

## Difference in Inhibitory Effects of Dexamethasone and Cyclosporin A on Sephadex Bead-Induced Airway Hyperresponsiveness and Inflammation in Rats

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*Received November 18, 1997 Accepted February 28, 1998*

**ABSTRACT**—We investigated the effects of dexamethasone and cyclosporin A on Sephadex bead (Sephadex G-200, Sephadex)-induced airway hyperresponsiveness (AHR) and inflammation in rats. Sephadex (0.5 mg/animal) was intravenously administered on days 0, 2 and 5. Bronchoalveolar lavage, histological study and measurement of AHR were performed on day 7. Dexamethasone (0.1, 1 and 10 mg/kg, p.o.  $\times 3$ ) and cyclosporin A (0.1, 1 and 10 mg/kg, s.c.  $\times 3$ ) clearly inhibited the increase in eosinophils in bronchoalveolar lavage fluid after Sephadex injection. On histological study, pulmonary eosinophilia, granulomatous arteritis with horseshoe-shaped multinuclear giant cell formation and goblet hyperplasia were observed after Sephadex injection. Both dexamethasone (10 mg/kg  $\times 3$ ) and cyclosporin A (10 mg/kg  $\times 3$ ) inhibited these findings and an increase in eosinophil peroxidase in the lung. Dexamethasone dose-dependently inhibited AHR induced by Sephadex, and completely suppressed it at a dose of 1 mg/kg ( $\times 3$ ). Cyclosporin A, however, did not inhibit AHR even at a dose of 10 mg/kg ( $\times 3$ ). These results show that there is a difference between dexamethasone and cyclosporin A in the inhibitory effect on Sephadex-induced AHR, and they suggest that eosinophils are not directly associated with the development of AHR after Sephadex injection.

**Keywords:** Sephadex bead, Dexamethasone, Cyclosporin A, Eosinophil, Airway hyperresponsiveness

Airway hyperresponsiveness (AHR) is a hallmark of asthma (1). This phenomenon is thought to be associated with airway inflammation and is characterized by a cellular infiltrate in the airway mucosa in which eosinophils and mononuclear cells are prominent (2). Although several mechanisms to explain the development of AHR have been proposed, the pivotal role of eosinophils is now accepted (3, 4).

Sephadex beads induce AHR with pulmonary and peripheral blood eosinophilia in rats and guinea pigs (5–7). Therefore, this model is invaluable for investigating the relationship between development of AHR and inflammatory changes (especially eosinophil infiltration in the airway). We have reported strain-related differences in Sephadex-induced AHR, in that Lewis rats exhibited reproducible AHR but Brown-Norway, Fischer 344 and Wistar-Kyoto rats did not (8). The use of Lewis rats is appropriate for investigation of the relationship between AHR and airway inflammation because they exhibit not only reproducible AHR, but also significant increases in eosinophils and mononuclear leukocytes in the airways

are recognized.

Glucocorticosteroids are anti-inflammatory agents which have been used in various inflammation models (9–12). They are highly effective in controlling chronic inflammatory diseases (13). Dexamethasone, a synthetic glucocorticosteroid, is a potent inhibitor of T-lymphocyte proliferation induced by antigens and mitogens (14). Corticosteroids such as dexamethasone are the most potent drugs for treating the underlying eosinophilic inflammatory process in the airways of asthmatics (15). Recently, some immunosuppressant agents have also been evaluated as therapy for chronic asthma (16–18). Cyclosporin A is an immunosuppressant with powerful T-lymphocyte inhibition (15). Additionally, apoptotic death of activated eosinophils is augmented by dexamethasone and cyclosporin A (19).

In the present study, we examined the effects of dexamethasone and cyclosporin A on leukocyte infiltration, inflammatory changes in the lung and the development of AHR, by bronchoalveolar lavage (BAL), histological study, assay of eosinophil peroxidase (EPO) activity and

measurement of airway responsiveness using Lewis rats.

## MATERIALS AND METHODS

### *Animals*

Male Lewis rats (6-week-old) were purchased from Charles River Japan (Tsukuba). These animals were acclimatized in an environmentally controlled room (temperature:  $23 \pm 2^\circ\text{C}$ , humidity:  $55 \pm 5\%$ , illumination time: 7:00 to 19:00), with food and water available ad libitum for at least 1 week before the experiment. Experimental procedures followed in this study adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals guideline.

### *Chemicals*

The following chemicals were used: Sephadex beads (Sephadex G-200, particle size: 40–120  $\mu\text{m}$ ; Pharmacia, Uppsala, Sweden); cyclosporin A (Sandimmun; Novartis, Basel, Switzerland); sterile physiological saline and distilled water (Otsuka Pharmaceuticals, Tokyo); urethane, HEPES, gallamine triethiodide, dexamethasone, hexadecyltrimethylammonium bromide (HTAB) and *o*-phenylenediamine dihydrochloride (OPD) (Sigma, St. Louis, MO, USA); serotonin creatinine sulfate (5-HT) and Giemsa's stain solution (Merck, Darmstadt, Germany); heparin (Mochida Pharmaceutical, Tokyo); Hanks' balanced salt (Nissui Pharmaceutical, Tokyo); Tween 80 (Kanto Chemical, Tokyo); hydrogen peroxide (Mitsubishi Gas Chemical, Tokyo); sulfuric acid and Tris (hydroxymethyl) aminomethane (Tris) (Nacalai Tesque, Kyoto); horseradish peroxidase (Boehringer Mannheim, Mannheim, Germany). These reagents were laboratory grade or better.

