The effect of allergen-induced airway inflammation on airway remodeling in a murine model of allergic asthma

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Abstract. Objective and design: We examined the effect of airway inflammation on airway remodeling and bronchial responsiveness in a mouse model of allergic asthma. Materials and methods: BALB/c mice were sensitized to ovalbumin (OA), and exposed to aerosolized OA (0.01, 0.1 and 1 %). Twenty-four hours after the final antigen challenge, bronchial responsiveness was measured, and bronchoalveolar lavage (BAL) and histological examinations were carried out. Results: Repeated antigen exposure induced airway inflammation, IgE/IgG1 responses, epithelial changes, collagen deposition in the lungs, subepithelial fibrosis associated with increases in the amount of transforming growth factor (TGF)-β1 in BAL fluid (BALF), and bronchial hyperresponsiveness to acetylcholine. The number of eosinophils in BALF was significantly correlated with TGF-β1 production in BALF and the amount of hydroxyproline. Furthermore, significant correlations were found between these fibrogenic parameters and the bronchial responsiveness. Conclusion: These findings demonstrated that in this murine model airway eosinophilic inflammation is responsible for the development of airway remodeling as well as bronchial hyperresponsiveness in allergic bronchial asthma.

Key words: Airway inflammation – Bronchial hyperresponsiveness – Goblet cells – Subepithelial fibrosis – TGF-β1

Introduction

Bronchial asthma has been suggested to be a reversible disorder, characterized by airway eosinophilic inflammation and bronchial hyperresponsiveness (BHR) [1], however, a recent study demonstrated the inefficacy of anti-inflammatory therapy in some patients with asthma [2]. As a consequence, enhanced attention has been paid to the structural changes referred to as “airway remodeling”, which are characterized by airway wall edema, deposition of collagen beneath the basement membrane, goblet cell hyperplasia/hypertrrophy and smooth muscle hyperplasia/hypertrrophy, especially in morphological studies [3–6]. All these changes are suggested to be responsible for thickening of airway walls, which appears to be the basis of the airway flow limitation and hyperresponsiveness in asthma [7, 8], although the precise mechanisms and the contribution of airway inflammation in the development of airway remodeling are unknown.

We previously established an allergic asthma model in mice by systemic sensitization and repeated allergen exposure [9, 10], however, in this model, airway remodeling was limited, especially goblet cell hyperplasia [11], and subepithelial fibrosis was not detected. Although recent investigations have shown that prolonged antigen inhalation can induce airway remodeling in qualitative morphological studies using animal models [12–15], it remains unclear whether the degree of airway inflammation influences these structural changes in asthma. Also, there are no known studies that have evaluated, quantitatively, the collagen content after allergen challenge.

In the present study, we examined the effect of airway inflammation induced by different concentrations of aeroallergen on cellular accumulation, IgE synthesis, cytokine production in the airways, collagen deposition, epithelial changes and bronchial responsiveness to cholinergic stimuli in a mouse model of allergic asthma.

Materials and methods

Animals

Seven-week-old female BALB/c mice were purchased from Japan SLC (Shizuoka, Japan). The animals were housed in plastic cages in an air-conditioned room at 22 ± 1°C with a relative humidity of 60 ± 1 %, fed a standard laboratory diet and given water ad libitum. Experiments were performed following the guidelines for the care and use of experimental animals of the Japanese Association for Laboratory Animals Science in 1987.
Agents
The following drugs and chemicals were purchased commercially and used: ovalbumin (OA, Seikagaku Kogyo, Tokyo, Japan), acetylcholine chloride (ACh, Nacalai Tesque, Inc., Kyoto, Japan), panconronium bromide (Sigma, St. Louis, MO, USA), sodium pentobarbionate (Abbott Lab., Chicago, IL, USA), disodium ethylenediaminetetraacetic acid (EDTA-2Na, Nacalai Tesque), Diff-Quick solution (International Reagent Corp., Kobe, Japan), monoclonal anti-mouse IgE antibody (MO-5E-3, Serotec Co., Ltd., Oxford, UK), polyclonal goat anti-mouse IgG1 antibody (STAR81, Serotec Co. Ltd.), peroxidase-conjugated monoclonal rat anti-mouse IgG2a antibody (MCA421P, Serotec Co.Ltd.), peroxidase-conjugated streptavidin (Dakopatts a/s, Glostrup, Denmark) and hydroxy-L-proline (Nacalai Tesque).

Sensitization and antigen challenge
Mice were actively sensitized by intraperitoneal injections of 50 µg OA with 1 mg alum on day 0 and day 12 as described previously [9–11]. Starting on day 22, they were exposed to OA (0.01, 0.1 or 1% w/v diluted in sterile physiological saline) for 30 min every day for 3 consecutive weeks. Control animals were sensitized and then exposed to saline in a similar manner. Negative control animals were injected with saline and exposed to saline in a similar manner. The aerosol (particle size; 2.0–6.0 µm) was generated by a nebulizer (Ultrasonic nebulizer UN-701, Azweli Co. Ltd., Osaka, Japan) driven by filling a perspex cylinder chamber (Diameter 5.5 cm, Height 12 cm) with a nebulized solution.

