HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDIES ON EXPERIMENTAL ASTHMATIC MODEL INDUCED BY AEROSOLIZED OVALBUMIN INHALATION IN GUINEA PIGS

Yasukazu SATO, Tetsuya KISHI* and Takashi Umemura**

Research Center, Kyorin Pharmaceutical Co., Ltd., 1848 Nogi, Nogi-machi, Shimotsuga-gun, Tochigi 329-0114, Japan
*Central Research Laboratories, Kyorin Pharmaceutical Co., Ltd., 2399-1 Mitarai, Nogi-machi, Shimotsuga-gun, Tochigi 329-0100, Japan
**Department of Comparative Pathology, Faculty of Veterinary Medicine, Hokkaido University, N18W9, Kita-ku, Sapporo-shi, Hokkaido 060-0818, Japan

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ABSTRACT — To establish an animal model of asthma, fully sensitized guinea pigs inhaled aerosolized ovalbumin after booster treatments. At 0, 1, 3, 6, 24 and 48 hr after provocation (hap), histopathological and immunohistochemical changes in the airways of guinea pigs were examined. From 1 to 6 hap, anaphylactic changes such as perivascular edema and bronchoconstriction were detected. After that, intensive infiltration of eosinophils appeared and lasted until 48 hap. Temporal increases in the number of apoptotic cells and proliferating cell nuclear antigen positive cells were detected in the alveoli after the provocation. These findings suggest that this animal model showing both immediate and late asthmatic responses may be useful as an asthmatic model.

KEY WORDS : Apoptosis, Asthmatic model, Guinea pig, Inhalation, Ovalbumin, PCNA-positive cell

INTRODUCTION

Asthma is a chronic disease characterized by reversible airway obstruction and airway hyperresponsiveness to a variety of physical and chemical stimuli. In asthmatic patients, provocation of the airways with inhaled allergens causes an immediate asthmatic response (IAR) and late asthmatic response (LAR). LAR has been known to be related to an increase in non-specific bronchial hyperresponsiveness (HR) (Cockcroft et al., 1977). Associated with these LAR and HR but not with IAR is an elevated bronchoalveolar eosinophilia (De Monchy et al., 1985, Wardlaw et al., 1988). It is hypothesized that in bronchial asthma, eosinophils in the airway release their toxic granule proteins, such as major basic protein (Frigas et al., 1980, Gleich et al., 1979) and eosinophil cationic protein (Motojima et al., 1989), and damage the epithelial cell lining (Gleich et al., 1988). A number of histopathological examinations have been conducted using animal models in which asthmatic response occurs in the airways, and these studies have also discussed the relevance and the significance of eosinophil in development of asthma (De Monchy et al., 1985, Dunn et al., 1988, Ishida et al., 1989).

In our previous study, we detected an increased number of terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end labeling (TUNEL) and proliferating cell nuclear antigen (PCNA) positive cells in the alveolar compartment of guinea pigs fully sensitized with aerosolized ovalbumin (OA) (Sato and Umemura, 1997). In this study, the fully sensitized guinea pigs inhaled challenging OA after booster treatments for provocation of asthma. We examined the time course of pathological changes in the airways and discussed the validity of this animal model for asthma.

Correspondence : Yasukazu SATO at the above address.
Table 1. Time-related histopathological findings in sensitized guinea pigs after provocation by OA inhalation.

<table>
<thead>
<tr>
<th>Time (hr) after OA provocation</th>
<th>Pre provocation</th>
<th>1</th>
<th>3</th>
<th>6</th>
<th>24</th>
<th>48</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of guinea pigs examined</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Grade of lesions</td>
<td>-</td>
<td>±</td>
<td>+</td>
<td>#</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>Anaphylactic lesions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perivascular edema in lungs</td>
<td>3²)</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Pulmonary bronchoconstriction</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Other lesions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alveolar hemorrhage</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Neutrophil infiltration in bronchial submucosa</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Foam cell aggregation in lungs</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

- : No pathological changes, ±: very slight changes, +: slight changes, ++: Moderate changes, a): Number of guinea pigs.
Experimental asthmatic model in guinea pigs.