### *Administration of Sephadex, dexamethasone and cyclosporin A*

A suspension of Sephadex beads (Sephadex) in sterile saline was prepared and stored for at least 48 hr at  $4^\circ\text{C}$  with gentle stirring. The suspension (0.5 mg/ml/animal) or an equivalent volume of vehicle (sterile saline, 1 ml/animal) was intravenously administered to animals via the tail vein. Administration of Sephadex or saline was performed on days 0, 2 and 5; and BAL, histological study, assay of EPO activity and measurement of airway responsiveness to 5-HT were performed on day 7.

Dexamethasone was suspended in distilled water containing 0.1% Tween 80. The drug at a volume of 5 ml/kg was orally administered 4 hr before each Sephadex injection. Cyclosporin A, prepared from Sandimmun solution at a volume of 5 ml/kg, was subcutaneously administered 2 hr before each Sephadex injection.

### *BAL*

Animals were sacrificed with an overdose of urethane anesthesia (2–3 g/kg, i.p.). A cannula was inserted in the trachea through a tracheotomy. BAL was independently performed with Hanks' balanced salt solution (HBSS, pH 7.2, volume: 2 ml  $\times$  5/animal) containing 25 mM HEPES and heparin (10 U/ml) warmed to  $37^\circ\text{C}$ , through the catheter in the trachea. BAL fluid (BALF) obtained from each animal was immediately cooled to  $4^\circ\text{C}$ , and then it was centrifuged at  $400 \times g$  for 10 min at  $4^\circ\text{C}$ . The obtained precipitate was suspended in 0.5 ml HBSS/animal, and the total number of cells in 50  $\mu\text{l}$  of suspension was counted by a hemocytometer (Celltac MEK-5158; Nihon Kohden, Tokyo). A 5- to 40- $\mu\text{l}$  aliquot of the cell suspension for differential counting was centrifuged by cytopspin (Cytospin 3; Shandon, Cheshire, UK) at 800 rpm for 3 min at room temperature. After making a smear preparation, cells on the slide glass were stained with Giemsa's stain. The numbers of neutrophils, eosinophils and mononuclear leukocytes (macrophages, monocytes and lymphocytes) were counted under a light microscope at a magnification of  $\times 1000$ .

### *Histological study*

The animals were sacrificed by urethane anesthesia (1.5 g/kg, i.p.) and exsanguination. The trachea and lung were isolated from the control and Sephadex-treated animals. Isolated trachea and lung were fixed in neutralized buffered formalin, embedded in paraffin, cut and stained with Giemsa, hematoxylin-eosin or PAS stain and observed under a light microscope.

### *Assay of EPO activity*

EPO was assayed in diluted lung homogenates. The animals were anesthetized with urethane (1 g/kg, i.p.) and were transcardially perfused with 50 mM Tris (pH 8.0) containing 0.5% HTAB for 5 min. The lung was then isolated, and it was minced. Samples of lung parenchyma were homogenized in 50 mM Tris (pH 8.0) containing 0.5% HTAB at 3% wet w/v weight with a homogenizer (Polytron®; Kinematica, Luzern, Switzerland) in a polypropylene tube. The homogenized samples were centrifuged at  $1,700 \times g$  for 20 min at room temperature. The obtained supernatant was filtered by a filter (pore size: 0.45  $\mu\text{m}$ , Steradisc 25; Kurabo, Osaka). An aliquot of clear supernatant, referred to as lung extract, was taken for assay of EPO activity.

The extracts were diluted 1/20 in 50 mM Tris (pH 8.0) containing 0.5% HTAB. A 100- $\mu\text{l}$  aliquot of diluted sample was incubated with 0.4 mg/ml OPD in 50  $\mu\text{l}$  of 50 mM Tris (pH 8.0) containing 0.5% HTAB and 0.013% hydrogen peroxide in a 96-well tissue culture plate for 5 min at room temperature. The reaction was terminated by

the addition of 4 M sulfuric acid (100  $\mu$ l). The absorbance was read at 492 nm using a microtiter plate reader (SPECTRAMax; Molecular Devices, Sunnyvale, CA, USA). The EPO activity was defined as peroxidase activity (U/g lung wet weight) calculated with a horseradish peroxidase standard curve.

#### Measurement of airway responsiveness

To evaluate airway responsiveness after Sephadex injection, animals were anesthetized with urethane (1.5 g/kg, i.p.). Then tracheotomy was performed and a polyethylene tube was passed through the stoma for a distance of about 1 cm into the tracheal lumen. The lateral saphenous vein was then cannulated for intravenous injection of 5-HT. The animals were paralyzed

with gallamine triethiodide (2 mg/kg, i.v.) and then mechanically ventilated (model 680-683; Harvard Apparatus, South Natick, MA, USA) at a constant tidal volume of 10 ml/kg and a frequency of 70 breaths/min at a pressure of 8 cmH<sub>2</sub>O. The changes in pulmonary mechanics were measured in accordance with the modified method of Konzett and Rössler (20) with a differential pressure transducer (TP-602, Nihon Kohden) connected to the tracheal cannula. The increase in ventilation overflow volume ( $\Delta$ VOFV) induced by 5-HT (12–384  $\mu$ g/kg, i.v.) administration was expressed as a percentage of the maximum bronchoconstriction obtained by clamping off the trachea. The provocative dose of 5-HT that caused 20% bronchoconstriction (PD<sub>20</sub>) was defined and log-transformed as an index of AHR (logPD<sub>20</sub>). When the

