Respiratory Physiology & Neurobiology 170 (2010) 76–82

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Airway responsiveness and bronchoalveolar lavage fluid profiling in individual rats: Effects of different ovalbumin exposures

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ARTICLE INFO

Article history:
Accepted 23 November 2009

Keywords:
Forced oscillations
Bronchial hyperreactivity
Allergy
Ovalbumin

ABSTRACT

We studied repeatedly the development of bronchial hyperreactivity (BHR) and bronchoalveolar lavage fluid (BALF) in rats undergoing different modes of ovalbumin exposures. Treatment was two intraperitoneal injections of ovalbumin in Groups 1–3, followed by one ovalbumin aerosolization in Groups 2 and 3, while rats in Group 4 received repeated ovalbumin aerosols after one single intraperitoneal injection. BHR was assessed longitudinally on day 0 (before treatment) and on day 14 (Groups 1 and 2) or 20 (Groups 3 and 4) and cellular influx was estimated from BALF. No BHR or change in BALF cellular profile was detected in Groups 1–3. However, the infiltration of inflammatory cells, associated with BHR (PC_{100} 8.9 ± 1.3 μg/kg vs. 4.2 ± 1.1 μg/kg), was observed in Group 4. The BHR was always associated with increased number of eosinophils in the BALF. The substantial interindividual variability confirmed the need for a technique that permits follow-up of lung responsiveness and BALF profile. This approach evidenced strong associations between the severity of BHR and the eosinophilia.

1. Introduction

Asthma is a chronic inflammatory lung disease characterized by infiltration of inflammatory cells (mostly eosinophils) into the respiratory mucosa leading to enhanced reactivity of the airways to various constrictor stimuli. Although the underlying pathophysiological mechanisms responsible for this bronchial hyperreactivity (BHR) have not been fully elucidated, animal models have contributed substantially to the understanding of this lung disease by demonstrating the key role of airway inflammation following exposure to various allergens (Chung, 1986; Chung et al., 1985; Elwood et al., 1991; Gundel et al., 1990).

Exposure to materials such as ozone (Chung, 1986), ragweed (Chung et al., 1985), ascaris suum extract (Gundel et al., 1990) or most commonly ovalbumin (OVA) (Bellofiore and Martin, 1988; Eidelman et al., 1988; Elwood et al., 1991; Kamachi et al., 2002) leads to chronic airway inflammation in animal models. Since such exposure causes an influx of various inflammatory cells into the mucosa and lamina, the inflammatory process in the airways has been assessed by analyzing the cellular content of the bronchoalveolar lavage fluid (BALF) (Chung, 1986; Chung et al., 1985; Elwood et al., 1991; Gundel et al., 1990; Kamachi et al., 2002). BHR has been characterized via the changes in lung responsiveness to nonspecific constrictor stimuli, such as histamine, exercise, adenosine and methacholine (MCh). Airway responsiveness measurements and the BALF analyses are commonly combined in human subjects (Reynolds, 1987) and animal models (Glaab et al., 2004; Parker and Townsley, 2004; Varner et al., 1999; Walters et al., 2000). Although the size of the lungs in humans (Reynolds, 1987) and in larger animals (Cohen and Batra, 1980; Weiss et al., 1983) allows repetition of the BALF procedure, small rodents (mice and rats) are generally sacrificed at the end of the experiments. We recently developed an experimental model that allows quantification of the airway responsiveness and characterization of the BALF cellular profile repeatedly within the same rat (Novak et al., 2006). Since the immune responses of animals exhibit considerable interindividual variability, the performance of longitudinal studies with repeated measurements of the airway and tissue mechanics and profiling of the BALF are particularly important following allergic sensitization. Various sensitization protocols have been applied in different animal models, with variable results, and the differences between these methods have not been fully characterized.

The aim of the present study was therefore to assess the BHR and the influx of cells into the lungs repeatedly in rats exposed to different modes of administration of the allergen, OVA. Longitudinal changes in airway responsiveness were deter-

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doi:10.1016/j.resp.2009.11.008

