Respiratory Physiology & Neurobiology 247 (2018) 43–51

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

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

Lung responses in murine models of experimental asthma: Value of house dust mite over ovalbumin sensitization

Camille Doras[a], Ferenc Petak[b], Sam Bayat[a], Aurélie Baudat[a], Christophe Von Garnier[c], Philippe Eigenmann[d], Walid Habre[a,*]

[a] Anaesthesiological Investigations Unit, University Hospitals of Geneva, Geneva, Switzerland
[b] Department of Medical Physics and Informatics, University of Szeged, Szeged, Hungary
[c] Respiratory Medicine Department, Inselspital, University of Bern, Bern, Switzerland
[d] Department of Paediatrics, University Hospitals of Geneva, Geneva, Switzerland

ARTICLE INFO

keywords:
Asthma
Airway hyperresponsiveness
Allergen exposure
Animal models
Mice

ABSTRACT

Ovalbumin (OVA) sensitization has limitations in modelling asthma. Thus, we examined the value of allergic sensitization using a purified natural allergen, house dust mite (HDM), over the sensitization performed with OVA. Mice were sham-treated, or sensitized with OVA- or HDM with identical chronology. Airway resistance, tissue damping and elastance were assessed under control conditions and after challenging the animals with methacholine (MCh) and the specific allergen. Inflammatory profile of the bronchoalveolar lavage fluid was characterized and lung histology was performed. While no difference in the lung responsiveness to the specific allergen was noted, hyperresponsiveness to MCh was observed only in the HDM-sensitized animals in the lung peripheral parameters. Lung inflammation differed between the models, but excessive bronchial smooth muscle remodelling occurred only with OVA. In conclusion, we demonstrate that a purified natural allergen offers a more relevant murine model of human allergic asthma by expressing the key features of this chronic inflammatory disease both in the lung function and structure.

1. Introduction

Increasing exposure to environmental allergens and pollution contribute to the dramatic rise in the incidence of chronic lung diseases such as asthma, which is a major public health concern (ELF, 2009; WHO, 2008). One of the main features of asthma is chronic airway inflammation with subsequent bronchial hyperresponsiveness to different stimuli (Bosse, 2014; Brutsche et al., 2006; Mannino and Buist, 2007). Various animal models contributed greatly to a better understanding of the underlying pathophysiological mechanisms and to the characterization of the complex interactions between allergic, neurologic and immunological pathways (Gern, 2008).

In the last decades, airway sensitization to ovalbumin (OVA) was considered as the experimental model of reference to investigate the pathogenesis of allergic lung diseases (Bayat et al., 2009; Habre et al., 2008). However, the value of OVA in these models to mimic human asthma has been challenged due to the lack of key mechanisms such as eosinophil migration, sustained chronic airway inflammation and the development of allergen tolerance leading to desensitization (DiGiovanni et al., 2009; Fattouh et al., 2005; Johnson et al., 2004; Swirski et al., 2002). Another important limitation of OVA exposure is related to the site of lung mechanical response. This is limited to the central conducting airways after an exposure to a nonspecific constrictor stimuli, such as methacholine or histamine (Bayat et al., 2009), despite the involvement of small airways in the lung function deteriorations during asthma exacerbations (Bjermer, 2014; Contoli et al., 2012).

Recently, there has been an increased interest towards using natural airborne allergens in the animal models of allergic respiratory diseases. Among them, dermatophagoides pteronissinus (*D. pter*) is the most widespread dust mite with Der p1 being one of the major proteins implicated in the allergic process induced by *D. Pter* (Tournoy et al., 2000; Tovey et al., 1981). It has been identified as having a cysteine protease activity capable of inducing epithelial desquamation, inflammatory cytokines release and allergens transport facilitation (Kauffman et al., 2006). It stimulates pulmonary inflammation, IgE humoral response (Gough et al., 2003; Kikuchi et al., 2006) and Th2 cell response (Comoy et al., 1998). Therefore, sensitization to house dust mite (HDM) appears the most clinically relevant asthma model in mice and is gaining interest in experimental research (Kikuchi et al.,

