Abstract. Allergic rhinitis (AR) and asthma belong to the category of type I allergic diseases, whose pathological features are airway remodeling of the lung and allergic inflammation. The aim of the present study was to evaluate inflammation and remodeling of lung tissue in a guinea pig model of AR in order to confirm consistent pathological changes of upper and lower airways in AR. Male guinea pigs were randomly divided into an experimental and a control group (n=10 in each). The AR model was established by sensitization through intraperitoneal injection of ovalbumin for three weeks and bilateral nasal local excitation for twelve weeks. All tissues of nasal mucosa and lung were subjected to hematoxylin and eosin as well as toluidine blue staining, and characteristics of remodeling of lung tissue, including thickness of bronchial wall, epithelial mucosa and smooth muscle were histologically determined. Collagen deposition in lung tissue was observed by Masson's trichrome stain. Severe paroxysmal nose scratching action, frequent sneezing, visible outflow of secretion from the anterior naris and frequent nose friction were observed in the AR model group within 30 min after local excitation. The total symptom scores were significantly increased in the AR model group compared with those in the control group. Obvious inflammatory cell infiltration was observed in the AR model group. Compared with those in the control group, the numbers of eosinophils and mast cells in nasal mucosa and lung tissue were significantly increased. Obvious airway remodeling of the lung was observed in the AR model group. Compared with those in the control group, bronchial wall thickness, epithelial layer thickness and smooth muscle thickness in the airways were significantly increased in the AR model group. Increased collagen deposition was found in the AR model group compared with that in the control group. The results of the present study revealed that inflammation and airway remodeling of lungs arose in guinea pigs with AR, suggesting that pathological changes of upper and lower airways are consistent in this AR model.

Introduction

Allergic rhinitis (AR) and asthma are common diseases with a high incidence rate and have a negative impact on the lives affected patients. AR and asthma used to be regarded as two different diseases, whose diagnosis and treatment were different (1). The AR and its impact on asthma (ARIA) initiative started during a workshop of the World Health Organization performed in 1999 and their guidelines were published in 2001 (2). Their review of 2010 provided guidelines for the diagnosis and treatment of AR and of clinical practices for the management of comorbidities of AR and asthma based on the Grading of Recommendations Assessment, Development and Evaluation method (3). ARIA has been popularized and implemented in >50 countries (4,5). An increasing amount of research on ‘airway consistency’ has been performed, including epidemiology, pathology, clinical diagnosis and treatment (6).

AR and asthma belong to type I allergic diseases. It is currently thought that AR and asthma not only interact with each other but also are a risk factor for the development of the other diseases (7). Management of AR improves the symptoms of asthma. AR and asthma require an integrated management (8). Early diagnosis and aggressive treatment of AR or asthma may prevent the development of other diseases. Bronchial hyper-reactivity (BHR) is a paramount feature of asthma and may be considered a strong risk factor for the onset of asthma in patients with AR. In a previous study, the bronchial methacholine test was performed in 342 patients with a moderate/severe degree of sustained AR. Of these, 22 (6.4%) had severe BHR, 74 (21.6%) had mild BHR and 192 (56.2%) had borderline BHR (9). Buslau et al (10) found that most patients with AR exhibited signs of BHR, and that ~30% of patients with AR may develop asthma later in life. Andiappan et al (11) found that neuropeptide S receptor 1 and cytotoxic T lymphocyte-associated antigen-4 were the genetic links between AR and asthma, indicating the presence of a certain genetic consistency among the
pathogeneses of the two diseases. The present study indicated that ongoing AR was associated with worsening of asthma by enhancing lower airway inflammation in patients with atopy (12). Chawes (13) also found that the nasal pathology in young children with allergic and non-allergic rhinitis exhibited marked differences, suggesting close association between upper and lower airway diseases partly through an allergy-driven process, but equally via non-allergic mechanisms. Regarding the effects of the pathogenetic process of AR on lung airway remodeling in patients with AR, only few studies have assessed this possible association (14,15).

Wagener et al (16) identified 1988 differentially expressed genes between healthy lower and upper airway epithelium, whereas only 40 and 301 genes were differentially expressed in AR with or without asthma, respectively. These results suggested that genes affected by AR with or without asthma may be associated with lung development and remodeling as well as normal epithelial barrier functions and regulation of peptidases. Airway remodeling of the lung and allergic inflammation are the pathological features of asthma. Lower airway inflammation and remodeling during the course of the pathogenesis of AR remain to be fully demonstrated. Therefore, the present study was designed to evaluate the inflammation and airway remodeling of lung tissue in a guinea pig model of AR.

Materials and methods

Animals. A total of 20 healthy male guinea pigs (weight, 150-220 g; age, 5-6 months) were obtained from the experimental animal center of the University of South China (Hengyang, China). The humidity of the rearing environment was 50±5% and the ambient temperature was 22.5±2.5°C. The guinea pigs were housed under a 12-h light/dark cycle with free access to water and food. The animal experiments were approved by the Committee on the Ethics of Animal Experiments of the University of South China (Hengyang, China). All efforts were made to minimize the number of animals and their suffering.