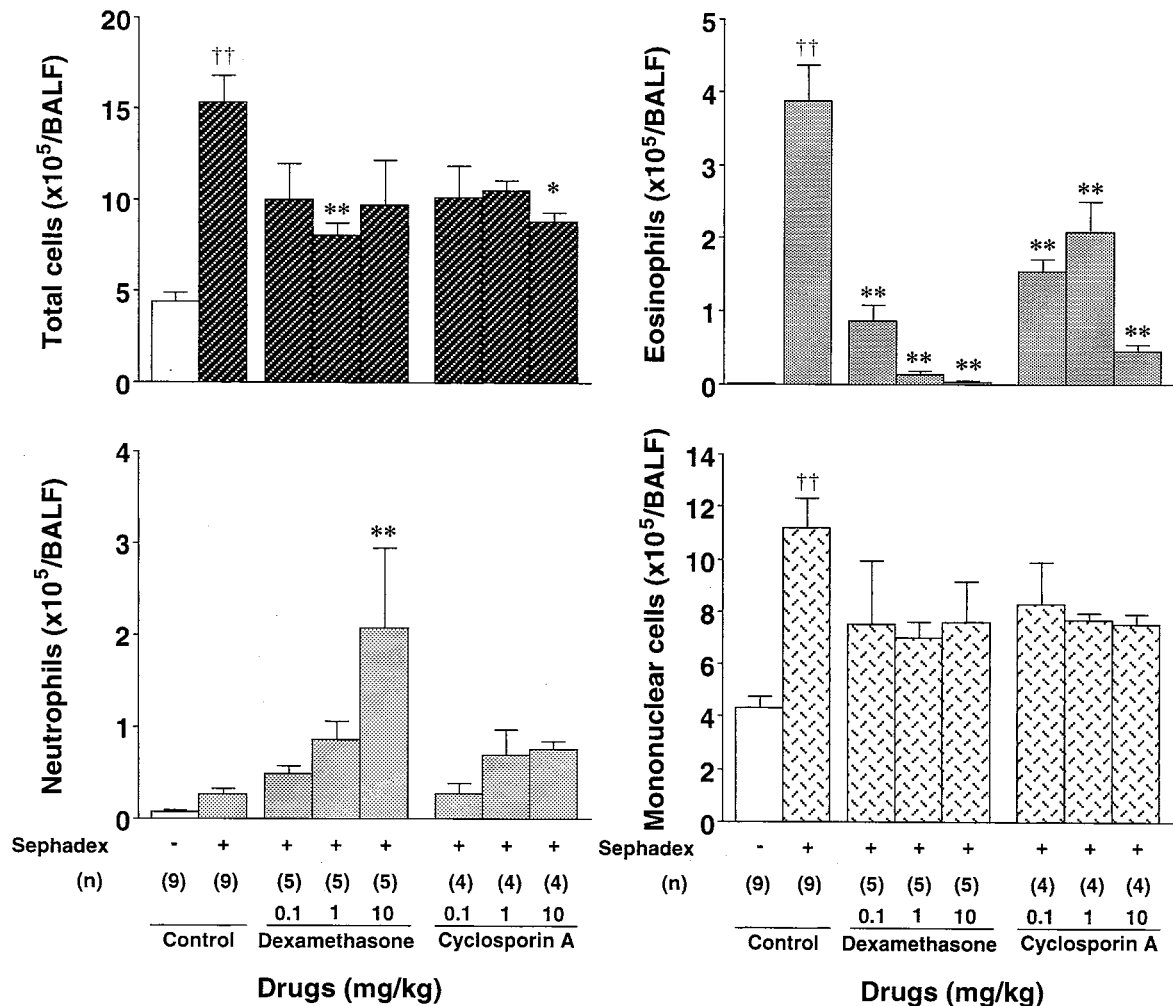


**Fig. 1.** Effects of dexamethasone and cyclosporin A on inflammatory cell infiltration after Sephadex G-200 (Sephadex) injection in Lewis rats. Saline (1 ml) or Sephadex (0.5 mg/ml/rat) was intravenously administered to rats on days 0, 2 and 5. Bronchoalveolar lavage was performed on day 7. Each column represents a mean value and vertical lines indicate S.E. (n=4–9). \*P<0.05 and \*\*P<0.01, statistically significant compared with the Sephadex-control group; ††P<0.01, statistically significant compared with the saline-control group by one-way ANOVA (Tukey-Kramer's method).

bronchoconstriction induced by maximum dose of 5-HT was lower than 20%,  $PD_{20}$  was calculated with linear plotting between maximum and submaximum dose of 5-HT.

#### Statistical analyses

All results are expressed as mean  $\pm$  S.E. except for the data of histological study. Comparisons of mean values were made by one-way analysis of variance (ANOVA) followed by Tukey-Kramer's method. Differences were considered statistically significant when  $P < 0.05$ .

## RESULTS

#### Effects of dexamethasone and cyclosporin A on Sephadex-induced inflammatory cell infiltration in BALF

To evaluate the effects of dexamethasone and cyclosporin A on Sephadex-induced inflammatory cell

infiltration in the airway lumen, BAL was performed. Figure 1 shows the results of the BAL experiments. Total leukocyte count was significantly increased by Sephadex injection ( $P < 0.01$  vs saline-control group). The increase in total leukocytes was significantly ( $P < 0.01$  vs Sephadex-control group) inhibited by dexamethasone and cyclosporin A at a dose of 1 and 10 mg/kg ( $\times 3$ ), respectively. Eosinophils and mononuclear cells were significantly increased by Sephadex injection ( $P < 0.01$  vs saline-control group). The increase in eosinophils was significantly ( $P < 0.01$  vs saline-control group) inhibited by dexamethasone and cyclosporin A at doses of 0.1 mg/kg ( $\times 3$ ) and higher. The increase in mononuclear cells was inhibited by both drugs, but this was not significant. Neutrophils were slightly increased by Sephadex injection, but the increase was not significant. Dexamethasone dose-dependently increased neutrophils in BALF of Sephadex-treated rats, and the potentiation was

