

Chapter 7

Experimental Model of Allergic Asthma

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Abstract The aim of the study was to prepare and evaluate the experimental model of allergic asthma. Changes in cough reflex, bronchoconstriction and the degree of inflammation were studied in ovalbumin (OVA) sensitized guinea pigs after 0, 7, 14, 21 days of exposure. The cough reflex was induced by citric acid inhalation in conscious animals in a double chamber body plethysmograph. Tracheal smooth muscle reactivity was assessed by examining the *in vitro* response to histamine (H) (10^{-8} – 10^{-3} mol/l) and *in vivo* to H nebulization (10^{-6} mol/l). BALF levels of IL-4, IL-5 and the eosinophil count were used as parameters of airway inflammation. After 7 days of OVA sensitization, there was an increase in tracheal smooth muscle contractility *in vitro* to cumulative concentration of H and an increase in cough parameters. After 14 days of OVA sensitization, there was a further increase in tracheal smooth muscle contractility to H, an increase in airway resistance, and a small increase in cough parameters. After 21 day of OVA sensitization, cough parameters were significantly reduced, airway resistance after H inhalation was increased, and there were significant increases in IL-4, IL-5, and eosinophils in BALF. In conclusion, progress in asthmatic inflammation during 21-day OVA sensitization caused a gradual increase in inflammatory mediators, a decline in cough reflex, and enhanced bronchoconstriction. This experimental model of allergic asthma can be used for pharmacological modulations of defense reflexes and inflammation.

Keywords Airway hyperreactivity • Allergic asthma • Guinea pigs • Cough reflex • Ovalbumin • Sensitization • Citric acid

7.1 Introduction

Allergic asthma is a chronic inflammatory disorder of the airways. Characteristic features of this disease are allergen-induced early and late bronchial obstructive reactions, airway inflammation, structural changes of the airway wall associated with progressive decline in lung function, and airway

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hyperresponsiveness. Airway hyperresponsiveness is a clinical hallmark of asthma and it is defined by an exaggerated obstructive response of the airways to a variety of pharmacological, chemical, and physical stimuli (Postma and Kerstjens 1998). Allergic asthma continues to be clinically important, since bronchoconstriction and airway hyperresponsiveness remain beyond our complete pharmacological control (Janssen 2009).

A major problem in experimental studies on the pharmacological approach to allergic asthma is lack of valid animal models which would mimic airway hyperreactivity, early and late allergen reaction, and inflammation all seen in human conditions. Different animal models of allergic asthma have been applied for the investigation of the mechanisms of acute and chronic airway hyperresponsiveness (Meurs et al. 2008). Currently, the most widely used experimental animal for modelling the allergic responses in the airways is the mouse, due likely to the availability of transgenic and gene targeted mice. The mouse also is easily sensitized to a number of antigens, including ovalbumin and human allergens. However, murine models appear to be less suited for the investigation of the mechanisms of airway hyperresponsiveness in relation to early and late asthmatic responses. Thus, airway hyperresponsiveness and early asthmatic reactions are usually observed only after repeated allergen challenges, and a late asthmatic reaction is rarely observed (Zosky and Sly 2007; Zosky et al. 2008). Difficulties in measuring these responses could be related to the anatomical structure of the mouse lung (Irvin and Bates 2003) and the unresponsiveness of airway smooth muscles to various bronchoconstrictors implicated in the pathophysiology of asthma (histamine, cysteinyl leukotrienes, neurokinins, bradykinin, and prostanoids) (Canning 2003).

The aim of the present study was to prepare and evaluate a model of late allergic asthmatic response in guinea pigs. Our experiments were focused on the assessment of airway reactivity and the degree of inflammation during 21-day allergen (ovalbumin) administration. The assessment consisted of tracheal smooth muscle reactivity *in vitro*, changes in specific airway resistance in response to histamine *in vivo*, the degree of inflammation evaluated by eosinophil count, and the estimation of the inflammatory cytokines IL-4 and IL-5 in bronchoalveolar lavage fluid (BALF).