* Corresponding author at: Anaesthesiological Investigations Unit, University Hospitals of Geneva, 6, rue Willy Donzé, 1205 Geneva, Switzerland.
E-mail address: walid.habre@hcuge.ch (W. Habre).

http://dx.doi.org/10.1016/j.resp.2017.09.001
Received 14 June 2017; Received in revised form 8 August 2017; Accepted 2 September 2017
Available online 08 September 2017
1569-9048/

2006). However, to date, the contribution of the conducting airways and the lung periphery to the lung mechanical responses following exogenous constrictor stimuli have not been compared between murine models of OVA and HDM sensitization. Additionally, such characterization is still missing following provocation with the specific allergen, which is of clinical relevance since asthma exacerbation occurs essentially following repeated airborne allergen exposures.

Therefore, we aimed at comparing the responses in the central conducting airways as well as in the lung periphery between mice sensitized and exposed to OVA with those sensitized and exposed to Der p1. Moreover, we aimed at investigating the differences between the 2 sensitization regimens following a lung constriction induced by non-specific cholinergic stimuli and during the early phase of allergic response.

2. Materials and methods

2.1. Animals

All experiments and procedures were conducted under the approval from the Swiss animal welfare committee (Geneva Cantonal Veterinary Office, registration number 1043/3924/2). Forty-four 10 week-old (22.0 ± 0.2 g) female BALB/cAnNRj mice purchased from Janvier Labs (St Berthevin, France) were involved in the study. Female BALB/c mice were chosen because of the most susceptible gender and strains to respiratory allergies including for HDM and OVA (Berndt et al., 2011; Blacquiere et al., 2010; Leme et al., 2010; Melgert et al., 2005).

2.2. Sensitization to allergens

Lyophilized total protein extracts of *D. pter* with known concentration of Der p1 were obtained from Stallergenes (Antony, France). Lyophilized ovalbumin from chicken egg white was purchased from SigmaAldrich (Buchs, Swizerland). Both substances were suspended in normal saline. Mice were sensitized to HDM with a protocol adapted from Tourdot et al. (2011). For an appropriate comparison, sensitizations with OVA and HDM were performed according to the same chronology. For both allergens, the procedure was initiated with two intraperitoneal injections of OVA (50 µg/200 µL) or HDM (dose equivalent to 10 µg Der p1 conveyed in 200 µL) supplemented with Al $(OH)_3$ as an adjuvant (2 mg) (Fig. 1A). 30-min long aerosol exposures to the allergens were then performed between days 21–25 with OVA (1 mg/mL) and HDM (dose equivalent to 0.1 mg/mL of Der p1), respectively. The same injections and aerosol exposures were carried out for the control group of mice with normal saline (SAL).

2.3. Anaesthesia and surgical preparation

Mice were anesthetized on day 28 or 29 with 5% isoflurane in a box until reflexes disappeared. Then a subcutaneous injection of a mixture of metomidate (Syndel Laboratories Ltd., Canada; 60 mg/kg) and fentanyl (60 µg/kg) was administered. After 5 min, a local anaesthesia by subcutaneous injection of 0.5% xylocaine (0.5 mL) was provided and a tracheostomy was performed. Mice were tracheotomised and mechanically ventilated with Flexivent system (Emka Technologies, Falls Church, VA, USA) in volume- controlled mode (tidal volume 8 mL/kg, 180 breaths/min, I:E 1/1.5, positive end-expiratory pressure (PEEP) 3 cmH_2O, FiO_2 50%). The mice were paralyzed thereafter with an intraperitoneal injection of muscle relaxant (5 mg/kg rocuronium bromide) to inhibit spontaneous breathing movements. This injection was repeated as needed (onset of respiratory movements during measurements). Similarly, the anaesthesia was maintained by additional intraperitoneal boluses of the anaesthetics at half doses of the concentrations used for the induction. Body temperature was maintained between 38 and 39 °C by using a heating mat and controlled rectally by a thermal sensor. ECG was continuously monitored by means of needle electrodes and PowerLab data acquisition system (ADinstrument, Dunedin, New Zealand).

