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Dexamethasone reduces lung eosinophilia, and VCAM-1 and ICAM-1 expression induced by Sephadex beads in rats

Akemi Ito[a], Mio Miyake[a], Masashi Morishita[b], Komei Ito[b], Shinpei Torii[c], Tatsuo Sakamoto[a,*]

[a] *Department of Pediatrics, Nagoya University Postgraduate School of Medicine, 65 Tsurumai-cho, Showa, Nagoya 466-8550, Japan*
[b] *Aichi Children's Health and Medical Center, Obu 474-0031, Japan*
[c] *The Faculty of Domestic Science of Aichi-Gakusen University, Okazaki 444-8520, Japan*

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Abstract

Airway eosinophilia is one of the key pathophysiologic features in asthma. The endothelial adhesion molecules, vascular cell adhesion molecule (VCAM-1) and intercellular adhesion molecule (ICAM-1), have previously been shown to play a crucial role in eosinophil recruitment into the inflamed airway. We have investigated the effects of dexamethasone on eosinophilia into the bronchoalveolar lavage fluid, and the upregulation of VCAM-1 and ICAM-1 expression, measured by immunoblotting, induced by i.v. injection of Sephadex beads into rats. The beads significantly increased the lung eosinophilia, and expression of VCAM-1 and ICAM-1 in the lung. Pretreatment with dexamethasone (0.1 to 2 mg/kg i.p.) strongly inhibited all the airway inflammatory events in a dose-dependent manner. In conclusion, glucocorticoids may be potent inhibitors of lung eosinophilia, at least in part, due to the prevention of the upregulation of VCAM-1 and ICAM-1 expression.

Keywords: Dexamethasone; Lung eosinophilia; ICAM-1 (intercellular adhesion molecule); Sephadex bead; TNF-α (tumor necrosis factor-α); VCAM-1 (vascular cell adhesion molecule)

1. Introduction

It is now recognized that chronic inflammation is an important aspect of asthma. A variety of inflammatory mediators, released through the activation of many inflammatory and structural cells, result in the typical pathophysiologic changes of asthma (Barnes, 1996; Barnes et al., 1998). Leukotrienes and highly basic and cytotoxic proteins such as major basic protein released from eosinophils, especially, may play a key role in inducing the pathohistologic changes of asthma and airway hyperresponsiveness (Wardlaw and Kay, 1987; Gleich et al., 1988; Bousquet et al., 1990).

Animal models of allergic lung eosinophilia have been widely used. Lung eosinophilia can also be induced by nonallergic stimuli such as intravenously or intratracheally administered particles of cross-linked dextran, Sephadex beads, in rats and guinea pigs (Walls and Beeson, 1972; Kubin et al., 1992; Maghni et al., 1993; Buyssens et al., 1995; Kleemann et al., 1996). Intravenously injected Sephadex beads become lodged in the small vessels of the lung. The subsequent vascular trauma leads to an inflammatory response characterized by granuloma formation, and eosinophilia in the lung tissues is accompanied by the development of lung granuloma. In addition, the pathohistologic changes induced by Sephadex beads have been shown to be associated with airway hyperresponsiveness (Laycock et al., 1987; De Francischi et al., 1993; Tramontana et al., 2002). Obviously, this model only partly represents the patterns of inflammatory characteristics of asthma. Nevertheless, the model is likely to be useful for defining the pathways involved in eosinophil recruitment.

The endothelial adhesion molecules, vascular cell adhesion molecule (VCAM-1) and intercellular adhesion molecule (ICAM-1), play an important role in eosinophil migration into the inflamed airway (Wegner et al., 1990; Schleimer et al., 1992; Nakajima et al., 1994). Expression of VCAM-1 and ICAM-1 is regulated transcriptionally by cytokines and mediators of inflammation, thus suggesting that glucocorticoids, which can inhibit the action of tran-

* Corresponding author. Tel.: +81-52-744-2294; fax: +81-52-744-2974.
E-mail address: tatsuos@med.nagoya-u.ac.jp (T. Sakamoto).