7.2 Methods

The protocol of this experimental study was approved by the Ethics Committee of the Jessenius Faculty of Medicine in Martin, Slovakia. Ovalbumin (OVA, egg albumin grade V), histamine (histamine $\times 2$ HCl), citric acid and other chemicals were purchased from Sigma Chemicals (Germany). Mouse IL-5, IL-4 ELISA Kits for protein array systems were purchased from RayBiotech, Inc. (Norcross, GA).

7.2.1 Sensitization of Guinea Pigs

Guinea pigs (200–250 g) were randomly divided into 2 experimental groups: Group 1 (Control) treated 21 days with saline only and Group 2 sensitized for 21 days with ovalbumin (OVA). There were 12 animals in each group.

Male Trik guinea pigs (200–250 g) were actively sensitized by administration of OVA (Grade V; Sigma Chemicals, Germany). Five milligrams of OVA (with 1 mg of aluminium hydroxide) were injected intraperitoneally together with 5 mg OVA in 1 ml of saline, subcutaneously, on Day 1. Subsequently, the guinea pigs were treated for 21 days with repeated i.p. OVA injections every 3 days. Additionally, the guinea pigs were exposed to nebulized OVA (1% in 0.9% sterile sodium chloride

solution) in an aerosol chamber for the last 5 days. This scheme of allergen sensitization has previously been found to evoke a sufficient degree of chronic inflammation in the airways of guinea pigs (Franova et al. 2009)

7.2.2 Tracheal Smooth Muscle Contraction *in vitro*

Reactivity of tracheal smooth muscle was assessed before OVA sensitization and then after 7, 14, 21 days of sensitization. Tracheal strips were placed in a 20 ml organ chamber containing Krebs-Henseleit buffer of the following composition in mM: NaCl 110.0, KCl 4.8, CaCl₂ 2.35, MgSO₄ 1.20, K₂PO₄ 1.20, and NaHCO₃ 25.0 in glass-distilled water. The chamber was maintained at 36.5°C ± 0.5°C and was aerated continuously with a mixture of 95% O₂ and 5% CO₂ to maintain pH 7.5 ± 0.1. The tracheal tissue strips were initially set to 4 g of tension (30-min loading phase). Afterward, tension was readjusted to the baseline of 2 g (30-min adaptation phase). The amplitude of muscle contraction (mN) to the cumulative doses of histamine (H) of 10⁻⁸–10⁻³ mol/l was used as a parameter of tracheal smooth muscle reactivity.

7.2.3 Evaluation of Airway Reactivity *in vivo*

Airway reactivity was evaluated *in vivo* using a rodent double chamber body plethysmograph (HSE type 855; Hugo Sachs Electronic-Harvard, with HSE Pulmodyn Pennock W software, Germany) before and after OVA sensitization according to the time schedule above outlined for the *in vitro* studies. Changes in specific airway resistance (RxW) after a 2-min inhalation of H (10⁻⁶ mol/l in saline) were considered an indicator of airways reactivity. There was an interval of 5 min between histamine exposure and airway resistance measurement. During this interval, fresh air was insufflated into the breathing chamber.

7.2.4 Chemically Induced Cough

Conscious guinea pigs, both control and OVA sensitized, were placed in a body plethysmograph box and was exposed to 0.3 M citric acid aerosol for 3 min. Cough detection and distinguishing of cough efforts from sneezing was based on expiratory airflow traces, recorded by a pneumotachograph connected to the head chamber of the plethysmograph, and typical cough movements and sounds recognized by a trained observer. The number of coughs was measured at 30 min intervals up to 120 min at a given day of sensitization above outlined.

7.2.5 Measurement of Inflammatory Cytokines

The left lung was lavaged with 0.9% NaCl (37°C) 2×10 ml/kg and BALF was centrifuged at 1,500 rpm for 10 min. The supernatant was used for subsequent biological analysis. Concentrations of IL-4 and IL-5 were determined by ELISA method in BALF, using commercially available kits (RayBiotech, Inc., Norcross, GA). The minimum detectable level of cytokines was 0.1 pg/ml.

