF- α , promote goblet cell metaplasia, and increase airway responsiveness in rats with asthma. Chronic exposure to *A. fumigatus* upregulates the expression of EGFR and its ligands in asthmatic rats. The EGFR pathway may play a role in asthma aggravation induced by exposure to *A. fumigatus*.

KEYWORDS airway, *aspergillus fumigatus*, asthma, epithelium, exposure, goblet cell metaplasia, hyperreactivity, injury, rat, remodeling

INTRODUCTION

Injury and shedding of airway epithelial cells, goblet cell metaplasia, and mucus hypersecretion are pathologic features of bronchial asthma that are closely related to airway responsiveness and disease severity. The epidermal growth factor receptor (EGFR) plays an important role in airway epithelial cell repair and airway remodeling. Activated EGFR activates the ERK1/2 signaling pathway, increases the expression of the mucin gene *MUC5AC*, and induces goblet cell metaplasia and airway mucus hypersecretion [1]. It has been found that increased EGFR expression in the airway epithelial cells of patients with asthma pos-

itively correlates with disease severity [2]. Spores of *Aspergillus fumigatus* are common inhalant allergens in both indoor and outdoor environments. Epidemiologic studies suggest that increased concentrations of airborne spores of *A. fumigatus* closely relate to asthma aggravation [3]. Our previous studies have found that chronic exposure to *A. fumigatus* aggravates Th2 airway inflammation, promotes airway remodeling, including goblet cell metaplasia, and upregulates mucin 5AC expression in asthmatic rats [4, 5]. The effects of chronic exposure to *A. fumigatus* on EGFR expression in the airway epithelial cells of asthmatic rats remain unclear. In this study, a rat model of chronic asthma was established using ovalbumin (OVA) sensitization and challenge. The rats with chronic asthma were then exposed to long-term inhalation of spores of *A. fumigatus*, and the dynamic changes in injury and shedding of airway epithelium, goblet cell metaplasia, and EGFR

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Address correspondence to Fu-Sheng Gao, Department of Respiratory Medicine, The Affiliated Hospital of Weifang Medical College, Yuhe Road 2428, Weifang 261031, China. E-mail: gaofs888@163.com

expression were observed to investigate the mechanism by which chronic exposure to *A. fumigatus* aggravates asthma.

METHODS

Animals

Healthy male Wistar rats approximately 5–6 weeks of age and weighing approximately 140–180 g were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. (SCXK (Shanghai); 2003–0003). The rats were housed under specific pathogen-free conditions and maintained on a 12-hour light–dark cycle, with food and water *ad libitum*. All experiments described in this study were approved by the Weifang Medical College Animal Investigational Committee and performed in accordance with the Guide for the Care and Use of Laboratory Animals published by Ministry of Health People's Republic of China.

Male Wistar rats were randomly divided into the following 7 groups (8 rats per group): the chronic asthma group (A) to establish a rat model of chronic asthma using OVA systemic sensitization and repeated challenges; (B) chronic asthma + 1-week inhalation of spores of *A. fumigatus*; (C) chronic asthma + 3-week inhalation of spores of *A. fumigatus*; (D) chronic asthma + 5-week inhalation of spores of *A. fumigatus*, with chronically asthmatic rats inhaling spores of *A. fumigatus* for 1, 3, and 5 weeks; (E) chronic asthma + normal saline inhalation, with chronically asthmatic rats inhaling normal saline for 5 weeks; (F) OVA sensitization and normal saline challenge, with rats sensitized by OVA and challenged with normal saline; (G) OVA sensitization and normal saline challenge + *A. fumigatus* spore inhalation, with rats sensitized by OVA and challenged by normal saline followed by nasal inhalation of spores of *A. fumigatus* for 5 weeks. Animal groups and corresponding treatments, see Table 1. The rats were sacrificed 24 hours after the last challenge or nasal inhalation.

Establishing the Chronic Asthma Model

The rat model of chronic asthma was established based on the method described by Palmans and colleagues. [6]. On the first day (d1) and the eighth day (d8), freshly prepared suspension consisting of 1 mg OVA (Grade V; Sigma-Aldrich, St. Louis, MO, USA) + 100 mg aluminum hydroxide (Shanghai Chemical Reagent, Shanghai, China) + 1 mL normal saline was subcutaneously injected into 4 spots on the rats including bilaterally in the groin, the abdomen, and the sole of a front paw; 0.2 mL of the suspension was in-