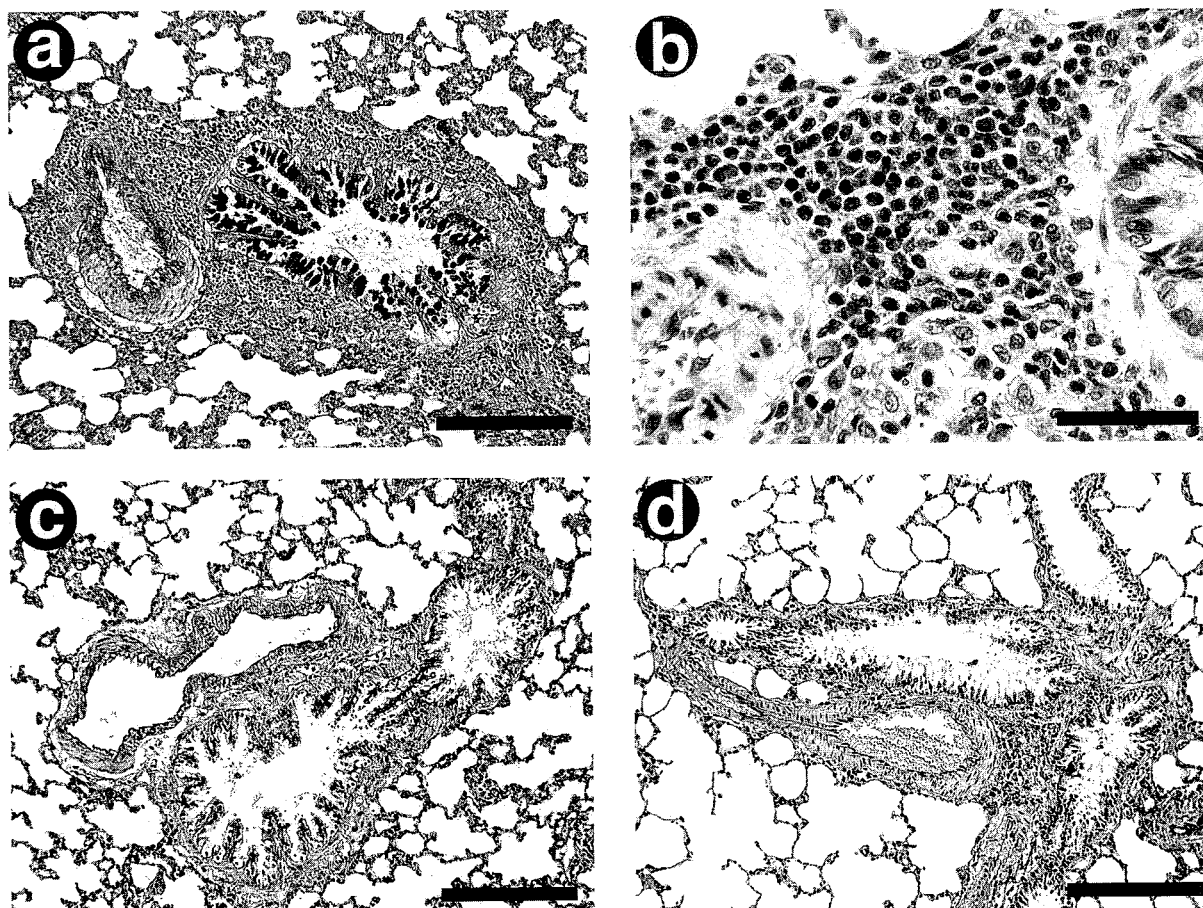


Fig. 2. Effects of dexamethasone and cyclosporin A on Sephadex-induced pulmonary eosinophilia in Lewis rats. Pulmonary eosinophilia appeared in the lung tissue around the airways and blood vessels in Sephadex-control group (a and b). Dexamethasone (10 mg/kg, p.o.  $\times 3$ ) (c) and cyclosporin A (10 mg/kg, p.o.  $\times 3$ ) (d) clearly inhibited the eosinophil accumulation in lung tissue induced by Sephadex injection. Giemsa stain. Bars = 200  $\mu$ m (a, c and d) and 50  $\mu$ m (b).

significant ( $P < 0.01$  vs Sephadex-control group) at a dose of 10 mg/kg ( $\times 3$ ). Cyclosporin A slightly potentiated the neutrophil infiltration in BALF, but the potentiation was not significant.

*Effects of dexamethasone and cyclosporin A on histological changes in lungs after Sephadex injection*

Inflammatory cell infiltration, especially of eosinophils, was observed in Giemsa-stained preparations and representative photographs are shown in Fig. 2. In the experiment, 10 mg/kg ( $\times 3$ ) dexamethasone or cyclosporin A was administered p.o. or s.c., respectively. The inflammatory cell infiltrate was marked and mainly consisted of mononuclear cells and eosinophils. Both dexamethasone and cyclosporin A inhibited the appearance of eosinophils in lung tissue.

Inflammatory changes in lung tissue were observed in hematoxylin-eosin-stained preparations, and representa-

tive photographs are shown in Fig. 3. Small diameter arteries were clogged with Sephadex particles in the control and drug-treated groups. The arteries displayed granulomatous arteritis. The appearance of huge horse-shoe-shaped multinuclear giant cells formed by fusion of mononuclear cells was observed in and around granulomas. A marked inflammatory cell infiltrate was observed in the mucosal layer and in or around granulomas. These findings were inhibited by administration of dexamethasone or cyclosporin A (10 mg/kg  $\times 3$ ).

Changes in the mucosal layer of the peripheral airways in the lung were observed in PAS-stained preparations and representative photographs are shown in Fig. 4. PAS-positive goblet cells were recognized in the Sephadex-control group. The goblet cell hyperplasia was also clearly inhibited by both 10 mg/kg ( $\times 3$ ) dexamethasone and cyclosporin A.

