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Vitamin A reduces lung granulomatous inflammation with eosinophilic and neutrophilic infiltration in Sephadex-treated rats

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

[a]*Department of Pediatrics, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, 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

Vitamin A is known to suppress the activity of the transcription factors, nuclear factor-κB (NF-κB) and activator protein-1 (AP-1), as do glucocorticoids. The possibility that vitamin A exerts various anti-inflammatory effects therefore seems likely. Sephadex beads were administered intravenously to anesthesized rats pretreated with a subcutaneous injection of vitamin A (3000, 10,000, or 30,000 IU/kg) or vehicle once daily for 3 days. After 16 h, the leukocyte differential, tumor necrosis factor (TNF)-α and eotaxin, and the DNA-binding activity of NF-κB were measured in bronchoalveolar lavage fluid (BALF). Additionally, lung histology was assessed using preparations stained with May–Giemsa stain. Sephadex beads caused histological granulomatous changes and eosinophilic and neutrophilic infiltration into the lung, and markedly increased cell counts of eosinophils and neutrophils, concentrations of TNF-α and eotaxin, and NF-κB binding to DNA in BALF. Vitamin A significantly inhibited all responses. Vitamin A may inhibit Sephadex-induced lung granulomatous formation, and eosinophilic and neutrophilic infiltration due to its suppression of TNF-α and eotaxin production, and NF-κB activation.

Keywords: Eotaxin; Lung granuloma; Nuclear factor-κB; Sephadex bead; Tumor necrosis factor-α; Vitamin A

1. Introduction

Vitamin A is known to display anti-inflammatory activity through genomic regulation of the expression of target genes (Pfahl, 1993; Minucci and Ozato, 1996). This activity is mediated by vitamin A receptors such as nuclear retinoic acid and retinoic X receptors. Vitamin A receptors bind with vitamin A, and this complex in turn binds with specific DNA sequences, known as retinoic acid-responsive elements, to modulate the activity of various transcription factors including nuclear factor-κB (NF-κB) and activator protein-1 (AP-1). This process is mediated by proteins called coactivators or corepressors (Minucci and Ozato, 1996; Germain et al., 2002). Indeed, vitamin A inhibits the transcriptional activity of AP-1 in synoviocytes stimulated by interleukin-1 and 12-*O*-tetradecanoyl-phorbol-13-acetate (Lafyatis et al., 1990), and also inhibits the transcriptional activity of NF-κB in monocytic cells stimulated by tumor necrosis factor (TNF)-α and lipopolysaccharide (Chen et al., 2002). Hisada et al. (1999) demonstrated that the DNA-binding activity of NF-κB increases in the lung tissues of rats exposed to ozone, and that vitamin A reduces this binding activity.

Many stimuli activate NF-κB, causing increased production of various cytokines, chemokines, adhesion molecules, and enzymes related to tissue infiltrates of eosinophils (Barnes and Karin, 1997). Because vitamin A suppresses the transcriptional activity of NF-κB (Hisada et al., 1999; Chen et al., 2002), as do glucocorticoids (Barnes, 1998), the possibility that vitamin A exerts various inhibitory effects against eosinophilic inflammation seems likely. However, whether vitamin A inhibits eosinophilic inflammation of the lung has not been clarified. Thus, the present study

* 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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investigated the effect of vitamin A in a rat model of lung eosinophilic infiltration caused by Sephadex bead treatment, which, when administered intravenously, can easily induce granulomatous changes with considerable accumulations of eosinophils and neutrophils in the lungs of rats and guinea pigs (Walls and Beeson, 1972; Sorden et al., 1990; Buyssens et al., 1995; Miyake et al., 2004). Such pathological changes are also common in human lung diseases caused by embolization of parasite eggs or larvae such as schistosomiasis and ascariasis (Kunkel et al., 1989), but differ significantly from those in asthmatic lung and allergen-sensitized, allergen-exposed animal models. However, previous studies have demonstrated that a fundamental commonality exists between the mechanisms causing eosinophilic infiltration into the lungs of Sephadex-treated and allergen-exposed animals [i.e., Th2 cytokines interleukin-4, interleukin-5, and interleukin-13 (Das et al., 1995; Haddad et al., 2002), and eotaxin (Guo et al., 1999; Harrington et al., 1999; Haddad et al., 2002)], and that vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) (Das et al., 1995; Ito et al., 2003) are principally mediated. Eosinophils play a key role in the pathophysiological changes of asthma (Wardlaw and Kay, 1987; Bousquet et al., 2000). Therefore, this experimental model is useful to study the mechanism of eosinophilic infiltration in asthmatic lung.