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scriptional factors such as activator protein-1 (AP-1) and nuclear factor-kappaB (NF-κB), may suppress the expression of these adhesion molecules (Carios and Harlan, 1994; Barnes and Karin, 1997). Das et al. (1995) have previously shown that both the very late activation antigen-4 (VLA-4)/VCAM-1 and CD18/ICAM-1 adhesion pathways contribute to the development of lung eosinophilia induced by Sephadex beads, and this was demonstrated using antibody blocking with VLA-4 and CD18 monoclonal antibodies. However, there is no evidence that the expression of VCAM-1 and ICAM-1 is upregulated in this model. In the present study, we have examined whether the expression of the two endothelial adhesion molecules increases in the rat lung following i.v. injection of Sephadex beads. In addition, we have investigated the effects of dexamethasone on the lung eosinophilia, and VCAM-1 and ICAM-1 expression in Sephadex-treated rats.

2. Materials and methods

2.1. Animals

The experimental procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the Institute for Laboratory Animal Research, Nagoya University Postgraduate School of Medicine (1989, Nagoya, Japan). Specific pathogen-free male Wistar rats at 8 weeks of age, weighing 250–280 g, were purchased from Japan SLC (Hamamatsu, Japan), and kept in a temperature-controlled environment with standard laboratory food and water freely available.

2.2. Materials

Sephadex beads (G-50 superfine; Pharmacia & Upjohn Diagnostics, Uppsala, Sweden) were prepared as previously described (Lemanske and Kaliner, 1982). Briefly, the beads were autoclaved for 30 min, and suspended in sterile 0.9% saline for at least 48 h at 4 °C. Dexamethasone 21–acetate (Sigma, St. Louis, MO, USA) was dissolved in pathogen-free 0.9% saline (Otsuka Chemical, Tokyo, Japan) containing 0.1% bovine serum albumin (BSA; Sigma) to a concentration of 0.05, 0.2, 0.5, or 1 mg/ml just before use. Pentobarbital sodium was purchased from Abbott Laboratories (North Chicago, IL, USA); phosphate-buffered saline (PBS) from Nissui Pharmaceutical (Tokyo, Japan); Hanks' balanced salt solution and formalin from Sigma; Turk reagent, Giemsa's stain solution, and May-Gruenwald's stain solution from Katayama Chemical Industries (Osaka, Japan); Diff-Quik staining kit from Kokusai Shiyaku (Kobe, Japan); and reagents for hematoxylin–eosin and periodic acid-Schiff (PAS) stains from Muto Pure Chemicals (Tokyo, Japan).

The following reagents were used for immunoblot analysis: molecular weight markers for proteins ranging from 10 to 250 kDa: Precision protein standards (Bio-Rad Laboratories, Hercules, CA, USA); blocking reagent, Blockace (Dainippon Pharmaceutical, Osaka, Japan); goat polyclonal immunoglobulin G antibodies (IgG) to rat VCAM-1, ICAM-1, and actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA); nonspecific purified goat IgG (Zymed Laboratories, South San Francisco, CA, USA); Vectastain avidin–biotinylated peroxidase complex (ABC) kit containing biotinylated rabbit polyclonal IgG to goat IgG and ABC, and Vector 3,3′-diaminobenzidine (DAB) substrate reagent kit (Vector Laboratories, Burlingame, CA, USA). The sample buffer for electrophoresis was composed of 1 ml of 0.5 M Tris aminomethane (Tris)–HCl (pH 6.8), 2 ml of 10% sodium dodecyl sulfate (SDS), 0.6 ml of β-mercaptoethanol, 1 ml of glycerol, 5.4 ml of distilled water, and a few drops of 1% bromophenol blue per 10 ml. The SDS-polyacrylamide gel electrophoresis (SDS-PAGE) buffer contained 25 mM Tris (pH 8.3), 192 mM glycine, and 0.1% SDS, and the electroblotting buffer comprised of 25 mM Tris (pH 8.3), 192 mmol glycine, and 20% methanol.

2.3. Bronchoalveolar lavage and leukocyte counts

Rats were administered an overdose of pentobarbital (120 mg/kg i.p.), and the lungs were lavaged 10 times with 2 ml aliquots of 0.9% saline via a tracheal cannula, 8 mm long with a 1.3-mm inner diameter, introduced through a tracheotomy. The lavage fluid was centrifuged at $800 \times g$ for 10 min at 4 °C, and the cell pellets were resuspended in 1 ml of Hanks' balanced salt solution. The cell suspension (100 μl) was added to Turk's reagent (100 μl), and the cells were counted under the light microscope in a Burker-Turk chamber (Erma Optical Works, Tokyo, Japan). Differential cell counts were made from cytospin preparations (Cytospin 3; Shandon Scientific, Cheshire, UK) stained using a Diff-Quik staining kit. Cells were identified as macrophages, neutrophils, eosinophils, lymphocytes and shed epithelial cells by standard morphologic techniques. Eight hundred cells were counted under 400× magnification and the percentage and absolute number of each cell type were calculated.