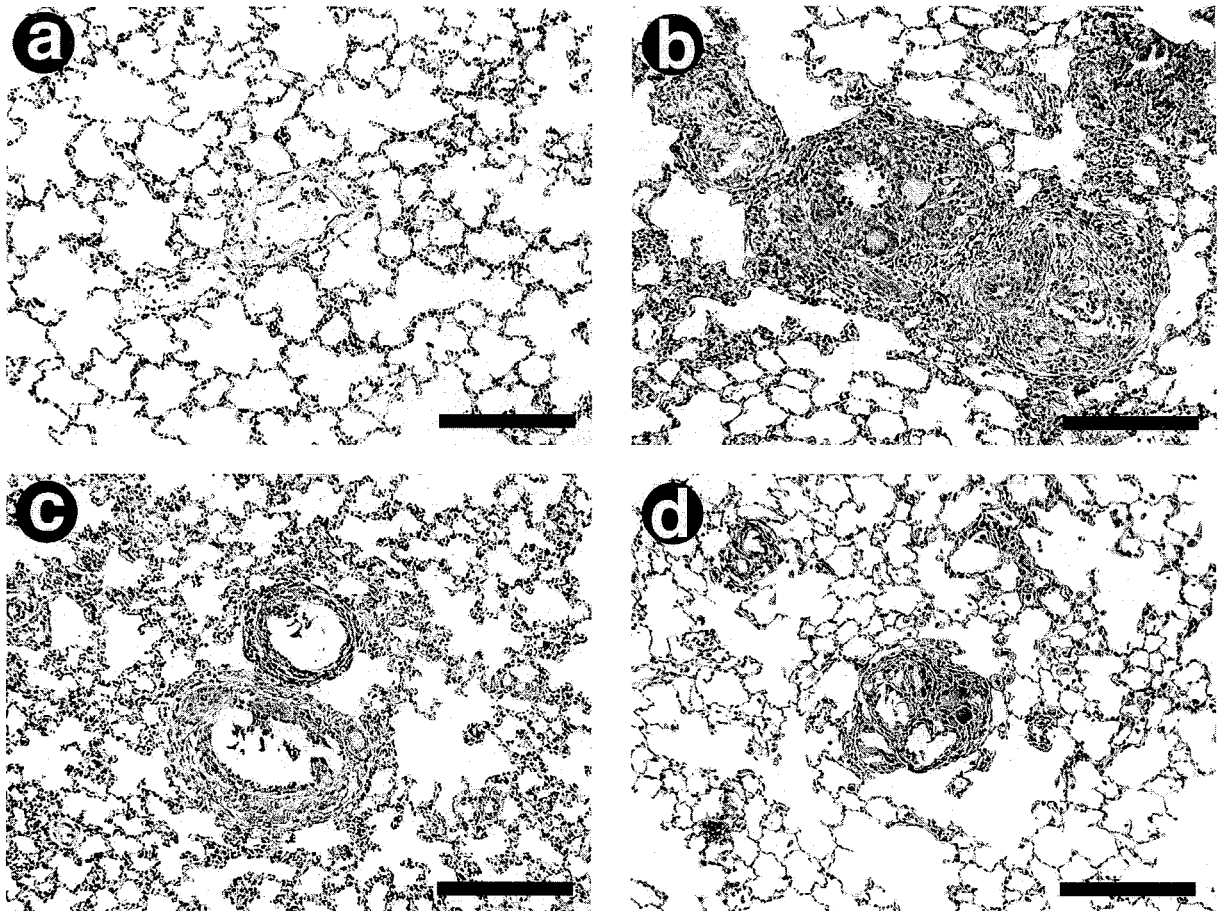


**Fig. 3.** Histopathological changes of Sephadex-induced pulmonary inflammation in dexamethasone- and cyclosporin A-treated rats. a: Saline-control group, b: Sephadex-control group, c: Sephadex-dexamethasone (10 mg/kg, p.o.  $\times 3$ ) group, d: Sephadex-cyclosporin A (10 mg/kg, p.o.  $\times 3$ ) group. Dexamethasone and cyclosporin A clearly inhibited the appearance of granulomas with multinuclear giant cells induced by Sephadex injection. Hematoxylin-eosin stain. Bars = 200  $\mu$ m.

*Effects of dexamethasone and cyclosporin A on increase in EPO in the lung after Sephadex injection*

To evaluate the effects of dexamethasone and cyclosporin A on eosinophil activation in the lung tissue after Sephadex injection, EPO activity was assayed as a parameter of eosinophil activation. Figure 5 shows the results of EPO assay. Non specific EPO activity in saline-treated group was  $0.37 \pm 0.00$  U/g lung wet weight. EPO activity was significantly increased after Sephadex injection, and the value was  $3.46 \pm 0.25$  U/g lung wet weight. Dexamethasone and cyclosporin A (10 mg/kg) significantly ( $P < 0.01$ ) inhibited the increase in EPO activity, and the inhibition was 99.7% and 43.4%, respectively.

*Effects of dexamethasone and cyclosporin A on Sephadex-induced AHR*

The effects of dexamethasone and cyclosporin A on Sephadex-induced AHR were investigated and the results

are shown in Fig. 6. Bronchoconstriction occurred following intravenous administration of 5-HT at doses of 24  $\mu$ g/kg and higher in the saline-control group, whereas after Sephadex injection, airway responsiveness significantly ( $P < 0.01$  vs saline-control group) increased at all doses of 5-HT. The increase in airway responsiveness was inhibited by dexamethasone and recovered to the control level at a dose of 1 mg/kg ( $\times 3$ ). However, cyclosporin A did not inhibit the increase in airway responsiveness to 5-HT at all.