2.4. Challenges and protocol groups

Mice were assigned into one or the other of the 7 protocol groups (Fig. 1B). Respiratory function was assessed in the anesthetized and ventilated animals by using the Flexivent system. In the mice challenged with the allergens, measurements were made during the baseline conditions and for 30 min following a single challenge of aerosolized OVA (1 mg/mL for 40s) in the Groups SAL/OVA (n = 6) and OVA/OVA (n = 8) or *D. pter* (0.1 mg/mL for 40 s) in the Groups SAL/HDM (n = 5) and HDM/HDM (n = 7). Measurements were made each 30 s until 2 min, then each 1 min for the following 3 min, every 2 min until 15 min followed by 20 and 30 min recording. In the other mice enrolled in groups SAL/MCh (n = 6), OVA/MCh (n = 8) and HDM/MCh (n = 6), basal respiratory mechanical measurements were repeated during an aerosolized challenge with a non-specific cholinergic constrictor agonist, methacholine (MCh) administered at increasing dose of 0.78, 1.56, 3.125, 6.25, 12.5 mg/mL (for 10 s each).

2.5. Measurement of respiratory mechanics

Lung volume history was standardized while inspiratory capacity (IC) was measured during the first deep inflation manoeuvre (slow inflation from PEEP to 30 cm H_2O with 3 s breath hold). Forced oscillatory respiratory mechanics were measured with Flexivent system. Respiratory system input impedance (Zrs) was derived from the signals detected by the ventilator piston volume displacement and cylinder pressure during 8 s oscillatory volume perturbations with 17 prime frequencies ranging from 0.5 and to 19.75 Hz. The impedance data were fitted with a model comprising an airway resistance (Raw) and inertance in series with a constant-phase model incorporating tissue damping (G) and elastance (H) (Hantos et al., 1992). Respiratory tissue hysteresivity (η) was calculated as G/H (Fredberg and Stamenovic, 1989).

2.6. Assessment of lung inflammation

At the end of the experiment, the animal was disconnected from the ventilator. Pre-warmed (38 °C) PBS was instilled via the endotracheal tube with a syringe containing a volume of 30 µL/g body weight (total lung volume of BALB/c mice (Thiesse, 2009)). Three gentle wash-in and wash-out maneuvers were made with the syringe to collect the bronchoalveolar lavage fluid (BALF); thereafter the animal was immediately euthanized. The BALF was centrifuged at 2000 rpm for 5 min at 5 °C and supernatant sampled and stored at −20 °C until use. The cell pellet was resuspended in BSA 1% in PBS, further dropped onto slides and centrifuged at 750 rpm for 7 min by cytospin. Then slides were fixed and stained with May Grünwald Giemsa for differential cell counting. Then stained slides were scanned by Mirax and the cells were counted by using an image acquisition software (Panoramic viewer, 3DHISTECH Ltd, Budapest, Hungary). Since the distribution of the cells was inhomogeneous, the cells were counted within rectangles with an edge length equivalent to the radius of the circular cytospin. The number of cells was normalized to the surface area of the rectangles.

2.7. Cytokine essays

The BALF supernatant and the blood serum were used to assess inflammatory cytokines. Blood samples were centrifuged at 4500 rpm for 3 min to collect serum, which was diluted 4× before the assays. Immuno assay with an appropriate software (Bio-Plex Manager™ Multiplex Reader) was used to measure fluorescence in the multiplex kit (Bio-Plex Pro™ Mouse Cytokine Th1/Th2 Assay #M6000003J7). This kit allowed the measurement of eight mice cytokines GM-CSF, IFN-γ, IL-