TNF-α may be closely involved in granuloma formation in a Sephadex-treated model (Kunkel et al., 1989; Ito et al., 2003) and promotes the transcriptional activity of NF-κB (Barnes and Karin, 1997). Eotaxin, being regulated mainly by NF-κB (Barnes and Karin, 1997), plays an important role in the recruitment of eosinophils in lung tissues (Barnes et al., 1998). Therefore, in the present study, the inhibitory effects of vitamin A on granuloma formation, NF-κB activation, and TNF-α and eotaxin production in the lung following Sephadex bead treatment were investigated.

2. Materials and methods

2.1. Animals

All experimental procedures were in accordance with guidelines for the care and use of laboratory animals at the Institute for Laboratory Animal Research, Nagoya University Graduate School of Medicine (Nagoya, Japan, 1989). Pathogen-free 8-week-old male Wistar rats (Japan SLC, Hamamatsu, Japan) weighing 240–260 g were used. Animals were kept in a temperature-controlled environment with standard laboratory food and water available ad libitum.

2.2. Materials

Sephadex beads (G-50 superfine; Pharmacia and Upjohn Diagnostics, Uppsala, Sweden) were prepared as previously described (Lemanske and Kaliner, 1982). Briefly, beads were autoclaved for 30 min, then suspended in 0.9% saline for at least 48 h at 4 °C. All-*trans*-retinol palmitate (Wako, Osaka, Japan) was dissolved in corn oil (0.6×10^5, 2×10^5, or 6×10^5 IU/ml; Honen Ajinomoto Oil Mills, Tokyo, Japan) and diluted in 0.15 M NaCl (1:39) to form three solutions with all-*trans*-retinol palmitate concentrations of 1500, 5000, or 15,000 IU/ml, respectively. Pentobarbital sodium was purchased from Abbott Laboratories (North Chicago, IL); phosphate-buffered saline (PBS) from Nissui Pharmaceutical (Tokyo, Japan); Hanks' balanced salt solution and formalin from Sigma (St. Louis, MO); Turk reagent, Giemsa stain solution, and May–Gruenwald stain solution from Katayama Chemical Industries (Osaka, Japan); and a thiazine–eosin staining kit (Diff-Quik stain) from Kokusai Shiyaku (Kobe, Japan).

2.3. Study design

Groups of rats (n=5) were pretreated with a subcutaneous (s.c.) injection of 0.5 ml of vitamin A suspension (1500, 5000, or 15,000 IU/ml) or solvent only (2.5% corn oil) once daily for 3 days. Within 15 min from their last pretreatment, rats were anesthesized using pentobarbital, and administered 1 ml of Sephadex bead suspension (12.5×10^4 particles/kg) via the tail vein. A sham-treated group (n=5) was pretreated with solvent for vitamin A once daily for 3 days, and administered 1 ml of 0.9% saline. Bronchoalveolar lavage fluid (BALF) was collected 16 h later for measurement of leukocyte differential and cytokine concentrations as described, and the lungs were removed for histological analysis.

Groups of rats (n=6) were pretreated with a s.c. injection of 0.5 ml of vitamin A suspension (5000 or 15,000 IU/ml) or its solvent only once daily for 3 days. Different treatment groups of rats were administered an intravenous injection of Sephadex beads as described above. A sham-treated group (n=6) was pretreated with solvent for vitamin A once daily for 3 days, and administered 1 ml of 0.9% saline. BALF was collected 16 h later for measurement of the active form of NF-κB as described below.