MATERIALS AND METHODS

Animals
A total of 17 male, 4-week-old Hartley guinea pigs (Japan Laboratory Animals Inc., Tokyo) weighing approximately 250g were used. They were maintained on a commercial diet (CG-7, Clea Japan, Tokyo) and tap water ad libitum, and housed at a temperature of 23±2°C, and a humidity of 50±10%, with 12 hr lighting (light on from 7:00 to 19:00).

Experimental Design
Active sensitization was induced by the inhalation of aerosolized antigen under the condition of spontaneous breathing without anesthesia (Arima et al., 1991, Sato and Umemura, 1997). Animals were placed in an acrylic exposure chamber (300x400x300 mm) and sensitized by exposure to aerosolized OA (Sigma, St. Louis, MO; 1% w/vol in 0.9% sodium chloride) administered at the rate of 1.5 ml/min via an ultrasonic nebulizer (Ne-U10B, Omron, Tokyo) for 10 min once a day. The establishment of sensitization was determined by the appearance of gasping and cyanosis.

The fully sensitized animals again inhaled 1% OA for 5 min a booster treatment at 7 and 14 days after sensitization. Fifteen min before inhalation, the animals were pretreated intraperitoneally with diphenhydramine (Sigma, St. Louis, MO; 60 mg/kg) in order to avoid histamine-induced fatal anaphylactic shock. One week after the 2nd booster treatment, the animals were provoked by the inhalation of 2% OA for 10 min. Fifteen min before the provocation, they were treated with diphenhydramine. The animals were sacrificed at pre-provocation (control), 1, 3, 6, 24 and 48 hr after provocation (hap).

Histopathological examination
Two to three animals were sacrificed under ether anesthesia on each occasion, and the trachea and lungs were then removed. The lungs were fixed by an infusion of 10% phosphate-buffered formalin via the trachea. The right posterior lobe was histologically examined in all cases. The specimens of the trachea and lungs were embedded in paraffin, cut serially into 4 μm sections in thickness, and stained with hematoxylineosin, Giemsa, Luna, alcian blue-PAS, and toluidine blue, respectively.

Detection of apoptotic cells
Apoptotic cells were detected by the method of TUNEL (Gavriel et al., 1992) using the Apop-Tag™ Plus-Peroxidase in situ Apoptosis Detection kit (Oncor Inc., Gaithersburg, MO). This method was based on the specific binding of TdT to 3'-OH ends of DNA. After pro-

teolytic treatment, TdT was used to incorporate biotinylated deoxyuridin at sites of DNA breaks. The binding sites were visualized by means of peroxidase-conjugated antidigoxigenin antibody and 3,3'-diaminobenzidine (DAB)(DAB Tablets S-300, DAKO Japan Inc., Kyoto). The sections were counterstained with methyl green.

Detection of PCNA positive cells
For immunohistochemical detection of PCNA positive cells, the paraffin sections were deparaffinized in xylol, then heated by autoclave (SS-320, TOMY SEIKO Co. Ltd., Tokyo) at 121°C for 15 min. Endogenous peroxidase was quenched by the introduction of freshly prepared 5% H2O2 for 5 min, followed by a rinse in tris buffered saline (TBS). The slides were incubated with anti-PCNA antibody (DAKO-EPOS Anti-PCNA/HPR) for 60 min at room temperature and rinsed with distilled water and TBS. DAB was applied for a PCNA single stain, then the sections were rinsed in distilled water and counterstained with Mayer's hematoxylin.

Criteria for tracheal epithelium damage
Three types of bronchi, different in size, were chosen randomly from the lungs of each specimen; i.e. bronchus circumscribed with bronchial cartilage, small bronchus scattered with cartilage around it, and terminal bronchus possessing a small amount of smooth muscular layer without cartilage.

<diagram>
Fig. 1. Degrees of eosinophil infiltration in lung tissue of sensitized guinea pigs after provocation by OA inhalation.

Vol. 23 No. 1
**photo 1.** No pathological changes at pre-provocation (0 hr). Luna stain. × 39.6

**photo 2.** Moderate to severe eosinophil infiltration at the small bronchiolar mucosa or submucosa at 6 hr after provocation. Luna stain. × 39.6
Experimental asthmatic model in guinea pigs.