Experimental protocol
Mice were divided into 5 groups. Mice were sensitized as previously, and were exposed to aeroallergen or saline for 3 consecutive weeks. Bronchial responsiveness to ACh, bronchoalveolar lavage and the measurement of hydroxyproline content in the right lungs were performed 24 h after the final exposure at week 3 (day 43).

Measurement of immunoglobulins
At week 3 (day 43), blood was collected and sera were obtained by centrifugation and stored at –80°C. Antigen-specific IgE, IgG1 and IgG2a in the mouse serum were measured using the enzyme-linked immunosorbent assay (ELISA) as previously described [16]. Briefly, serum OA-specific IgE was measured by coating monoclonal rat anti-mouse IgE antibody (MO-5E-3, Serotec Co., Ltd.) with OA and peroxidase-conjugated streptavidin. Serum OA-specific IgG1 was measured by coating polyclonal goat anti-mouse IgG1 antibody (STAR81) at a concentration of 2 µg/ml. After blocking with 1% BSA, serum dilutions were incubated for 1 h followed by biotinylated-OA and peroxidase-conjugated streptavidin. Serum OA-specific IgG2a was measured by coating OA solution at a concentration of 20 µg/ml. After blocking with 1% BSA, serum dilutions were incubated for 1 h followed by peroxidase-conjugated monoclonal rat anti-mouse IgG2a antibody. Sequentially diluted monoclonal anti-OA IgE, IgG1 and IgG2a (donated by Dr. Kiniwa, Taiho Pharmaceutical Co. Ltd.) were used as a standard.

Bronchoalveolar lavage study
To evaluate airway inflammation, we examined the accumulation of inflammatory cells in bronchoalveolar lavage fluid (BALF). Experiments were carried out according to a method previously described with modifications [11, 17]. Animals were sacrificed with an intraperitoneal injection of sodium pentobarbinate (100 mg/kg). The trachea was cannulated and the left bronchi were tied for histological examination. Then, the right air lumen was washed 4 times with 0.5 ml calcium- and magnesium-free phosphate-buffered saline (PBS) containing 0.1% BSA and 0.05 mM EDTA-2Na. This procedure was repeated three times (total volume; 1.3 ml, recovery >85%). BALF from each animal was pooled in a plastic tube, cooled on ice and centrifuged (150 x g) at 4°C for 10 min. Cell pellets were resuspended in the same buffer (0.5 ml). BALF was stained with Türk solution and the number of nucleated cells was counted in a Bürker chamber. A differential count was made on a smear prepared with a cytocentrifuge (Cytospin II, Shandon, Cheshire, England) and stained with Diff-Quick solution (based on standard morphologic criteria) of at least 300 cells (magnification x 500). The supernatant of BALF was stored at –30°C for determination of IL-13, IFN-γ and TGF-β1.

Cytokine levels in BALF
The amount of cytokine in the supernatant of BALF was measured using ELISA (Endogen Inc., Woburn, USA for IFN-γ, R&D Systems Inc., Minneapolis, MN, USA for IL-13). The TGF-β1 content in BALF was also measured using ELISA (Genzyme Tecne, Minneapolis, MN, USA), which can detect mouse TGF-β1 protein, because of the high homology of TGF-β1 across species. The assay detects only the active form of TGF-β1. Each sample was activated before measuring according to the manufacturer’s recommendations. The detection limit of each kit is 1.5 pg/ml for IL-13, 10 pg/ml for IFN-γ or 7 pg/ml for TGF-β1, respectively.

Measurement of airway function
Measurements of bronchial responsiveness to intravenous ACh were performed as previously described [11, 17]. Briefly, to measure bronchial responsiveness to ACh, mice were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and the jugular vein was cannulated for intravenous injection of ACh. Mice were injected with panconronium bromide (0.1 mg/kg, i.v.) to stop spontaneous respiration, and animals were ventilated using a rodent ventilator (New England Medical Instruments Inc., Medway, MA, USA) with oxygen supplemented air at 60 strokes/min, at a stroke volume of 0.6 ml/animal. Bronchoconstriction was measured according to the overflow method, using a bronchospasm transducer (Ugo Basile 7020, Milan, Italy) connected to the tracheal cannula. To measure bronchial responsiveness to ACh, changes in respiratory overflow volume were measured using increasing doses of ACh. The increase in respiratory overflow volume induced by ACh was represented as a percentage of the maximal overflow volume (100%) obtained by clamping the tracheal cannula. The bronchial responsiveness to ACh was evaluated as a provocative dose of ACh required to cause a 50% bronchoconstriction (PD50), which was calculated from each dose-response curve.

Measurement of hydroxyproline content in right lungs
Whole collagen content of the right lung was evaluated by determining hydroxyproline content [18]. Briefly, after recovery of BALF, the right lung lobes were removed and cut into sections (1 mm thick). The chopped lungs were dried with acetone. Then, the dried lung samples were hydrolyzed with 2 ml of 6 N HCl at 120°C for 24 h in sealed glass tubes. The amount of hydroxyproline in the hydrolysate was measured...