TABLE 1. The Animal Groups and the Corresponding Treatments

Group	Treatment
Group A	Rat model of chronic asthma using OVA sensitization and challenges
Group B	Chronic asthma rats given 1-week inhalation of <i>A. fumigatus</i> spores
Group C	Chronic asthma rats given 3-week inhalation of <i>A. fumigatus</i> spores
Group D	Chronic asthma rats given 5-week inhalation of <i>A. fumigatus</i> spores
Group E	Chronic asthma rats given 5-week inhalation of normal saline
Group F	Rats given OVA sensitization and normal saline challenge
Group G	Rats given OVA sensitization and normal saline challenge followed by 5-week inhalation of <i>A. fumigatus</i> spores

jected at each spot and intraperitoneally. An intraperitoneal injection of 1 mL inactivated *Bordetella pertussis* strain (Shanghai Institute of Biological Products, Shanghai, China) was also performed. From d15 to d57, the rats were placed in sealed, custom-made Plexiglas boxes (50 × 27 × 25 cm³), which were connected to a nebulizer (PARIBOY N037; PARI, Germany). The rats inhaled nebulized 1% OVA normal saline solution 3 times per week for 30 minutes each. For groups F and G, 0.9% normal saline was used instead of OVA for the challenge.

Preparation of *A. Fumigatus* Spore Suspension

A strain of isolated *A. fumigatus* from a patient with invasive aspergillosis were used in the study. According to the method of our previous study [4], the strain was inoculated into the medium of Sabouraud agar plates (Biomérieux, Marcy Etoile, France) and incubated at 37°C for 7 days. A volume of 10 mL normal saline containing 0.1% Tween 80 (Sigma-Aldrich, St. Louis, MO, USA) was used to flush the agar surface to collect the conidia suspension, which was then filtered through 8 layers of sterile gauze to remove the mycelium. The spore suspension was centrifuged at 1000 rpm for 10 minutes, the supernatant was discarded, and the spores were resuspended in normal saline. The spore number was determined using a hemocytometer, and the spore concentration was adjusted to 1×10^5 cfu/mL. The concentration of spores was again measured before administration. Bacterial contamination was excluded by the absence of colony-forming units after plating an aliquot on an agar plate. Endotoxin was measured by Limulus Amebocyte Lysate Assay

(Westang Co. Ltd., Shanghai, China) and shown to be <1 EU/mL. The virulence was confirmed by invasive pulmonary infection caused by inoculating the spores to cyclophosphamide-treated rats.

A. *Fumigatus* Spore Inhalation

Starting from d64, the following procedures were performed 2 times per week according to the method of Denis and colleagues [7]. The rats were anesthetized through intraperitoneal injection of 1% pentobarbital (Sigma-Aldrich, St. Louis, MO, USA) and placed in the vertical position. Each side of the nostril was administered 50 μ L of the *A. fumigatus* spore suspension in drops, and the rats were maintained in the vertical position for 5–10 minutes. The rats in group E inhaled 100 μ L saline using the same method.

Measurement of Airway Resistance

Based on the method of Glaab and colleagues [8], the rats were anesthetized with 1% pentobarbital (Sigma-Aldrich, St. Louis, MO, USA) at the corresponding time points and fixed on a blanket with a constant temperature of 37°C followed by an incision in the middle of the neck skin to expose the external jugular vein, trachea, and esophagus. An incision was made in the trachea and esophagus, and the catheters were inserted. The endotracheal tube was connected to the respiratory rate plethysmometer (Hans Rudolph, Shawnee, KS, USA) to measure the rate of airflow. The distal esophageal tube was placed in the middle lower part of the thoracic esophagus, and the insertion depth was determined based on the acquisition of maximum negative pressure. The proximal end was connected to the pressure transducer. The measured intraesophageal pressure was used to replace intrathoracic pressure. The signal input was entered into the computer, and the airflow velocity and intraesophageal pressure were converted into airway resistance through software processing (Smup-B bio-signal processing system, Medical Equipment Research center of Shanghai medical college, Shanghai, China). The samples were collected after the data stabilized. After measuring the basal airway resistance (R_{aw}), acetylcholine chloride (Sigma-Aldrich, St. Louis, MO, USA) (300 μ g/kg) was injected via the external jugular vein to observe changes in R_{aw} and to calculate the rate of change for R_{aw} .

Specimen Processing

After determining airway resistance, the rats were sacrificed by bleeding from the abdominal aorta, and the chest was opened to dissect the right upper lobe of

the lung, which was stored in liquid nitrogen for later use. The right lower lobe of the lung was fixed in 10% formalin for 48 hours, dehydrated, embedded in paraffin, sectioned into 5- μ m thick slices, which were subjected to hematoxylin–eosin (HE), periodic acid–Schiff (PAS), and EGFR immunohistochemical staining procedures. Left lung bronchoalveolar lavage was performed by injecting a total of 10 mL PBS via endotracheal intubation 3 times. The recovered bronchoalveolar lavage fluid (BALF) was centrifuged at 1000 rpm for 10 minutes, and the supernatant was stored at -80°C .

Measurement of EGF and TGF- α Levels in BALF Supernatant

ELISAs were used for the measurements; the procedures were performed according to instructions specified in the kit manuals (Shanghai Westang Biotech Inc., Shanghai, China).