7.2.6 Histological Examination

The trachea was fixed in 10% buffered formaldehyde. Sections of 10 μ m were made and stained with haematoxylin and eosin for light microscopy at a magnification 240x. On the basis of eosinophils counted, the degree of inflammation was divided into mild – up to 50, medium – up to 100, and strong – over 100 eosinophils.

7.2.7 Data Analysis

All results were expressed as means $\pm$ SE. Statistical analysis was performed using one-way ANOVA. Differences were considered statistically significant when p value was below 0.05.

7.3 Results

The effects of allergen sensitization on guinea pig tracheal smooth muscle reactivity were evaluated before OVA application and after 7, 14, 21 days OVA sensitization by *in vitro* responses to cumulative H. The results show progressive significant increases in contraction amplitude of tracheal strips to cumulative doses of H after 7 days of OVA sensitization, with a clear plateauing of the increase at the highest concentration of H (Fig. 7.1a). After another week of OVA sensitization, there was an enhancement of this progressive increase in contraction amplitude along all increasing H concentrations employed; the plateauing of the effect at the highest H concentration was no more observed (Fig. 7.1b). The contraction amplitude of tracheal strips remained at the same enhanced level after the 3rd week of OVA sensitization (Fig. 7.1c).

OVA sensitization caused gradual increases in specific airway resistance (RxW) after H nebulization (10^{-6} mol/l); the increase assumed significance as of the 14th day and was further appreciably augmented after 3 weeks of sensitization (Fig. 7.2a–c).

Concerning the cough reflex, the results were different. The number of chemically induced cough efforts was the greatest already after 7 days of OVA sensitization. From that point on, cough gradually

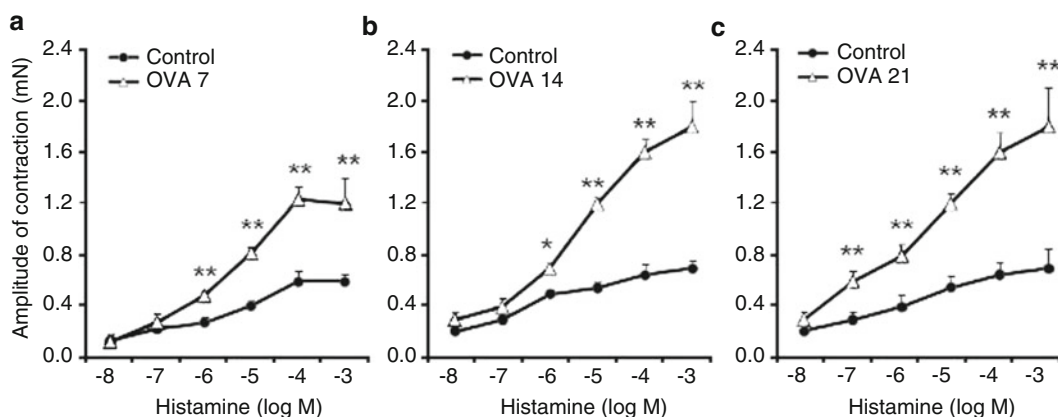


Fig. 7.1 Changes in amplitude of tracheal smooth muscle contraction in response to cumulative doses of histamine after (a) 7, (b) 14, and (c) 21-day of OVA sensitization; *p<0.05 and **p<0.01 for control vs. OVA