2.4. Histologic analysis

After bronchoalveolar lavage, the removed lungs were inflated with 10% phosphate-buffered formalin to a pressure of 25 cm H_2O. Paraffin-embedded sections (4 μm thick) of the tracheobronchi and lung were stained with hematoxylin–eosin, PAS or May-Giemsa's stain. The lung histology was assessed by light microscopy.

2.5. Measurement of tumor necrosis factor (TNF-α)

The concentration of TNF-α in bronchoalveolar lavage fluid was measured using an enzyme-linked immunosorbent assay with a commercially available kit (Bio Source International, Camarillo, CA, USA). The detection limit of this kit for TNF-α was 4 pg/ml.

2.6. Preparation of lung extracts

Rats were sacrificed by administration of an overdose of pentobarbital (120 mg/kg i.p.). The thoracic cavity was opened and a cannula was inserted into the aorta through the left ventricle. Perfusion was performed with 30 ml of 0.9% saline at a pressure of 120 mm Hg. Blood and perfused liquid were expelled through an incision in the right atrium. The pulmonary circulation was also perfused with 20 ml of 0.9% saline at the same pressure. The lung was excised after the vessels and connective tissues were removed, and then divided into segments. All the segments obtained from an animal and PBS (5 ml/g wet weight, 4 °C) were homogenized together for about 90 s using a homogenizer (Nissei Biomixer; Nihonseiki Kaisha, Tokyo, Japan). The homogenate was mixed with an equal volume of the electrophoresis sample buffer, followed 15 min later by boiling at 100 °C for 5 min. Thirty minutes later, the lung mixture was centrifuged for 15 min (15,000 × *g*, 4 °C). The supernatant was stored at − 80 °C until use.

2.7. Immunoblot analysis

An equal volume of the lung extract from each different treatment animal and protein molecular weight markers were applied to a polyacrylamide gradient gel (10–20% Ready Gels; Bio-Rad Laboratories) placed in a Mini-Protean II Cell (Bio-Rad Laboratories). After SDS-PAGE (20 mA), the proteins in the gel were transferred to a polyvinylidene difluoride membrane (Immobilon Transfer Membranes; Millipore, Bedford, MA, USA) using a Trans-Blot SD Semi-Dry Transfer Cell (Bio-Rad Laboratories) at 20 mA for 1 h. The membrane was kept in blocking reagent for 1 h at room temperature with gentle shaking. After washing with PBS, the membrane was divided into two pieces: the upper part was incubated with the anti-rat VCAM-1 IgG (1: 300) or the anti-rat ICAM-1 IgG (1: 100) for 1 h at room temperature, and the lower part was incubated with the anti-rat actin IgG (1: 1000). The goat-specific IgGs to VCAM-1, ICAM-1, and actin were diluted in PBS. Another membrane, onto which the lung protein extract was blotted, was incubated with the nonspecific purified goat IgG at a concentration corresponding to that of the anti-rat ICAM-1 IgG. Then each piece was washed with PBS, and incubated with the biotinylated anti-goat IgG diluted 1:1000 in PBS for 1 h at room temperature. After washing with PBS, the avidin–biotinylated peroxidase complex was added according to the instructions of the manufacturer of the Vectastain ABC Kit. Development was performed using a Vector DAB substrate reagent kit.

The density of VCAM-1, ICAM-1 and actin bands on the membrane was measured using an image analyzer, Bioinstrument (ATTO, Tokyo, Japan), and a densitometer, ATTO Lane Analyzer ver. 3 (ATTO). We calculated the relative intensity unit (%), which was defined as the ratio of the density of VCAM-1 or ICAM-1 to the density of the "housekeeping" protein actin.

2.8. Protocols

2.8.1. Effects of dexamethasone on lung inflammation induced by Sephadex beads

Animals were divided into five groups ($n = 5–6$) to study the effect of dexamethasone on lung eosinophilia induced by i.v. injection of Sephadex beads. Dexamethasone at a dose of 0.1, 0.4 mg/kg, 1 or 2 mg/kg, or its vehicle was administered intraperitoneally. One hour later, animals were anesthetized with pentobarbital (40 mg/kg i.p.), and 1 ml of 0.9% saline with Sephadex beads (3.5×10^4 particles/kg) was administered via the tail vein. A sham group of animals was pretreated with the vehicle for dexamethasone, and challenged with 0.9% saline (1 ml/animal i.v.). Sixteen hours later, the bronchoalveolar lavage fluid was collected for measurements of the leukocyte count and TNF-α concentration as described above. After bronchoalveolar lavage, the lungs were removed for histologic analysis as described above. In our preliminary study, the effect of different doses of the beads from 0.5×10^4 to 50×10^4 particles/kg i.v. on the eosinophil count in the bronchoalveolar lavage fluid was investigated. We subsequently used a dose of 3.5×10^4 particles/kg i.v., inducing a submaximal response.