Animal grouping and induction of AR animal models. The guinea pigs were randomly divided into a normal control group and an AR model group. The animals were allowed to adapt to the experimental environment for one week. The AR model was established according a previous method (17). In brief, the guinea pigs were administered a suspension of 0.3 mg ovalbumin (OVA) and 30 mg aluminum hydroxide (both from Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) in 1 ml normal saline by intraperitoneal injection every other day for 4 weeks. Furthermore, in order to induce local excitation, 50 µl 2% OVA solution was dripped into each side of the nasal cavity of the guinea pigs once a day for twelve weeks. Additional 50 µl 2% OVA solution was dripped into each side of the nasal cavity 5 times. The guinea pigs were housed under a 12-h light/dark cycle with free access to water and food. The animal experiments were approved by the Committee on the Ethics of Animal Experiments of the University of South China (Hengyang, China). All efforts were made to minimize the number of animals and their suffering.

Standard for evaluation of nasal symptoms. Each local nasal excitation in guinea pigs was observed and recorded, including scratching nose action, frequency of sneezing and condition of the nose. Nasal symptoms were evaluated according to the standard scoring system presented in Table I. The total score was recorded and a total score of >5 points indicated the successful establishment of the AR model in the guinea pigs.

Preparation of nasal mucosa and lung tissues and histological analysis. The guinea pigs were deeply anesthetized with 10% chloral hydrate solution (300 mg/kg; Sigma-Aldrich; Merck KGaA) by intraperitoneal injection. The nasal bone of each guinea pig was removed and the nasal mucosa was gained. The lung was removed. Nasal mucosa and lung tissues were fixed in 4% paraformaldehyde at 4°C for 48 h. Nasal mucosa was decalciified in 10% EDTA (Sigma-Aldrich; Merck KGaA). The tissues were embedded in paraffin and sliced at a thickness of 4-5 µm. The sections were stained with hematoxylin and eosin (H&E) or toluidine blue (Sigma-Aldrich; Merck KGaA). Collagen deposition in lung tissues was observed by Masson's trichrome stain. Five microscopic fields were randomly selected, and the eosinophils and mast cells in each high-power microscopic field (magnification, x400) were counted under a light microscope.

Analysis of airway remodeling in lung tissue. Image analysis software Image Pro Plus V6 (Media Cybernetics, Inc., Rockville, MD, USA) was utilized to measure the circumference of the bronchial basement membrane, total area of bronchia, total area of bronchial lumen, area of the bronchia within the outer or inner edge of the epithelial layer and area of the bronchia within the outer or inner edge of the smooth muscle of lung tissue sections after H&E staining. The thickness

| Table I. Standard for evaluation of nasal symptoms. |
|-----------------------------------------------------|
| Symptoms score | Scratching nose action | Frequency of continuous sneezing | Condition of nose |
|----------------|------------------------|-------------------------------|------------------|
| 0              | None                   | 0                             | Normal           |
| 1              | Light touch            | 1-3                           | Flow to the nostrils |
| 2              | Strong scratching       | 4-10                          | Flow beyond the nostril |
| 3              | Full rubbing           | >11                           | Flow to the lips |

Figure 1. Total symptom scores in guinea pigs with AR. AR was induced by ovalbumin challenge. The nasal symptoms were evaluated according to scoring criteria listed in Table I. Values are expressed as the mean ± standard deviation (n=10 per group). *P<0.05 compared with the control group. AR, allergic rhinitis.
of the bronchial wall, epithelial layer and bronchial smooth muscle was then calculated as characteristics of airway remodeling. The parameters were calculated as follows: Bronchial wall thickness=(total area of bronchia-total area of bronchial lumen)/bronchial basement membrane size. Epithelial layer thickness=(area of the bronchia within the outer edge of the epithelial layer-area of the bronchia within the inner edge of the epithelial layer)/bronchial basement membrane diameter. Bronchial smooth muscle thickness=(area of the bronchia within the outer edge of the smooth muscle-area of the bronchia within the inner edge of the smooth muscle)/bronchial basement membrane diameter.

Statistical analysis. Values are expressed as the mean ± standard deviation. Differences between groups were analyzed using the Student's t-test. All statistical analyses were performed by using SPSS 17.0 statistical software (SPSS, Inc., Chicago, IL, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Nasal symptoms and total symptom scores in guinea pigs with AR. Nasal symptoms such as severe paroxysmal nose scratching action, frequent sneezing, visible outflow of
secretions from the anterior nares and frequent nose friction were observed in the AR model group within 30 min after local excitation. The guinea pigs in the AR model group refrained from eating and drinking, and cried within 2 h after local excitation. The total symptom scores were significantly increased in the AR model group compared with those in the control group (Fig. 1).

Influenza cell infiltration in nasal mucosa and lung tissues. As presented in Figs. 2 and 3, obvious inflammatory cell infiltration in the nasal mucosa and lung tissues was observed in the AR model group. Compared with those in the control group, the numbers of eosinophils and mast cells in the nasal mucosa and lung tissues were significantly increased (all P<0.05).