LogPD<sub>20</sub> values as an index of AHR are shown in Table 1. LogPD<sub>20</sub> was significantly ( $P < 0.01$  vs saline-control group) reduced in the Sephadex-treated group. The reduction of logPD<sub>20</sub> value recovered following administration of dexamethasone, and the inhibitory effect was significant at 1 mg/kg ( $\times 3$ ) (Table 1). On the other hand, cyclosporin A did not cause recovery of the reduction of logPD<sub>20</sub> even at 10 mg/kg ( $\times 3$ ).

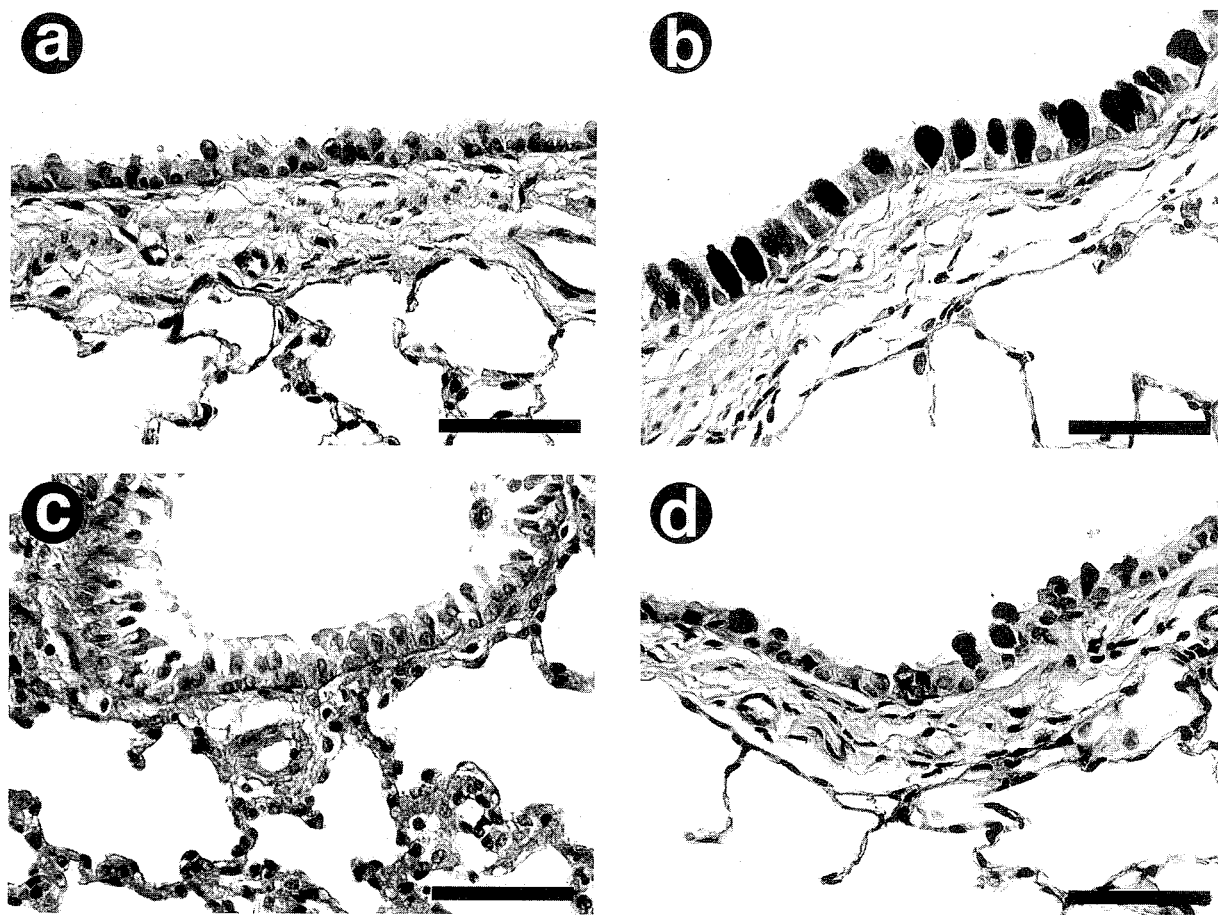


**Fig. 4.** Effects of dexamethasone and cyclosporin A on Sephadex-induced goblet cell hyperplasia in the airways. a: Saline-control group, b: Sephadex-control group, c: Sephadex-dexamethasone (10 mg/kg, p.o.  $\times 3$ ) group, d: Sephadex-cyclosporin A (10 mg/kg, p.o.  $\times 3$ ) group. Dexamethasone and cyclosporin A inhibited the increase in PAS-positive goblet cells induced by Sephadex injection. PAS stain. Bars = 100  $\mu$ m.

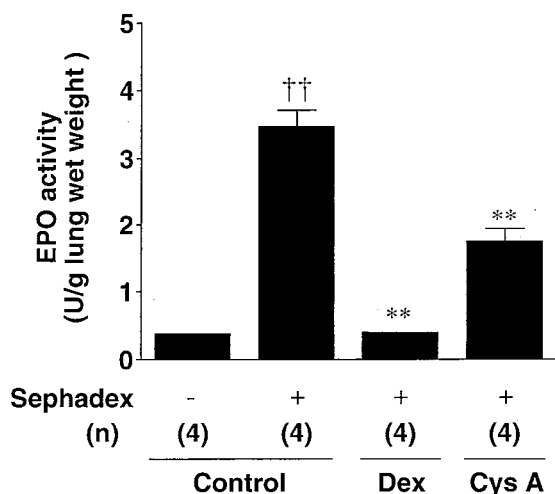


**Fig. 5.** Effects of dexamethasone (Dex) and cyclosporin A (Cys A) on increase in eosinophil peroxidase (EPO) activity in the lung after Sephadex injection. Sephadex (0.5 mg/ml/rat) was intravenously administered to rats on days 0, 2 and 5. Dexamethasone (10 mg/kg, p.o.) and cyclosporin A (10 mg/kg, s.c.) were administered 4 hr and 2 hr before each Sephadex injection, respectively. Assay of EPO was performed on day 7. Each column represents a mean value and vertical lines indicate S.E. (n=4). ††P<0.01 and \*\*P<0.01, statistically significant compared with the saline-treated group and Sephadex-control group, respectively, by one-way ANOVA (Tukey-Kramer's method).