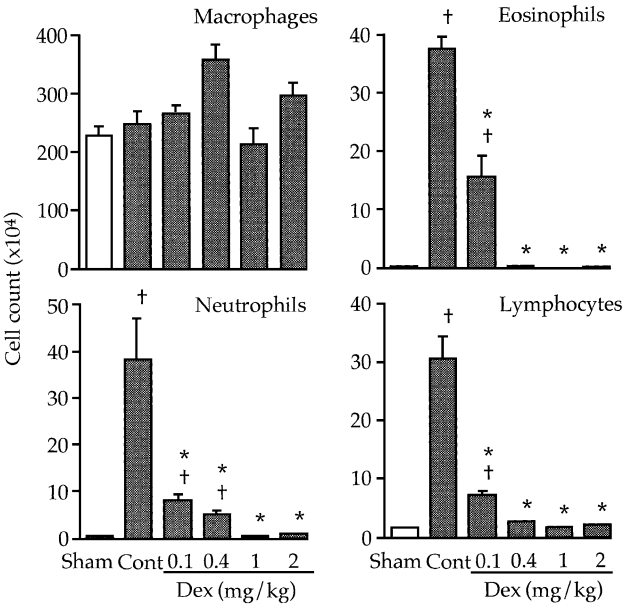

Fig. 1. Effects of different doses of dexamethasone (Dex: 0.1, 0.4, 1, 2 mg/kg) on leukocyte counts in the bronchoalveolar lavage fluid (BALF) 16 h after administration of Sephadex beads to Wistar rats. The beads (3.5×10^4 particles/kg) were suspended in 1 ml of 0.9% saline, and administered via the tail vein. A control group was pretreated with the vehicle for Dex, and given the dose of the beads intravenously. Sham-treated animals were pretreated with the vehicle for Dex, and given 0.9% saline intravenously. Dexamethasone or its vehicle was administered 1 h before the Sephadex bead injection. Results are expressed as means ± S.E.M. ($n = 5–6$). Statistical significance: †$P < 0.01$ compared with the sham group, assessed by the unpaired Student's *t*-test or Welch's test; *$P < 0.05$ compared with the control group, assessed with ANOVA and the Bonferroni/Dunn test.

2.8.2. Effects of dexamethasone on the VCAM-1 and ICAM-1 expression in the lung

Animals were divided into three groups ($n = 6$) to study the effect of dexamethasone on the VCAM-1 and ICAM-1 expression in the lung after Sephadex bead administration. A single dose of dexamethasone (1 mg/kg i.p.) or its vehicle was given to the animals, followed 1 h later by i.v. injection of the Sephadex beads (3.5×10^4 particles/kg) via the tail vein. A sham group was pretreated with the vehicle for dexamethasone, and challenged with 0.9% saline (1 ml/animal i.v.). Sixteen hours later, the lungs were excised for immunoblot analysis as described above.

2.9. Statistical analysis

All values were expressed as the mean ± S.E.M. To evaluate the significance of the difference between two independent groups with equal variance, which was assessed with the F test, we used the unpaired Student's t-test (two-tailed). Otherwise, the Welch's test (two-tailed) was employed. One-way analysis of variance (ANOVA) and the Bonferroni/Dunn test were used for multiple comparisons with the controls. A level of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Cell counts in the bronchoalveolar lavage fluid

There was no significant difference in the average body weight among the different treatment groups used in this study (data not shown). The recovery rate of the bronchoalveolar lavage fluid was about 95% and bronchoalveolar lavage fluid was hardly contaminated by red blood cells, as assessed by microscopy, in all cases. The total cell count of bronchoalveolar lavage fluid increased significantly after i.v. injection of Sephadex beads from $2.32 \pm 0.18 \times 10^6$ to