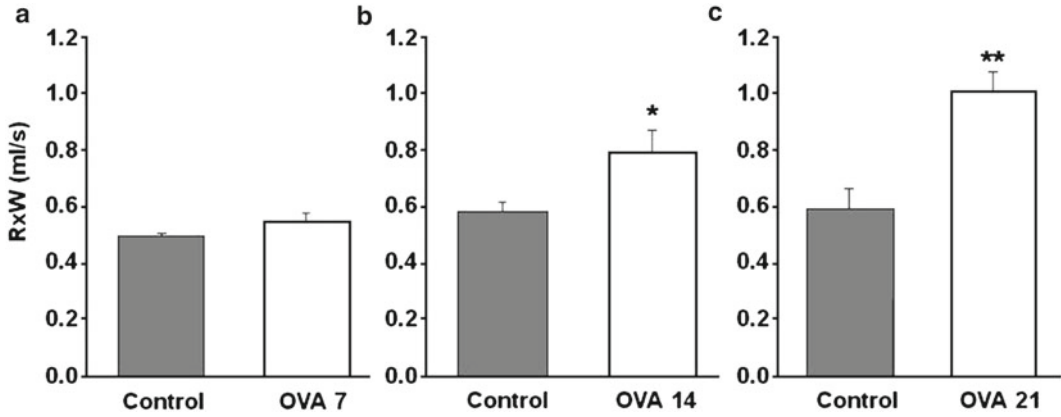


Fig. 7.2 Changes in specific airway resistance after inhalation of histamine (10^{-6} mol/l) between control healthy guinea pigs and then after (a) 7, (b) 14, (c) 21 days of OVA sensitization; * $p < 0.05$ and ** $p < 0.01$ for control vs. OVA

Fig. 7.3 Comparison of the number of citric-acid-induced coughs in control healthy guinea pigs and then after 7, 14, 21 days of OVA sensitization; ** $p < 0.01$ for control vs. OVA 7 days

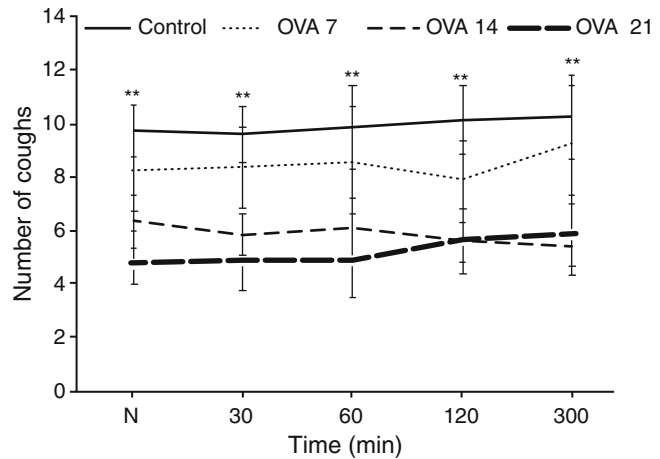
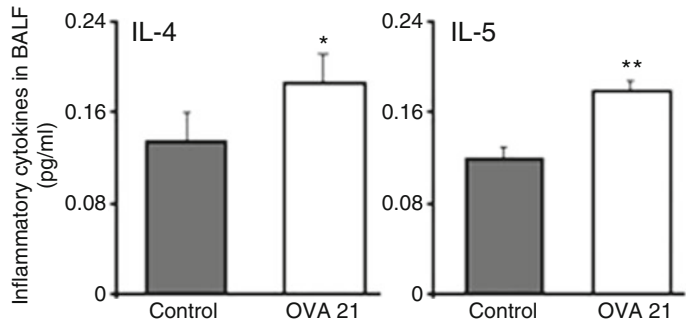


Fig. 7.4 Changes in the inflammatory cytokines IL-4, IL-5 in BALF between control healthy guinea pigs and after 21 days of OVA sensitization (OVA 21); * $p < 0.05$ and ** $p < 0.01$ for control vs. OVA 21



decreased with the extension of OVA sensitization time and actually dropped below the control level recorded before commencement of sensitization after 21 days (Fig. 7.3).

OVA sensitization also influenced the levels of airway inflammatory cytokines measured in BALF, as typified by IL-4 and IL-5. Both cytokines significantly increased; the increases were however noted not until the 21st day of sensitization (Fig. 7.4).

Histological examinations of tracheal mucosa of guinea pigs after OVA sensitization revealed, in general, eosinophilic infiltrations of the epithelium and submucosa. The intensity of inflammation in 12 animals after 21 days of sensitization was as follows: mild – 3, medium – 4, and strong – 3 cases. Eosinophils were organized into microabscess-like entities in the epithelium.