Morphometric Analysis

Digital image analysis were performed on histologic sections using Image-Pro Plus software (Image Pro-Plus software 5.0; Media Cybernetics, Silver Springs, MD, USA) as previously described [9–12]. In the HE-stained sections, the inner perimeter of the of airway epithelium and the length of the part uncovered with intact ciliated cells or goblet cells were measured. The ratio of the length of the detached epithelium to inner perimeter was used to assess the extent of epithelial detachment. In the PAS-stained sections, the PAS-stained area within the basal border and the inner border of airway epithelium was measured. The extent of goblet metaplasia was assessed as percentage of PAS-stained area to total epithelial area. For each lung compartment, image data were averaged from a total of 5 sections, each taken from a separate paraffin block. In each set of 5 sections, measurements were taken from all available bronchioles and small bronchi, ranging from 200 to 2000 μ m in diameter (calculated on basement membrane perimeter), which were cut in transverse section and free of branching.

Immunohistochemical Staining for EGFR

Paraffin sections (with a thickness of 5 μ m) of the right lower lobe of the lung were used, and the Streptavidin–Biotin Complex (SABC) method was applied for the measurement. The primary anti-EGFR antibody was a rabbit polyclonal antibody (Cell Signaling Co., Boston, MA, USA) with a working concentration of 1:200; the working

concentration of the secondary antibody (Jackson laboratory, Bar Harbor, ME, USA) was 1:1000, and the DAB chromogenic method was used followed by hematoxylin double staining. In negative controls, a normal rabbit serum was used instead of the primary antibody. The results were determined as follows. Positive staining was represented as brown particles on the membrane and in the cytoplasm. The images under the microscope were acquired using an image analysis system (Image Pro-Plus software 5.0; Media Cybernetics, Silver Springs, MD, USA). Five fields of view ($\times 400$ magnification) were randomly selected in the bronchiole area with a diameter approximately 500–1000 μm (minimum lumen diameter/maximum lumen diameter ≥ 0.6) for each rat. The integrated optical density (IOD) value of positive EGFR staining was used to represent the EGFR protein expression levels.

Immunoblot Assay to Determine EGFR Expression

Soluble protein was extracted using radio immunoprecipitation assay (RIPA) reagent (Shanghai Westang Biotech Inc., Shanghai, China). The lung tissue was placed in the grinder, ground after the addition of liquid nitrogen, and homogenized after adding RIPA. After being on ice for 30 minutes, the mixture was centrifuged at 4°C and 14,000 rpm for 15 minutes. The supernatant was transferred to a sterile centrifuge tube and stored at -20°C ; a 20- μL volume of lung tissue protein lysate containing 50 μg of protein and 10 μL of $5\times$ sample loading buffer were added to 10% sodium dodecyl sulfate-polyacrylamide (10% SDS-PAGE) gels for electrophoresis and then transferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was blocked in blocking solution containing 5% fat-free milk for 1 hour, and the primary rabbit polyclonal anti-EGFR antibody (1:1000; Cell Signaling Technology, Boston, MA, USA) was added, followed by overnight incubation at 4°C . The membrane was washed in Tris-Buffered-Saline with Tween (TBST) buffer and incubated with the anti-rabbit horseradish peroxidase (HRP)-conjugated secondary antibody (1:2000; Jackson laboratory, Bar Harbor, ME, USA) at room temperature for 1 hour followed by TBST buffer washes and detection via the chemiluminescence method.

Statistical Analyses

Each set of data were expressed as mean $\pm$ SEM. The SAS6.12 statistical package was used for data processing. Statistical analyses were performed using one-way analysis of variance (ANOVA); the compar-

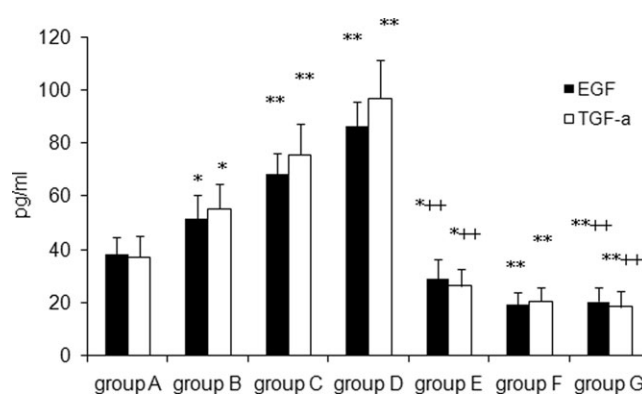


FIGURE 1. EGF and TGF- α levels in the BALF of each group of rats. Data are expressed as means $\pm$ SEM of 8 individual samples. * $P < .05$, ** $P < .01$ versus group A; + $P < .05$, ++ $P < .01$ versus group D.

ison of means between groups was conducted using the SNK test, and $P < .05$ was considered significant. Linear correlation analysis was used to analyze different indicators.