In our previous study (Miyake et al., 2004), the dose of Sephadex beads used submaximally increased eosinophil and neutrophil counts in BALF. We used doses of vitamin A that inhibited neutrophilic infiltration and DNA-binding activity of NF-κB in lung tissues of rats exposed to ozone, as described by Hisada et al. (1999).

2.4. Bronchoalveolar lavage and measurement of leukocyte counts

Rats were sacrificed by an overdose of pentobarbital (150 mg/kg, i.p.), and lungs were lavaged 10 times with 2-ml aliquots of 0.9% saline via a tracheal cannula (8 mm long, 1.3 mm inner diameter) introduced through tracheostomy. Lavage fluid was centrifuged at $800\times g$ for 10 min at 4 °C,

and the cell pellet resuspended in 1 ml of Hanks' balanced salt solution. After adding 20 μl of cell suspension to 80 μl of Turk's reagent, cells were counted under light microscopy 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 mononuclear cells, neutrophils, eosinophils, and shed epithelial cells according to standard morphological techniques; 1000 cells were counted under ×200 magnification, and the percentage and absolute number of each cell type were calculated.

2.5. Histological 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 tracheobronchus and lung were stained using May–Giemsa stain. Lung histology was assessed under light microscopy.

Granuloma areas were measured quantitatively using the National Institutes Image program (National Institutes of Health, Bethesda, MD) as described in detail elsewhere (Goldfarb, 1999; Mahy et al., 2003). Areas of at least 10 granulomas with visible central Sephadex beads from different May–Giemsa-stained sections of each lung were measured, and a microscope image (magnification ×200) was projected onto a charge-coupled device camera (HC-300Z/DL; Olympus, Tokyo, Japan).

2.6. Cytokine assay

Concentrations of TNF-α and eotaxin in BALF were measured using an enzyme-linked immunosorbent assay with commercially available kits (TNF-α: BioSource International, Camarillo, CA; eotaxin: R&D Systems, Minneapolis, MN). Detection limits of these kits for TNF-α and eotaxin were 4 and 3 pg/ml, respectively.

2.7. Preparation of whole cell extract samples and measurement of NF-κB DNA-binding activity

Cellular components (3×10^6 per sample) were separated from BALF by centrifugation at $800\times g$ for 10 min at 4 °C and resuspended in 100 μl of a lysis buffer (20 mM Hepes, 350 mM NaCl, 20% glycerol, 1% Igepal-CA630, 1 mM $MgCl_2$, 0.5 mM EDTA, 0.1 mM EGTA, 5 mM dithiothreitol, and protease inhibitors) for 20 min at 4 °C. The cell suspension was centrifuged at $14{,}000\times g$ for 20 min at 4 °C and the supernatant stored in aliquots at −80 °C until measurement of NF-κB. The active form of NF-κB in the whole cell extract was measured quantitatively using an enzyme-linked immunosorbent assay with a commercially available kit (Trans-AM™ NF-κB kit; Active Motif North America, Carlsbad, CA). The kit contained a 96-well plate to which was attached an oligonucleotide containing the NFκB consensus site (5′ -GGGACTTTCC-3′). The primary antibodies used to recognize an epitope on p65 that is accessible only when NF-κB is activated and bound to its target DNA were used to detect NF-κB. A horseradish peroxidase-conjugated secondary antibody provided a sensitive colorimetric readout easily quantified by spectrophotometry at 450 nm.

In a preliminary experiment to evaluate the specificity of the assay kit, double-stranded oligonucleotides encoding the consensus target sequence of NF-κB and the mutated ones were provided as positive and negative competitors, respectively, for NF-κB binding to the solid-phase consensus oligonucleotide. Excess doses of wild consensus oligonucleotides completely inhibited the DNA-binding activity of NF-κB included in the whole cell extract obtained from BALF in Sephadex-treated rats. On the other hand, the mutated ones had no effect on NF-κB binding.