7.4 Discussion

In the present study we evaluated a model of the late allergic asthmatic response in guinea pigs, based on OVA sensitization. The results confirm the progression of airway inflammation after sensitization. The inflammatory cytokines IL-4 and IL-5 in BALF were gradually increasing throughout the period of sensitization, from the 7th up to the 21st day, reaching a significantly higher level at the end of that period. Sensitization also enhanced airway readiness for bronchoconstrictive responses. The cough reflex, on the other hand, after an early strong enhancement, clearly declined in the later part of the sensitization process.

Allergic asthma is defined in as a chronic inflammatory disorder characterized by reversible airways obstruction and airway hyperresponsiveness (AHR). Inflammation is thought to cause symptoms of asthma directly and indirectly by inducing contraction of airway smooth muscles, enhancing airway responsiveness to various stimuli and by inducing changes in structural components of the airway wall, including ASM cells, leading to airway remodeling (Zuyderduyn et al. 2008). Airway smooth muscle cells contribute to airway hyperresponsiveness, remodelling, and inflammation by virtue of their increased sensitivity to bronchoconstrictive stimuli (e.g., histamine, prostaglandins, kinins, or tachykinins) and increased proliferation (Meurs et al. 2008).

In this study we confirmed the development of an increase in tracheal smooth muscle reactivity during a 21-day period of OVA sensitization. Allergen application increased the amplitude of contraction of the tracheal smooth muscle strips, prepared from OVA sensitized guinea pigs, to cumulative doses of histamine. Concerning the *in vivo* airway reactivity, we observed a gradual increase in specific airway resistance measured in a double-chamber body plethysmograph over the sensitization period.

Another reflex which is modulated during inflammatory conditions is cough. The cough response is thought to be regulated by a neuronal network in the brainstem which receives signals from sensory receptors in the larynx and lower respiratory tract. Inflammation is a powerful stimulus for coughing. It has recently been determined that tachykinins, substance P, and the neurokinins A and B are released from non-myelinated C-fiber endings and have a role in airway inflammatory responses such as cough (Widdicombe 1995). It follows that depletion of tachykinins due to destruction of airway C-fibre receptors during inflammation might weaken the cough reflex. Karlsson (1996) has indeed reported a decrease in cough induced by capsaicin and citric acid due to inflammation. The results of the present study also confirm that inflammatory changes in the airways decrease cough reflex sensitivity.

In allergic asthma, it is assumed that the early-phase reaction is due to antigen cross-linking of immunoglobulin IgE molecules bound to specific cell-surface receptors on mast cells (Fc epsilon RI receptors). Subsequent activation of IgE leads to the release of bronchoconstricting mediators, notably leukotrienes and histamine. The allergic late-phase reaction is, in addition to the effects above outlined, linked with the infiltration of the airway wall by inflammatory cells (eosinophils, lymphocytes, and, particularly, CD4-positive T-cells). The immunological processes involved are characterized by proliferation and activation of Th2 lymphocytes (Van Oosterhout and Bloksma 2005). Th2-derived cytokines play a key role in orchestrating the eosinophilic inflammatory response in asthma. The Th2-mediated allergic inflammation is accomplished with cytokines, such as IL-4 and IL-13 the role of which is to make B lymphocytes synthesize IgE, IL-5 which has a role in maturation and activation of eosinophils, and IL-13 which has a role in mast cell genesis (Kips 2001). The estimation of inflammatory cytokines is thus a good marker of the advancement of asthmatic inflammation.

In conclusion, we can summarize that progress in asthmatic inflammation after 21 days sensitization of guinea pigs with OVA is associated with increase in inflammatory cytokines in BALF and amount of eosinophils in tracheal tissue, with the decline of cough reflex and strengthen of bronchoconstriction. From pharmacological point of view sensitised guinea pigs may be preferable as an animal model for investigating of late asthmatic reactions and AHR in asthma.

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