**Table 1.** Effects of dexamethasone and cyclosporin A on Sephadex-induced airway hyperresponsiveness in Lewis rats

| Drugs, mg/kg  | LogPD <sub>20</sub> |      |                  |      |
|---------------|---------------------|------|------------------|------|
|               | Saline-treated      | (n)  | Sephadex-treated | (n)  |
| Control       | 2.32±0.06           | (10) | 1.86±0.05††      | (11) |
| Dexamethasone | 0.1                 | -    | 2.17±0.06        | (5)  |
|               | 1                   | -    | 2.79±0.19**      | (5)  |
|               | 10                  | -    | 2.65±0.34**      | (4)  |
| Cyclosporin A | 0.1                 | -    | 1.81±0.07        | (5)  |
|               | 1                   | -    | 1.92±0.05        | (4)  |
|               | 10                  | -    | 1.82±0.08        | (4)  |

The provocative dose ( $\mu\text{g/kg}$ , i.v.) of 5-HT that caused a 20% increase in bronchoconstriction is expressed as PD<sub>20</sub>. As an index of AHR, logPD<sub>20</sub> values were determined. Each value represents the mean±S.E. of 4–11 experiments. ††P<0.01 and \*\*P<0.01, statistically significant compared with the saline-control and Sephadex-control group, respectively, by one-way ANOVA (Tukey-Kramer's method).

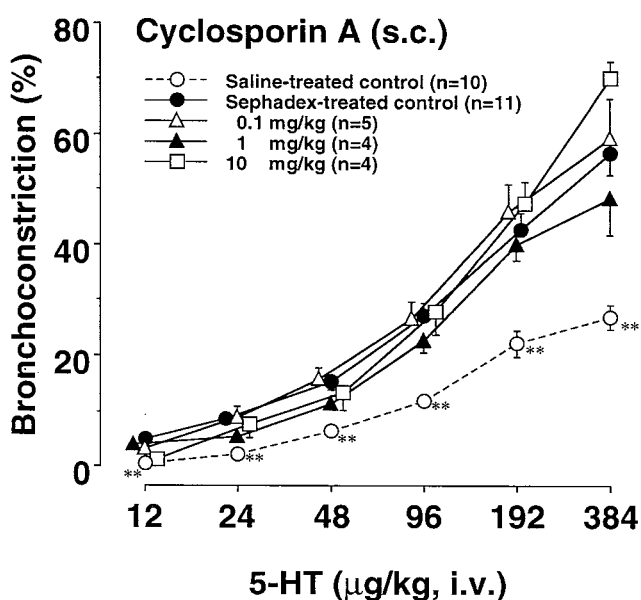
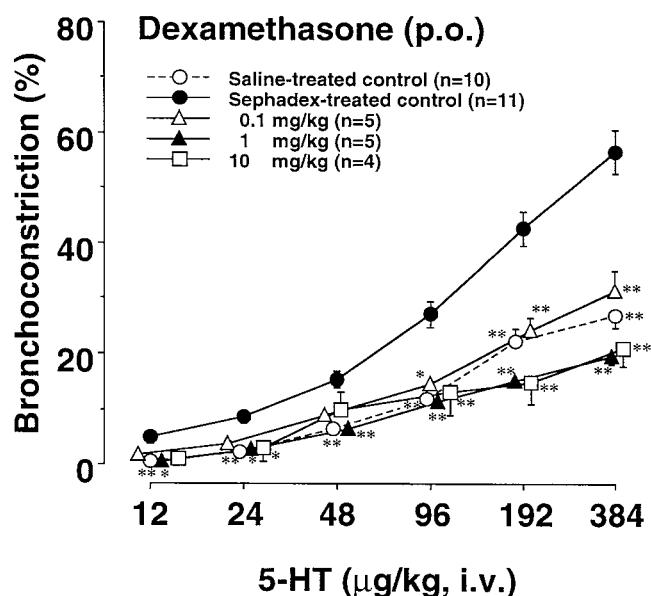


**Fig. 6.** Effects of dexamethasone and cyclosporin A on increase in airway responsiveness to 5-HT in Sephadex-treated rats. Saline (1 ml/rat) or Sephadex (0.5 mg/ml/rat) was intravenously administered to rats on days 0, 2 and 5. Measurement of airway responsiveness to 5-HT (12–384  $\mu\text{g/kg}$ , i.v., every 5 min) was performed on day 7. Each symbol represents a mean value and vertical lines indicate S.E. (n=4–11). \*P<0.05 and \*\*P<0.01, statistically significant compared with the Sephadex-control group by one-way ANOVA (Tukey-Kramer's method).