RESULTS

EGF and TGF- α Levels in the BALF of Each Rat Group

The EGF and TGF- α levels in the BALF supernatant were higher in group A than group F (both with $P < .01$); the rats in groups B, C, and D showed progressive increases in EGF and TGF- α levels in the BALF supernatant, and the levels of the 3 groups were all increased compared with before spore inhalation ($P < .05$ or $P < .01$). After inhalation of normal saline, the EGF and TGF- α levels in the BALF supernatant of group E rats decreased ($P < .05$ or $P < .01$). The EGF and TGF- α levels in the BALF supernatant of group G rats showed no significant change compared with the levels before spore inhalation ($P > .05$; Figure 1).

R_{aw} and R_{aw} Rate of Change in Each Rat Group

The basal R_{aw} , R_{aw} after application of Ach, and R_{aw} rate of change were all higher in the group A rats than in the group F rats ($P < .05$). After inhaling *A. fumigatus* spores for 1 week, the group B rats showed no significant changes in basal R_{aw} , the R_{aw} after application of Ach, or the R_{aw} rate of change ($P > .05$); the group C rats showed increased basal R_{aw} , R_{aw} after application of Ach, and R_{aw} rate of change compared with before spore inhalation (both with $P < .01$).

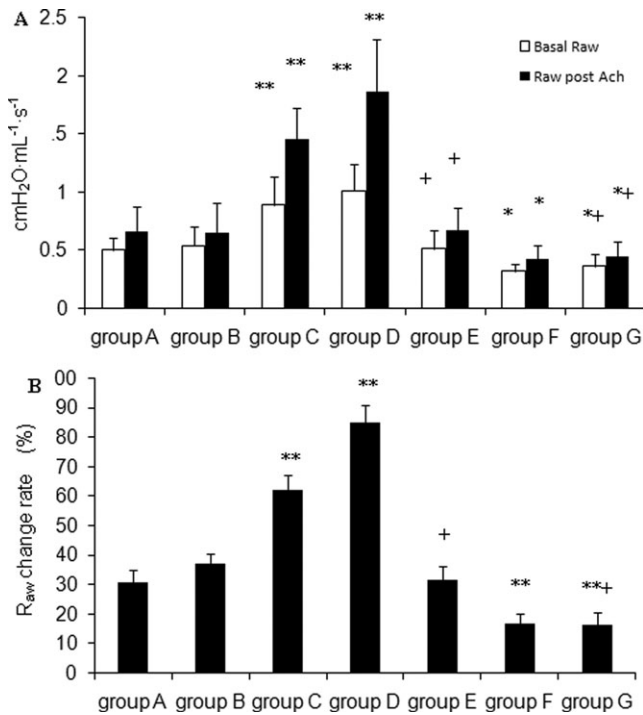


FIGURE 2. The basal airway resistance (R_{aw}), R_{aw} after acetylcholine chloride (A) and R_{aw} rate of change (B) in each group of rats. Data are expressed as means $\pm$ SEM of 8 individual samples. * $P < .05$, ** $P < .01$ versus group A; + $P < .05$, ++ $P < .01$ versus group D.

After inhaling normal saline for 5 weeks, the rats in group E did not show significant changes in basal R_{aw} , the R_{aw} after application of Ach, or the R_{aw} rate of change ($P > .05$) compared with before normal saline inhalation. After inhaling *A. fumigatus* spores for 5 weeks, the rats in group G showed no significant changes in basal R_{aw} , the R_{aw} after application of Ach, or the R_{aw} rate of change compared with before spore inhalation ($P > .05$; Figure 2).

Extent of injury and shedding of airway epithelial cells and goblet cell metaplasia in each rat group

The rats in group A showed scattered airway epithelial cell injury and shedding as well as goblet cell metaplasia; the rats in group F did not show significant airway epithelial shedding or goblet cell metaplasia, and the difference between the two groups was significant ($P < .01$). The rats in groups B, C, and D showed progressively severe airway epithelial cell shedding and goblet cell metaplasia, and all showed more severe conditions compared with before spore inhalation ($P < .05$ or $P < .01$). After a 5-week inhalation of normal saline, the group E rats showed a reduced degree of airway epithelial cell shedding compared with before inhalation ($P < .05$ or $P < .01$) and no significant changes in goblet cell metaplasia compared with

before inhalation ($P > .05$). After a 5-week inhalation of *A. fumigatus* spores, the rats in group G showed no significant injury and shedding of airway epithelium or goblet cell metaplasia, which did not differ significantly from the conditions before spore inhalation ($P > .05$; Figures 3, 4 and 5).