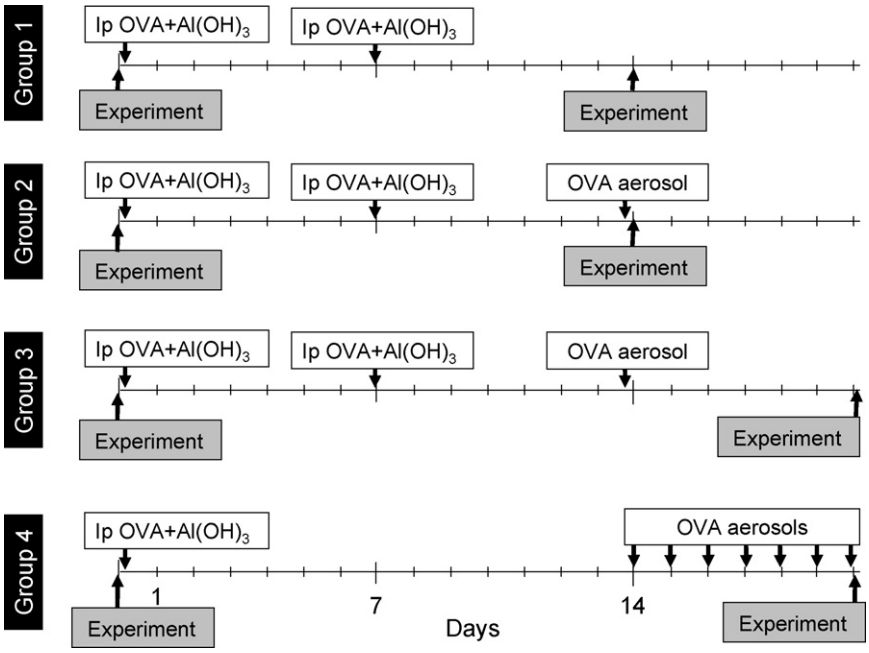

Fig. 1. Experimental and treatment protocols in the four groups of rats.

mined by applying the low-frequency forced oscillation technique, which allows reliable separation of the airway and respiratory tissue mechanics (Hantos et al., 1992; Petak et al., 1997, 1998), while the cellular influx was estimated by collecting partial BALF, which does not preclude the recovery of the animal from the anesthesia (Novak et al., 2006). By using these highly selective and sensitive experimental approaches, we questioned whether the intraperitoneal (ip) injection of the allergen, or its combination with single or multiple topical exposures of the airway epithelium, alters the lung responsiveness and/or BALF profile.

2. Materials and methods

The experimental protocol was approved by the Institutional Animal Care Committee of the University of Szeged School of Medicine, and was performed in accordance with the National Institutes of Health guidelines for animal use. The animals were kept in a healthy colony in the animal housing facility of the University of Szeged, and were allowed access to food and water *ad libitum*.

2.1. Treatment procedures and protocol groups

Four groups of Wistar rats were studied (weight range 350–500 g). After the first assessment of lung responsiveness by performing iv MCh challenges (detailed below) and BALF, the rats were assigned into one or the other of the following protocol groups (Fig. 1).

The animals in Group 1 ($N = 6$) received an ip injection of 1 mg OVA and 50 mg aluminium hydroxide (Sigma–Aldrich Ltd, Budapest, Hungary) on days 0 (after completing the first experiment) and 7. The experiments in these rats were performed on days 0 and 14.

The rats in Groups 2 ($N = 7$) and 3 ($N = 7$) similarly received an ip injection of 1 mg OVA and 50 mg aluminium hydroxide on days 0 (after completing the first experiment) and 7. These rats were then exposed to aerosolized (Voyage Mefar Jet Nebulizer, Italy) OVA (25 mg/ml in saline, driven by a flow rate of 8 l/min of compressed air) during a 20-min period prior to the experiments. The second set of experiments was than performed on day 14 in the animals of Group 2, and on day 20 in the rats involved in Group 3.

The Group 4 ($N = 10$) rats received an ip injection of 1 mg OVA and 50 mg aluminium hydroxide on day 0 (after completing the first experiment). Aerosolized OVA was then administered to these animals on seven consecutive days (days 14–20). Experiments were performed on day 0 and on day 20.

2.2. Animal preparations

On the days of the experiments, anesthesia was induced with an ip injection of 5% chloral hydrate (400 mg/kg). This dose can keep rats fully anesthetized for 50–60 min. Intubations were performed in the same manner as described by Brown et al. (1999). Briefly, the rat was suspended at an angle of 45° by its two front upper teeth, by a rubber band attached to a Plexiglas support. A 150-W halogen light source (Nicon Volpi Cold Light Illuminator) with two flexible fibre-optic arms allowed transillumination of the trachea just below the vocal cords. During this direct visualization, a 7.0-cm-long (ID 1.5 mm, OD 2.0 mm) polyethylene catheter was inserted with the help of a Draeger baby laryngoscope into the trachea via the oral cavity. To avoid tissue damage in the trachea, the tip of the catheter was rounded. The rat was then removed from the Plexiglas support, placed in a supine position on a special holder, attached to a small animal ventilator (Model 683, Harvard Apparatus, South Natick, MA, USA), and mechanically ventilated with room air (70 breaths/min, 7 ml/kg tidal volume). The tail vein was cannulated with a 24-gauge cannula (Vygonüle V 24G) and muscle relaxation was achieved by administering pancuronium bromide (0.2 mg/kg iv).

2.3. Measurement of respiratory mechanics

The input impedance of the respiratory system (Zrs) was next measured during short end-expiratory pauses interposed in the mechanical ventilation. The measurement set-up used to collect Zrs data (Petak et al., 1997) and its adaptation for repeated measurements were described in detail previously (Novak et al., 2006). Briefly, a three-way tap was used to