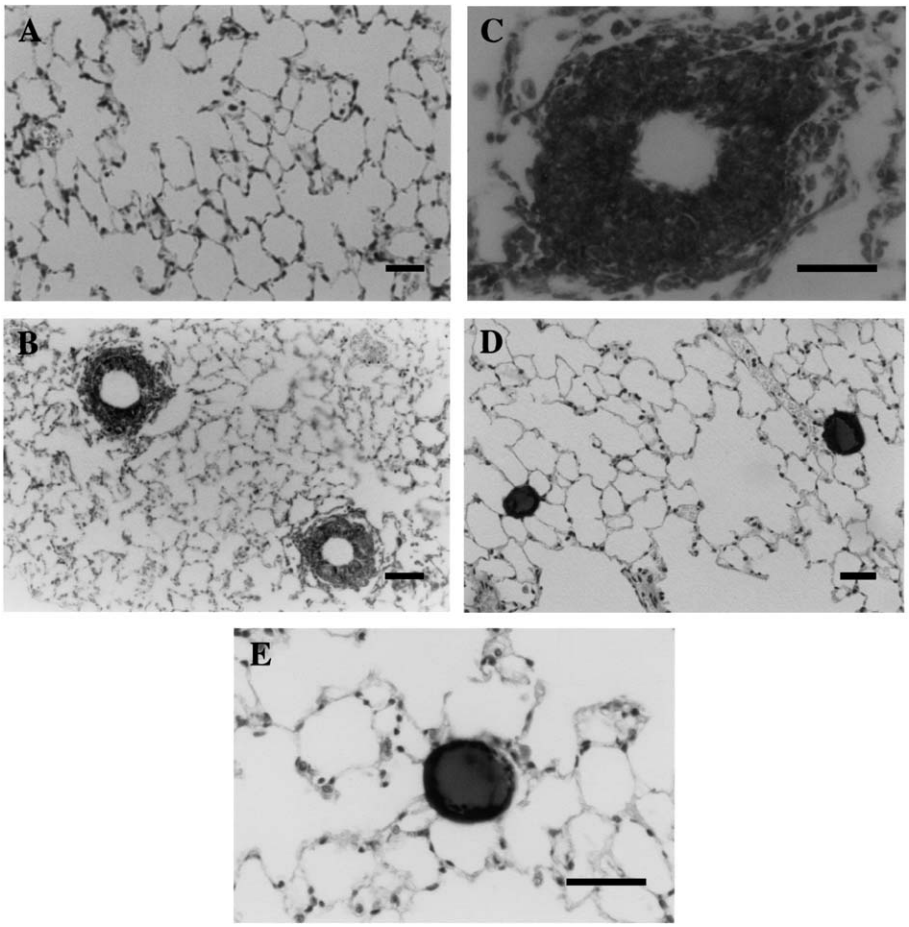

Fig. 2. Histopathologic features of pulmonary inflammation induced by i.v. injection of Sephadex beads (3.5×10^4 particles/kg) in two groups of Wistar rats, which were pretreated with dexamethasone (Dex, 1 mg/kg i.p.) or its vehicle. Sham-treated animals were pretreated with the vehicle for Dex, and given 0.9% saline intravenously. The lungs were removed 16 h after treatment with the beads or 0.9% saline, and paraffin-embedded sections (4 μm thick) were stained with May-Giemsa's or PAS stain. (A) Lung section from the sham group; (B), (C) lung sections from the control group; (D), (E) lung sections from the dexamethasone-treated, Sephadex bead-stimulated group. (A, B, C): stained with May-Giemsa's stain; (D, E): stained with PAS stain. Bars indicate 100 μm.

$3.58 \pm 0.28 \times 10^6$ cells. The cell counts of eosinophils, neutrophils, and lymphocytes, but not macrophages, increased significantly. Shed epithelial cells were hardly detected in the bronchoalveolar lavage fluid from either group. Dexamethasone significantly inhibited the recruitment of inflammatory cells in a dose-dependent manner (Fig. 1). Doses more than 1 mg/kg of dexamethasone completely inhibited the responses, since there was no significant difference in the cell counts between the two groups pretreated with 1 or 2 mg/kg dexamethasone or its vehicle.

3.2. Histologic analysis of the lung preparation

Inflammatory responses to Sephadex beads in the lung were observed in hematoxylin–eosin, May-Giemsa's, and PAS stained preparations. Mononuclear cells, especially macrophages, were packed tightly around the beads, and eosinophils and neutrophils were conspicuous in the periphery of the lesions (Fig. 2B, C). The number of cells in the interstitium was increased by macrophages, eosinophils, neutrophils and lymphocytes. However, little inflammatory change in response to Sephadex beads was observed in the airway lumen and wall which were not adjacent to the granulomatous lesions (data not shown). Dexamethasone (1 mg/kg i.v.) almost completely inhibited the granulomatous changes, and the infiltration of eosinophils and neutrophils into the periphery of the lesion (Fig. 2D, E).