Airway remodeling in lung tissues. As displayed in Fig. 4 and Table II, obvious airway remodeling of lung tissues was observed in the AR model group. Compared with that in the control group, the thickness of bronchial wall, epithelial layer...
Table II. Airway remodeling of lung tissue in guinea pigs with AR.

| Group    | n  | Bronchial wall thickness (µm²/µm) | Epithelial layer thickness (µm²/µm) | Smooth muscle thickness (µm²/µm) |
|----------|----|----------------------------------|-------------------------------------|----------------------------------|
| Control  | 10 | 16.60±1.00                       | 11.44±0.87                          | 4.15±0.30                        |
| AR model | 10 | 18.82±0.91                       | 13.52±1.10                          | 6.19±0.78                        |

AR, allergic rhinitis.

Discussion

Airway remodeling leads to reversible airflow obstruction and persistent airway hyperresponsiveness, and is also an important feature of asthma in addition to chronic airway inflammation (18). Airway remodeling directly affects the outcome of asthma. It refers to the pathological changes of the airway, which include airway epithelial injury, epithelial fibrosis, hypertrophy, proliferation and migration of airway smooth muscle (ASM), an increased number of activated and muscle fibroblasts, goblet cell proliferation, mucus secretion, angiogenesis, decreased cartilage integrity and increased airway vascularity (19). Numerous types of inflammatory cell, including eosinophils, T lymphocytes, macrophages, mast cells and neutrophils, as well as airway structural cells, including ASM cells, epithelial cells and fibroblasts, release a variety of cytokines, including chemokines, growth factors and extracellular matrix protein. A variety of cytokines form a complex signaling environment participating in and strengthening airway remodeling (20). In a mouse model of chronic asthma, Stellari et al (21) confirmed that metalloproteinase was significantly increased at 8 and 11 weeks, which coincided with hypertrophy of the airway epithelium and with collagen deposition, as histologically evaluated by Masson's trichrome staining. Increases in blood vessel density and vascular area are now recognized as important features of remodeled airways in asthma. In a sheep model of chronic asthma, van der Van der Velden et al (22) found that blood vessel density and the percentage of airway vascularity in the bronchi were significantly increased following 24 weeks of house dust mite challenge compared with those in untreated controls. Fibrosis is also one of the key pathological features of airway remodeling in asthma. In normal airways, the amount of collagen and other extracellular matrix components is maintained in equilibrium by regulation of synthesis and degradation. In asthma, this homeostasis is disrupted due to genetic and environmental factors (9). Airway remodeling contributes to the thickening of airway walls and consequently leads to airflow narrowing.

Allergen challenge of the nose induces the influx of inflammatory cells in the lower airways and vice versa. Treatment of the nose, in various ways, is likely to affect inflammation in the lower airways (12). In a study by Hovland et al (23), BHR was assessed by metacholine bronchial challenge, and airway inflammation was assessed by fractional exhaled nitric oxide (FeNO), revealing that boys with asthma and AR had significantly more severe BHR and higher FeNO than those with other asthma phenotypes. Kim et al (24) evaluated 3 characteristics in AR using measurement of FeNO, a methacholine challenge test and impulse oscillometry (IOS), and found reversible lower airway obstruction on IOS and elevated FeNO levels in children with AR. Elevated FeNO levels indicated airway inflammation, suggesting that chronic inflammation may lead to BHR in AR. Chawes (13) confirmed that BHR was increased and the level of FeNO was elevated in children with AR, suggesting different endotypes of asthma symptoms in young children with allergic- and non-allergic rhinitis. They also found bronchial hyperresponsiveness and increased values of FeNO in children with AR without asthma, suggesting sub- clinical bronchial inflammation and supporting that the allergic disease process involves the upper as well as lower airways. An experimental study by Bonay et al (25) suggested that eosinophils participate in bronchial inflammation in patients with seasonal AR following allergic nasal challenge outside the pollen season and may result in airway responsiveness. In conclusion, the abovementioned studies objectively demonstrated that the nasal pathology in AR may have an important role in inflammation and BHR of the lower
airways. However, it remains to be fully demonstrated whether AR affects the remodeling of lung tissue.

The present study revealed obvious inflammation, including infiltration of eosinophils and mast cells in the upper and lower airway tissues of guinea pigs with AR, which indicated airway inflammation consistency. The remodeling of lung tissues in guinea pigs with AR was evaluated by measuring the thickness of the bronchial wall, epithelial mucosa and smooth muscle, which changed during the pathogenesis of asthma. Remodeling of lung tissue of guinea pigs was observed after twelve weeks of local ovalbumin excitation and more collagen deposition was found in the AR model group compared with that in the control group. The process of local excitation represents an AR attack. The present study indicated that repeated attacks of AR may lead to the occurrence of pulmonary airway remodeling in guinea pigs with AR. The integrity of the upper and lower airways in this process was therefore indicated. The mechanisms underlying the consistency of upper and lower airway pathology in guinea pigs with AR requires further study.

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