EGFR expression in the airway epithelial cells of each rat group

Immunohistochemical staining showed that the airway epithelium of the rats in group A had scattered EGFR expression, the rats in group F did not have significant EGFR expression, and the two groups showed a significant difference ($P < .01$). The EGFR expression levels in the airway epithelial cells of the rats in groups B, C, and D were all increased compared with those prior to spore inhalation ($P < .05$ or $P < .01$). After a 5-week inhalation of normal saline, the EGFR expression levels in the airway epithelium of group E rats showed no significant change compared with before inhalation ($P > .05$). After a 5-week inhalation of *A. fumigatus* spores, the EGFR expression levels in the airway epithelium of group G rats showed no significant change compared with before inhalation ($P > .05$; Figures 6 and 7).

EGFR immunoblot assays of lung tissues from each rat group

Immunoblot assays showed that the EGFR expression levels in the lung tissue of the rats in group A were higher than in the rats in group F ($P < .01$). With increasing spore inhalation time, the EGFR expression levels in the lung tissues of groups B, C, and D rats showed progressive increases, and all significantly differed from the levels before spore inhalation ($P < .05$ or $P < .01$). After inhaling normal saline for 5 weeks, the EGFR expression levels in the lung tissues of group E rats did not show significant changes compared with before inhalation ($P > .05$). After inhaling *A. fumigatus* spores for 5 weeks, the EGFR expression levels in the lung tissues of group G rats did not show significant changes compared with before inhalation ($P > .05$; Figures 8 and 9).

Correlation analysis between indicators

The EGF and TGF- α levels in BALF positively correlated with the EGFR expression level (IOD value) in airway epithelial cells ($r = 0.712$, $P < .01$; $r = 0.735$, $P < .01$). The EGFR IOD values of airway epithelial cells positively correlated with exfoliated epithelial length/epithelial inner circumference (%; $r = 0.692$, $P < .01$) and PAS staining area/epithelial cell area (%; $r = 0.657$, $P < .01$). The PAS staining

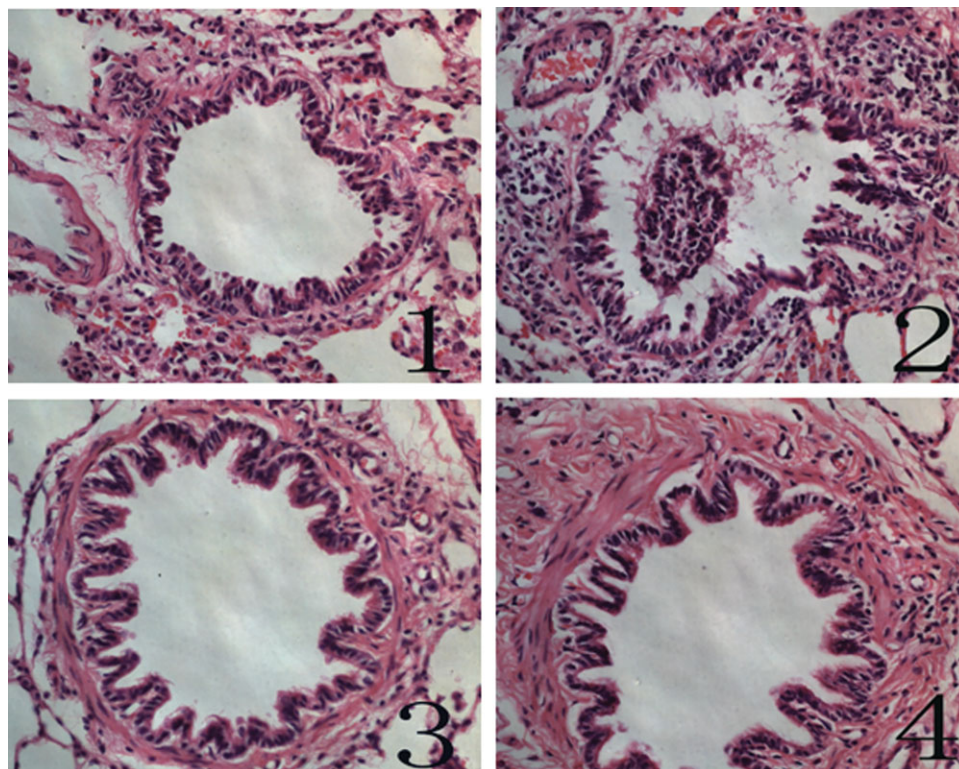


FIGURE 3. The extent of airway epithelial cell injury and shedding in each group of rats. Hematoxylin and eosin-stained lung sections from group A (A), group D (B), group F (C), and group G (D). Original magnification, $\times 200$.

area/epithelial cell area (%) showed a positive correlation with R_{aw} rate of change (%; $r = 0.528$, $P < .05$).