The degree of epithelial change and inflammatory cell infiltration was categorized into 5 groups, and the degree of eosinophil infiltration was scored 0 to 4 according to the following grading: −; no remarkable changes (Score 0), ±; very slight changes (Score 1), +; slight changes (Score 2), ++; moderate changes (Score 3), and +++; Severe changes (Score 4).

A total number of apoptotic cells and PCNA-positive cells were also counted in each lung specimen in whole visual fields at magnification ×400.

Statistical analysis

All experimental values except for histopathological findings were expressed as mean ± SD, and were analyzed using the Student’s t-test with p<0.05 as minimum significance.

RESULTS

Histopathological examination

Perivascular edema and bronchoconstrictions were found in several animals sacrificed at 1 to 6 hap. On day 2, no pathological changes were found in the tra-
chea and lungs except for eosinophil infiltration (Table 1). The degree of eosinophil infiltration in the tracheal mucosa or submucosa showed a tendency to intensify with time, and eosinophil infiltration in bronchial mucosa or submucosa was most intense at 6 hap (Fig. 1, Photo 1, 2).

![Graph](image)

Fig. 2. Number of apoptotic cells in lung tissue of sensitized guinea pigs after provocation by OA inhalation.

* **: Significantly different from the value at pre-provocation (0 hr) at p<0.05 and p<0.01, respectively.

![Photo](image)

photo 3. Many TUNEL positive, apoptotic cells in alveolar compartments at 24 hr after provocation. TUNEL stain, ×132.
Labeling of apoptotic cells

The number of apoptotic alveolar epithelial cells appeared to gradually decrease until 6 hap. At 24 hap, however, the number of apoptotic cells markedly and significantly increased (Photo 3). On day 2 after provocation, this transient increase returned to control levels (Fig. 2).

Labeling of PCNA

A transient 2-fold increase in the number of PCNA-positive alveolar epithelial cells occurred at 3 and 48 hap (Fig. 3).

![Graph](image)

**Fig. 3.** Number of PCNA positive cells in lung tissue of sensitized guinea pigs after provocation by OA inhalation.

*: Significantly different from the value at pre-provocation (0 hr) at p<0.05.

DISCUSSION

We previously examined the airway of guinea pigs fully sensitized with aerosolized OA and found anaphylactic changes in the lungs and bronchi (Sato and Umemura, 1997). In the present study, the fully sensitized animals were further provoked by OA inhalation after booster treatment and showed perivascular edema, bronchoconstriction and infiltration of eosinophils in the airways. These pathological changes concurred well with IAR and LAR of asthmatic patients (De Monchy et al., 1985, Kay, 1991). Asthmatic patients show airway obstruction by inhalation challenge with aerosolized allergen. The allergen-induced IARs are reversible, and many of the IARs progress to LARs exhibiting eosinophil infiltration in the airways 4 to 12 hr after IAR (Booij-Noord et al., 1971). The present animal models showed the pathological findings of both IAR and LAR after inhalation of OA. Animal models of asthma showing both IAR and LAR are not many in the literature (Iijima et al., 1987, Yukawa et al., 1987). Therefore the present animal model may be one of the useful animal models for asthma.

In our previous report, we observed not only an increased number of apoptotic and PCNA-positive cells, but also vascular leak syndrome-like lesions in the alveolar compartments of the sensitized guinea pigs. We suggested that the appearance of these morphological changes was possibly associated with interleukin-2. Zhang and co-workers (1995) observed that the pulmonary morphological alteration in rats treated with human recombinant interleukin-2 consisted of endothelial cell damage, interstitial edema, and injury to bronchial and alveolar epithelial cells, especially type II alveolar epithelial cells. These damaged cells showed nuclear and cytoplasmic features indicative of apoptosis. Similar findings were also observed in the present experiment and confirmed our previous finding.

To the best of our knowledge, there has been no report describing the elevated apoptotic and proliferative activities of alveolar epithelial cells in asthmatic patients and animal models for asthma. The significance and cause of the alveolar changes in our animal model remain to be elucidated in the future.

REFERENCES


Experimental asthmatic model in guinea pigs.