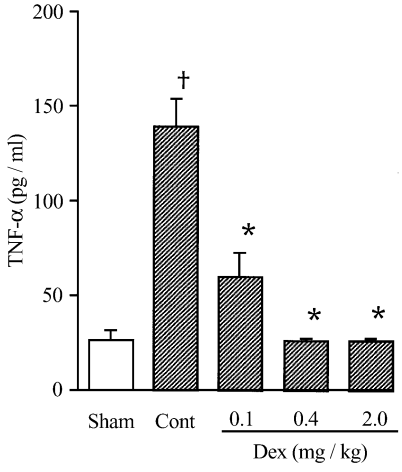

Fig. 3. Effects of different doses of i.p. dexamethasone (Dex: 0.1, 0.4, 2 mg/kg) on the concentration of TNF-α in the bronchoalveolar lavage fluid (BALF) 16 h after administration of Sephadex beads to Wistar rats. The beads (3.5×10^4 particles/kg) were suspended in 1 ml of 0.9% saline, and administered via the tail vein. The control group was pretreated with the vehicle for Dex, and given the dose of the beads intravenously. Sham-treated animals were pretreated with the vehicle for Dex, and given 0.9% saline intravenously. Dexamethasone or its vehicle was administered 1 h before the Sephadex bead injection. Results are expressed as means ± S.E.M. ($n = 5–6$). Statistical significance: †$P < 0.01$ compared with the sham group, assessed by the unpaired Student's t-test or Welch's test; *$P < 0.05$ compared with the control group, assessed with ANOVA and the Bonferroni/Dunn test.

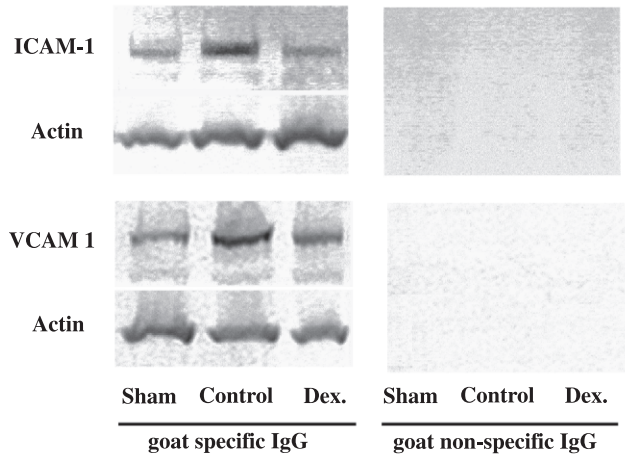

Fig. 4. Immunoblot analysis for VCAM-1 and ICAM-1 expression in the whole rat lung homogenate, which was obtained 16 h after i.v. injection of Sephadex beads (3.5×10^4 particles/kg) or 0.9% saline as a sham stimulation. Animals were pretreated with dexamethasone (Dex., 1 mg/kg i.p.) or its vehicle 1 h before the challenge. The presence of VCAM-1 and ICAM-1 was indicated by bands at approximately 100 and 90 kDa, respectively. Equal protein loading was confirmed by probing the same blot with actin (45 kDa). The photographs are the results from a representative experiment.

3.3. TNF-α in the bronchoalveolar lavage fluid

The concentration of TNF-α in the bronchoalveolar lavage fluid increased significantly from 30.6 to 138.8 pg/ml after Sephadex bead injection. Dexamethasone strongly reduced