## DISCUSSION

A close relationship has been reported between the development of AHR and eosinophilia induced by administration of Sephadex (7, 21). Accordingly, if eosinophils infiltrating the airways and lungs are involved in the development of AHR, inhibition of eosinophilia might prevent AHR. In the present study using Lewis rats, which exhibit reproducible AHR, we investigated the effects of dexamethasone and cyclosporin A on Sephadex-induced airway inflammation and AHR. As the results, dexamethasone inhibited both AHR and pulmonary eosinophilia induced by Sephadex. In contrast, cyclosporin A did not inhibit AHR, although it inhibited pulmonary eosinophilia. Furthermore, both the drugs inhibited the increase in EPO activity as a parameter of eosinophil activation. Thus, inhibitory effects of the drugs on eosinophil activation did not necessarily result in the inhibition of AHR. Francisci et al. (22) reported the effects of dexamethasone, cyclosporin A and rapamycin on inflammatory cell infiltration and the development of AHR induced by Sephadex (G-50) in guinea pigs. Their results showed that dexamethasone and cyclosporin A significantly inhibited the increase in number of lymphocytes and eosinophils, but both drugs failed to suppress the AHR, and that rapamycin, an immunosuppressant, significantly inhibited both phenomena. There is a discrepancy in the effect of dexamethasone between their study using guinea pigs and our study using rats, although their results and ours both arrived at the conclusion that inhibition of pulmonary eosinophilia does not produce inhibition of AHR. It is unknown how the different effects of dexamethasone occurred. There is, however, a possibility of a species difference in the effect of dexamethasone. Generally speaking, the rat is sensitive to anti-allergic drugs such as disodium cromoglycate in inflammation models, but the guinea pig is insensitive to such drugs and steroids (23). Thus, the report by Francisci et al. (22) and our present results may reflect the species difference in the effect of dexamethasone.

The Lewis rat strain exhibited significant AHR induced by Sephadex, but Brown-Norway, Fischer 344 and Wistar-Kyoto strains did not, although the number of eosinophils in BALF was increased in all the strains (8). Additionally, Kubin et al. (24) reported pulmonary eosinophilia without AHR induced by intratracheal application of Sephadex beads in Brown-Norway rats. These findings also support that there is hardly any causal relationship between pulmonary eosinophilia and the development of AHR. Together with the present results, we can conclude that the mechanism of pulmonary eosinophilia is not involved in the development of AHR in this model. The time course of Sephadex-induced AHR

and airway inflammation showed that pulmonary eosinophilia was accompanied by the development of AHR (25). Therefore, these findings are consistent with the proposition that pulmonary eosinophilia and the development of AHR induced by Sephadex are parallel, rather than sequential processes in the pathogenesis of this model.

The present results also indicate the possibility that T-lymphocyte activity is associated with the pulmonary eosinophilia but not with the development of AHR after Sephadex injection, because cyclosporin A, a specific T-lymphocyte inhibitor, was not effective against Sephadex-induced AHR. Similar to cyclosporin A, corticosteroids such as dexamethasone have an inhibitory effect on lymphocyte activation and production of various cytokines (14, 26). Furthermore, corticosteroids inhibit the activation of macrophages, monocytes and eosinophils (27). Inhibition of activation of mononuclear cells such as macrophages and monocytes may be involved in the mechanism of the inhibition of Sephadex-induced AHR by dexamethasone.

Both dexamethasone and cyclosporin A inhibited the findings such as appearance of granulomas with horse-shoe-shaped multinuclear giant cells and goblet cell hyperplasia in Sephadex-treated rats, similar to their inhibition of pulmonary eosinophilia in the present study. Therefore, these findings, as well as pulmonary eosinophilia, may not be directly associated with the development of AHR induced by Sephadex. In the time course study of Sephadex-induced AHR and airway inflammation, the findings of pulmonary inflammatory changes were accompanied by the development of AHR as well as pulmonary eosinophilia in Lewis rats (25). Therefore, inflammatory changes such as formation of granulomas with giant cells and goblet cell hyperplasia appear to occur in parallel with the development of AHR, rather than as sequential processes in the development of AHR in the pathogenesis of this model. Further studies are needed to investigate the trigger of the development of AHR induced by Sephadex.

Neutrophils were slightly increased by Sephadex injection, but the increase was not significant. Interestingly, there was significant potentiation of the increase in neutrophils in BALF by administration of dexamethasone (10 mg/kg  $\times$  3) in Sephadex-treated rats. Cyclosporin A also tended to potentiate the increase in neutrophils in BALF, but this was not significant. These findings indicate that the existence of neutrophils in the airway is not associated with the development of AHR in this model. In our preliminary examination, dexamethasone significantly inhibited airway neutrophilia on day 2 when the increase in neutrophils in the airway reached a peak without dexamethasone, and the airway neutrophilia

reached a peak on day 7 or later during dexamethasone treatment (S. Matsubara et al., unpublished data). Namely, dexamethasone delayed the peak of neutrophil infiltration in the airway from day 2 to day 7 or later. This explains the apparent increase in neutrophils on day 7 that was caused by administration of dexamethasone in Sephadex-treated rats. It has been reported that dexamethasone caused a profound, acute, short-lived down-regulation of L-selectin and CD18 on cow neutrophils (28). Consequently, down-regulation of adhesion molecules on neutrophils by dexamethasone may produce delay of migration of neutrophils in Sephadex-treated rats.

In conclusion, the present study shows that dexamethasone significantly inhibited Sephadex-induced AHR and airway inflammation such as pulmonary eosinophilia, granulomatous arteritis with horseshoe-shaped multinuclear giant cells and goblet cell hyperplasia. In contrast, cyclosporin A, a specific T-lymphocyte inhibitor, which significantly inhibited the airway inflammation as described above, failed to reduce AHR in Sephadex-treated rats. The findings of airway inflammation observed in this study are thought not to be directly associated with AHR induced by Sephadex. The mechanisms of Sephadex-induced AHR and pulmonary eosinophilia are parallel, rather than sequential processes in the pathogenesis of this model.

#### Acknowledgments

The authors wish to thank Dr. K. Kawashima and Dr. T. Iwasaki of Tanabe Seiyaku Co., Ltd. for their support throughout the study. Thanks are also due to Dr. Y. Kawai, Dr. K. Takashima, Dr. K. Kitamura, Dr. M. Kikuchi, Ms. K. Hashimoto and Ms. A. Sakai for their valuable discussion and technical assistance.

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