DISCUSSION

In the present study, the OVA-sensitized asthmatic rats were exposed to prolonged intranasal inhalation

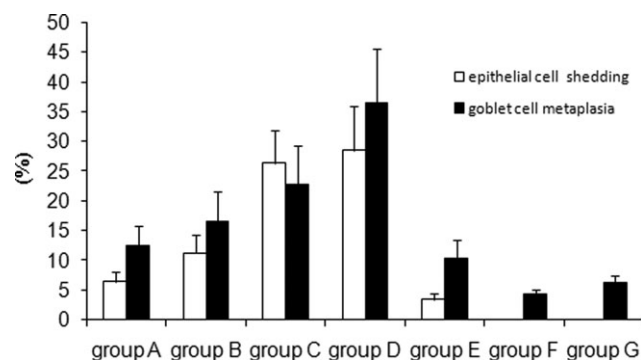


FIGURE 4. Morphometric analysis of the extent of airway epithelial cell injury and shedding and goblet cell metaplasia in each group of rats. Data are expressed as means $\pm$ SEM of 8 individual samples. * $P < .05$, ** $P < .01$ versus group A; + $P < .05$, ++ $P < .01$ versus group D.

of *A. fumigatus* spores, aimed at exploring the effects of chronic *A. fumigatus* exposure on EGFR expression in the airway epithelial cells in asthmatic rats. The main findings of the study is that chronic *A. fumigatus* exposure aggravates epithelial cells injury and remodeling, upregulates EGFR expression, and increases airway resistance in a dose-dependent fashion in OVA-sensitized asthmatic rats. As we know, this is the first study focusing on the influence of chronic *A. fumigatus* exposure on EGFR expression in the airways of asthmatic rats. The findings of the study enhances our understanding of the deleterious effects of chronic *A. fumigatus* exposure on airway epithelial cells injury and remodeling in asthma patients.

A. fumigatus is a common saprophytic fungus that is widely distributed in both indoor and outdoor environments. Englehart and colleagues measured the indoor and outdoor concentrations of *A. fumigatus* spores in the air, which reached 8.1 and 9.4 cfu/m³, respectively [13]. The diameter of the *A. fumigatus* spore is approximately 2–3 μ m, the spore surface has a highly hydrophobic structure, and the spore can remain suspended in the air for a long time and be easily inhaled into the lower respiratory tract, with an estimated daily inhalation of up to 2000 spores to the terminal airways [14]. Epidemiologic data show

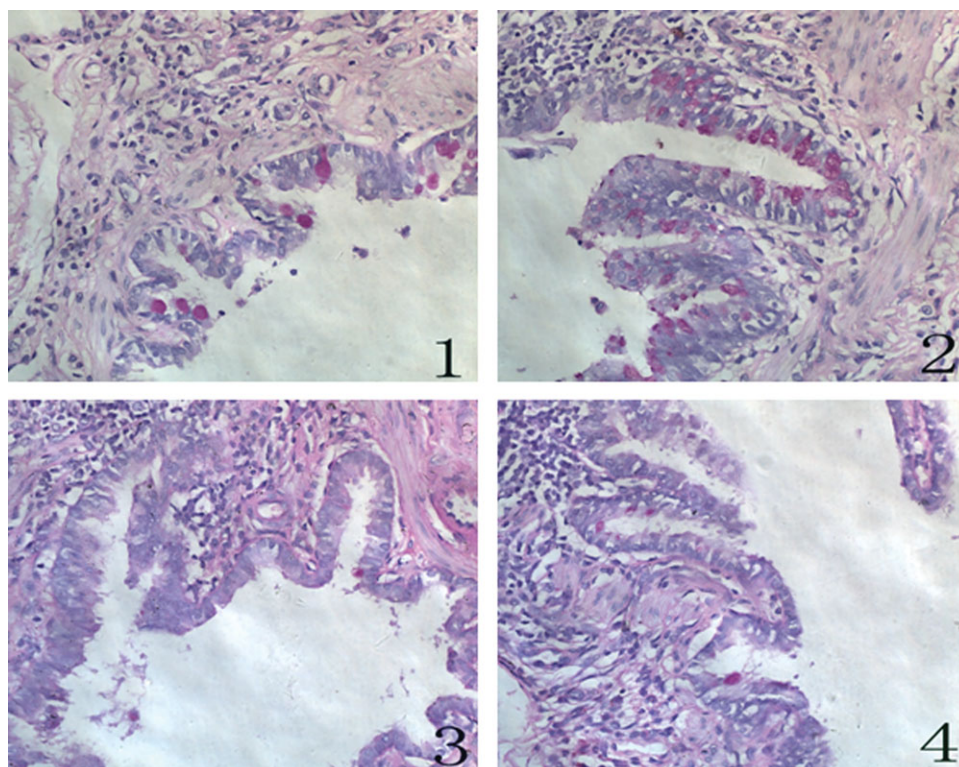


FIGURE 5. The extent of airway epithelial goblet cell metaplasia in each group of rats. Periodic acid Schiff (PAS)-stained lung sections from group A (A), group D (B), group F (C), and group G (D). Original magnification, $\times 400$.