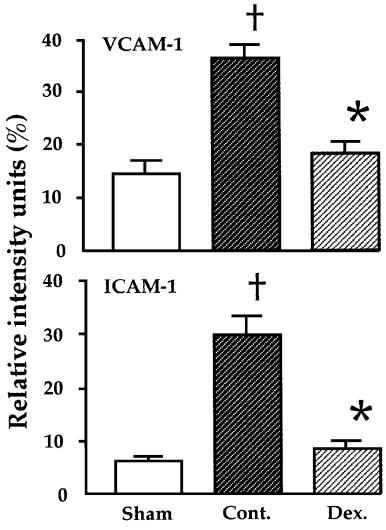

Fig. 5. Effects of dexamethasone (Dex.: 1 mg/kg i.p.) on VCAM-1 and ICAM-1 expression in the lung homogenate obtained 16 h after i.v. injection of Sephadex beads (3.5×10^4 particles/kg) or 0.9% saline as a sham stimulation. Animals were pretreated with dexamethasone (1 mg/kg i.p.) or its vehicle 1 h before challenge. Immunoblot analysis was performed for the detection of VCAM-1 and ICAM-1, and their relative intensity units were compared among the three different treatment groups. The results are expressed as means ± S.E.M. ($n = 6$). Statistical significance: †$P < 0.05$ compared with the sham group; *$P < 0.05$ compared with the control group (Cont.), assessed by the unpaired Student's t-test or Welch's test.

the TNF-α production in a dose-dependent fashion, and doses of more than 0.4 mg/kg completely prevented it (Fig. 3).

3.4. Immunoblot analysis of VCAM-1 and ICAM-1 in the lung

The presence of rat VCAM-1, ICAM-1 and actin was indicated by bands at approximately 100, 90, and 45 kDa, respectively. There were no significant differences in the densities of the actin bands among the different treatment groups used in this study (control group: 4883 ± 588, dexamethasone-treated group: 4437 ± 524, sham group: 6032 ± 775), indicating that the protein contents of the lung extracts applied for immunoblot analysis were almost equal. Sephadex beads significantly increased the relative intensity units of both VCAM-1 and ICAM-1 in the lung extract (Figs. 4 and 5). As shown in Fig. 5, dexamethasone completely inhibited the responses.

4. Discussion

In the present study, Sephadex beads caused marked granulomatous lesions around the beads clogging the small vessels in the lung, and macrophages constituted the principal part of the lesions, although eosinophils, neutrophils and lymphocytes also accumulated appreciably in the lesions and their surroundings. Such pathohistologic changes most likely paralleled the numbers of eosinophils, neutrophils and lymphocytes, but not macrophages, in the bronchoalveolar lavage fluid. These results are consistent with the previous reports (Walls and Beeson, 1972; Buyssens et al., 1995). In addition, we have provided new evidence for the upregulation of VCAM-1 and ICAM-1 expression in the lung in Sephadex-treated rats, and the result that the TNF-α level in the bronchoalveolar lavage fluid was increased by i.v. Sephadex beads confirms the finding of Williams et al. (1997) that the beads elevate the mRNA levels of TNF-α in the lung tissues.

Das et al. (1995) have previously reported that monoclonal antibodies to VLA-4 and CD18 caused significant suppression of eosinophilia in the bronchoalveolar lavage fluid in Sephadex-treated guinea pigs, and that co-administration of the two kinds of monoclonal antibodies resulted in total inhibition of the response. Thus, the VLA-4/VCAM-1 and CD18/ICAM-1 adhesion pathways may complementarily mediate the lung eosinophilia in this model. The initial steps of eosinophil migration from blood vessels have been extensively investigated (Carios and Harlan, 1994; Marik and Lo, 1996), and the regulation of endothelial and leukocyte adhesion molecules involves both quantitative changes in surface expression and qualitative changes in avidity. For the endothelial adhesion molecules such as VCAM-1 and ICAM-1, quantitative alterations in surface expression may predominate. Indeed, we have revealed that Sephadex beads upregulate the expression of VCAM-1 and ICAM-1 on endothelial cells in the initial stages of eosinophil migration into lung tissues.

Das et al. (1995) have also shown that blockade of CD18 inhibits neutrophilia in the bronchoalveolar lavage fluid in Sephadex-treated guinea pigs whereas blockade of VLA-4 had no effect, indicating the contribution of the CD18/ICAM-1 adhesion pathway to lung neutrophilia. The β2 integrin subfamily such as CD11a/CD18 and CD11b/CD18, which bind to ICAM-1 on endothelial cells, are expressed extensively not only on eosinophils but also on macrophages, neutrophils, and lymphocytes (Carios and Harlan, 1994; Marik and Lo, 1996). Thus, ICAM-1-mediated mechanisms presumably underlie the granuloma formation induced by the Sephadex beads, as well as leukocyte accumulation in the bronchoalveolar lavage fluid. In the present study, dexamethasone abolished the upregulation of VCAM-1 and ICAM-1 expression in the lung, as well as the lung injury with eosinophilia and neutrophilia, suggesting that dexamethasone may prevent the lung injury, at least in part, by inhibition of the VCAM-1 and ICAM-1 expression. However, we have not attempted to examine whether anti-VCAM-1 and ICAM-1 monoclonal antibodies inhibit the lung inflammatory events in this model, and this experiment may help to confirm the role of the upregulation of VCAM-1 and ICAM-1 expression in the development of lung injury with eosinophilia and neutrophilia.