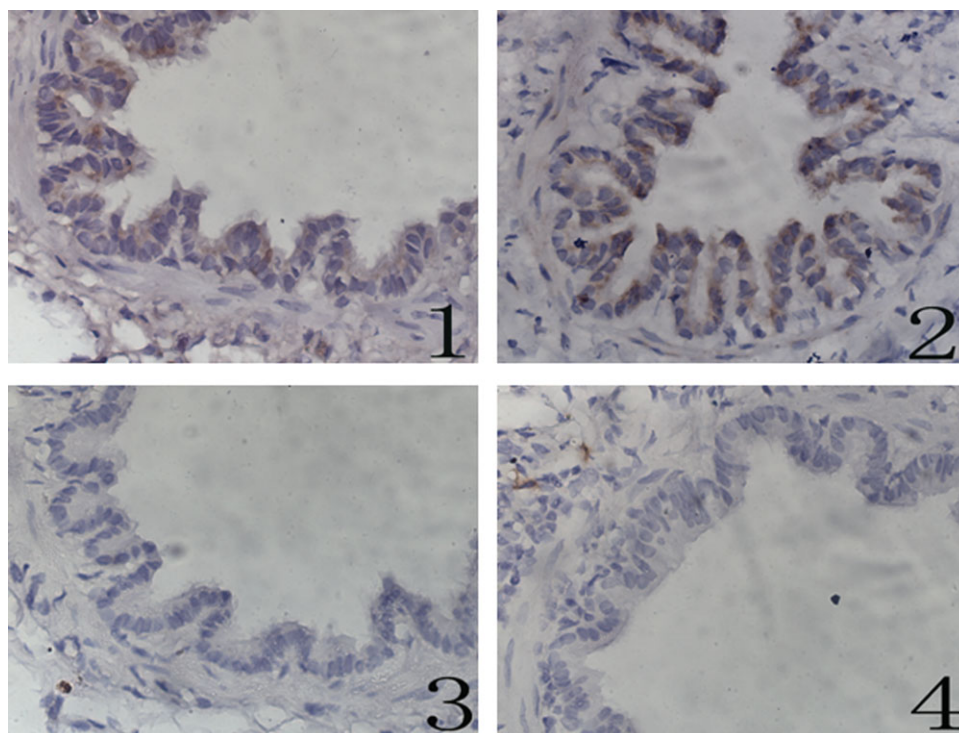


FIGURE 6. EGFR expression in airway epithelial cells in each group of rats. Immunohistochemistry-stained lung sections from group A (A), group D (B), group F (C), and group G (D). Original magnification, $\times 400$.

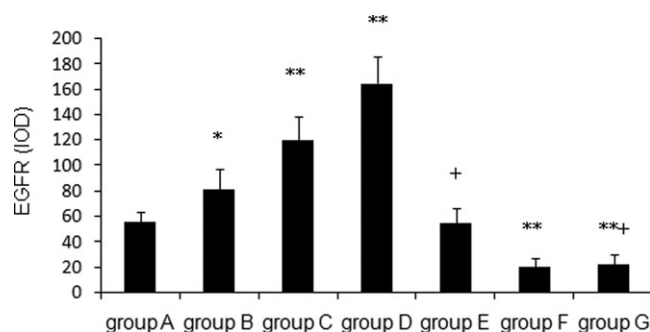


FIGURE 7. Integrated optical density (IOD) of EGFR staining as assessed through image analysis in each group of rats. Data are expressed as means $\pm$ SEM of 8 individual samples. * $P < .05$, ** $P < .01$ versus group A; + $P < .05$, ++ $P < .01$ versus group D.

that elevated ambient concentrations of *A. fumigatus* spores are closely related to asthma exacerbation in patients [3]. Using a rat model of chronic asthma, our previous study found that, under the basis of chronic airway inflammation, a prolonged exposure to *A. fumigatus* aggravates Th2 airway inflammation, promotes airway remodeling including goblet cell metaplasia, and upregulates mucin 5AC expression in asthmatic rats [4,5].

Airway epithelial cells are the barriers of the respiratory tract and are exposed to various stimuli, including allergens. Airway epithelial cells in asthma can have a variety of pathologic changes including injury and shedding of epithelial cells, goblet cell metaplasia, and mucus hypersecretion, and the extent of epithelial cell damage positively correlates with airway responsiveness and epithelial permeability [15]. Injured or activated airway epithelial cells can produce proinflammatory cytokines and growth factors, which are involved in the occurrence and development of asthma and promote the progressive development of airway inflammation and remodeling [16]. Kauffman and colleagues found that *A. fumigatus* extracts have protease activity and can lead to injury and shedding of airway epithelial cells and IL-6 and IL-8 release [17]. This study found that, with increasing inhalation time of *A. fumigatus* spores, the severity of

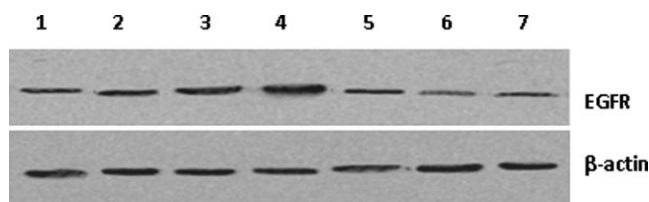


FIGURE 8. Western blot detection of EGFR in each group of rats. Lanes 1–7 represent groups A, B, C, D, E, F, and G, respectively.

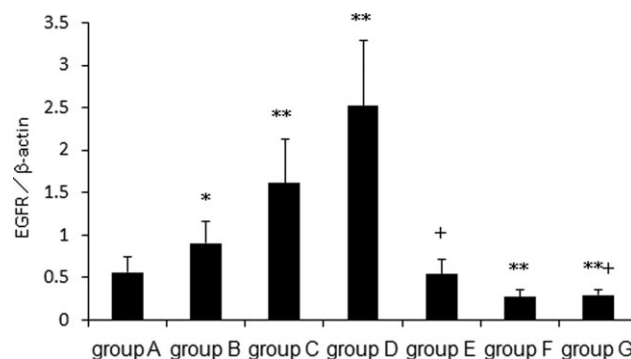


FIGURE 9. Relative gray scale value of EGFR as assessed through image analysis in each group of rats. Data are expressed as means $\pm$ SEM of 8 individual samples. * $P < .05$, ** $P < .01$ versus group A; + $P < .05$, ++ $P < .01$ versus group D.

airway epithelial damage and shedding as well as goblet cell metaplasia was gradually enhanced and positively correlated with airway responsiveness. The results suggest that progressive exacerbation in airway epithelial cell damage and reconstruction caused by prolonged inhalation of *A. fumigatus* spores may be involved in increased airway responsiveness.

EGFR is a transmembrane glycoprotein with tyrosine kinase (PTK) activity. EGFR binding to its ligands induces conformational changes in the receptor ectodomain to generate activated receptor dimers, thus activating the PTK in the intracellular region, stimulating receptor autophosphorylation and tyrosine phosphorylation of other intracellular proteins, and exerting its biologic effects. It has been found that mechanical damage, oxidative stress, TNF- α , IL-13, LPS, and allergens with enzyme activity can all increase EGFR expression in bronchial epithelial cells [18–22]. Puddicombe and colleagues found that, in normal subjects, EGFR was expressed mainly in the junction between the basal cells of airway epithelium and the mast cells, and there was no EGFR expression in the apical side of epithelial cells. Furthermore, different from normal subjects, EGFR showed a full-thickness expression in the airway epithelium of patients with bronchial asthma, the expression intensity significantly increased, and the intensity of EGFR expression correlated with the severity of asthma [1].

Using a rat model of chronic asthma, our previous study found chronic *A. fumigatus* exposure leads to aggravation of airway epithelial cell injury and remodeling and upregulation of mucin 5AC expression in the airways of asthmatic rats [4,5]. However, the signal pathways through which *A. fumigatus* exposure leads to asthma deterioration were not clear. Using the same animal model of asthma, the present study first focus on the influence of *A. fumigatus* exposure on EGFR expression in the airway epithelial

cells in asthmatic rats, trying to explore the role of EGFR signal pathway in asthma aggravation induced by fungal exposure. EGFR is involved in the regulation of goblet cell metaplasia. Injured and activated airway epithelial cells can produce EGF, TGF- α , and other EGFR pro-ligands, which bind to and activate EGFR, activate the ERK signaling pathway, increase the expression of the airway mucin protein MUC5AC, and induce airway goblet cell metaplasia and mucus hypersecretion [23, 24]. The study found that inhalation of *A. fumigatus* spores caused aggravated airway epithelial cell injury in asthmatic rats and a gradual increase in EGF and TGF- α levels in the BALF. Immunohistochemistry showed that EGFR was expressed in airway epithelial cells, and expression levels gradually increased as the inhalation time of *A. fumigatus* spores extended. Western blot detection of EGFR protein expression showed a similar trend. Image analysis showed that the intensity of EGFR expression positively correlated with the levels of its ligands EGF and TGF- α and also positively correlated with the extent of epithelial cell injury and goblet cell metaplasia. The results suggest that chronic exposure to *A. fumigatus* can upregulate the expression of EGFR and its ligands EGF and TGF- α in the airway epithelium of asthmatic rats and that EGFR activation after binding to its ligands leads to the activation of downstream signaling molecules, promotes goblet cell metaplasia, and is involved in the progressive increase in airway responsiveness.

CONCLUSIONS

The results of the present study showed that chronic exposure to *A. fumigatus* could aggravate airway epithelial cell damage in rats with asthma, upregulate the expression of EGFR and its ligands EGF and TGF- α , promote goblet cell metaplasia, and increase airway responsiveness. Regulation of EGFR function may be important for the prevention and treatment of *A. fumigatus* exposure-induced asthma exacerbation.

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

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