TNF-α may activate NF-κB, resulting in coordinated expression of numerous genes whose products mediate the recruitment of eosinophils, such as VCAM-1, ICAM-1 and eotaxin (Barnes and Karin, 1997). In previous studies, TNF-α has been shown to progressively increase ICAM-1 expression on endothelial cells (Pober et al., 1986), and display synergistic effects with IL-4 to increase VCAM-1 expression on endothelial cells (Thornhill et al., 1991). In addition, TNF-α has been shown to produce eotaxin and exert synergistic effects with IL-4 and IL-13 to augment production of eotaxin on BEADS-2B human bronchial epithelium (Fujisawa et al., 2000). Pretreatment with a soluble receptor comprising human p55 TNF receptor and human heavy-chain IgG_1, Ro 45−2081, significantly inhibited eosinophil and neutrophil infiltration into the bronchoalveolar lavage fluid after antigen challenge in sensitized rats (Renzetti et al., 1996). However, Ro 45−2081 at the doses used in the Renzetti's (1996) study only reduced neutrophilia into the bronchoalveolar lavage fluid in Sephadex-treated rats (Gater et al., 1996). TNF-α may thus play a role in the promotion of neutrophil recruitment into lungs in Sephadex-treated rats, and mediators other than TNF-α may be involved in eosinophil responses to Sephadex beads. Our study provided no evidence to determine whether TNF-α contributes to the development of lung eosinophilia in Sephadex-treated rats. TNF-α augments ICAM-1-mediated inflammatory responses such as neutrophil recruitment and granuloma formation, as previously described (Horgan et al., 1991; Lo et al., 1992). In the present study, we have demonstrated that dexamethasone completely inhibits the elevated TNF-α

concentration in the bronchoalveolar lavage fluid by i.v. Sephadex beads, verifying the findings presented by Williams et al. (1997) that dexamethasone reduces upregulation of TNF-α mRNA expression by bronchoalveolar lavage fluid cells after Sephadex bead treatment. In this Sephadex-treated model, dexamethasone may inhibit the ICAM-1-mediated inflammatory responses through the prevention of TNF-α production. Intratracheal Sephadex beads, which have been shown to induce lung inflammatory responses similar to those induced by i.v. Sephadex beads (Kubin et al., 1992; Buyssens et al., 1995), also increase the mRNA and protein levels of TNF-α in rat lung tissues (Haddad et al., 2002), and the protein level was strongly suppressed by dexamethasone or budesonide (Birrell et al., 2000; Haddad et al., 2002).

Sephadex beads have been shown to evoke lung eosinophilia associated with increased gene and protein expression of the Th2 cytokines IL-4, IL-5 and IL-13, and eotaxin in lung tissue of rats (Haddad et al., 2002). Das et al. (1995) demonstrated a requirement for IL-5 in the development of lung eosinophilia after Sephadex bead treatment using anti-IL-5 monoclonal antibodies. Antibodies blocking eotaxin suppresses eosinophilia in the bronchoalveolar lavage fluid from guinea pigs treated with Sephadex beads (Guo et al., 1999). In addition, the time course for eotaxin expression after Sephadex bead administration is related to the appearance of eosinophilia in the lungs (Harrington et al., 1999; Haddad et al., 2002). These studies reveal the important role played by IL-5 and eotaxin for eosinophil recruitment in lung tissue, although we did not measure levels of protein or gene expression for IL-5 or eotaxin in the bronchoalveolar lavage fluid. Glucocorticoids markedly inhibit increased protein and gene expression of Th2 cytokines IL-5 and IL-13, and eotaxin (Haddad et al., 2002). It therefore seems likely that dexamethasone in our study completely abolished the eosinophil recruitment induced by Sepahadex beads.

In conclusion, we have shown that i.v. Sephadex beads cause lung injury characterized by granuloma formation and eosinophilia, upregulation of VCAM-1 and ICAM-1 expression, and TNF-α release. Glucocorticoids may suppress the lung injury with eosinophilia, at least in part, due to the prevention of the upregulation of VCAM-1 and ICAM-1 expression.

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