2.8. Statistical analysis

All values are expressed as mean±S.E.M. An unpaired Student's *t* test (two-tailed) was utilized to evaluate the significance of the difference between two independent groups with equal variance, as assessed using the *F* test. In

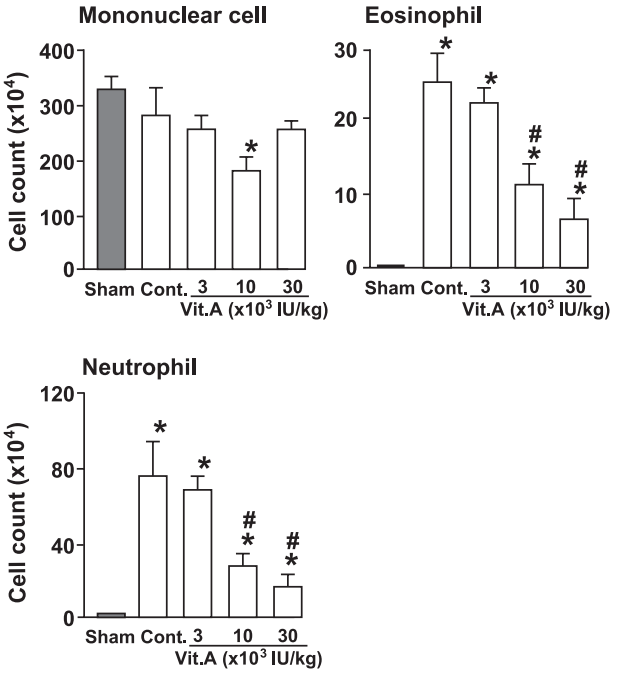

Fig. 1. Effect of different doses of vitamin A (3000, 10,000, or 30,000 IU/kg) on leukocyte counts in BALF 16 h after administration of Sephadex beads to Wistar rats. Beads (12.5×10^4 particles/kg) were suspended in 1 ml of 0.9 % saline and administered via the tail vein. Pretreatment with a s.c. injection of vitamin A or solvent (0.5 ml of 2.5% corn oil) was performed once daily for 3 days. A control group of rats was pretreated with solvent and administered the beads. Sham-treated rats were pretreated with solvent and administered 0.9 % saline intravenously. Results are expressed as mean±S.E.M. (n=5). Statistical significance: *P<0.01 compared with the sham group, assessed by the unpaired Student's *t* test or Welch's test; $^{\#}P$<0.01 compared with controls, assessed with ANOVA and Fisher's test.

all other cases, Welch's test (two-tailed) was employed. One-way analysis of variance (ANOVA) and Fisher's test were used for multiple comparisons with controls. Values of $P<0.05$ were considered statistically significant.

3. Results

3.1. Leukocyte counts in BALF

No significant difference in mean body weight was observed among the different treatment groups used in this study (data not shown). No symptoms of poisoning such as behavioral inactivity or diarrhea were noted in groups pretreated with vitamin A. The recovery rate of BALF was about 95% and, in all cases, BALF was barely macroscopically contaminated by red blood cells. Mononuclear cells were quite dominant cellular components of BALF in sham-stimulated group. Cell counts of eosinophils and neutrophils increased significantly after Sephadex bead treatment, but mononuclear cell count did not (Fig. 1). Shed epithelial cells were barely detected in BALF from either group. Vitamin A significantly inhibited increases in eosinophil and neutrophil counts in a dose-dependent manner (Fig. 1). Eosinophil and neutrophil counts in BALF were inhibited by the maximal dose of vitamin A (30,000 IU/kg, s.c.) by 74.7% and 80.7%, respectively. On the other hand, vitamin A at any of the three doses used did not alter mononuclear cell counts in BALF from Sephadex-treated rats.

3.2. Histological investigations of lung preparations

Inflammatory responses to Sephadex beads in the lung were observed in preparations stained using May–Giemsa stain. Mononuclear cells, particularly macrophages, were packed tightly around beads, and eosinophils and neutrophils were conspicuous in the periphery of lesions (Fig. 2A and D). Cell counts in the interstitium were increased for macrophages, eosinophils, neutrophils, and lymphocytes. However, few inflammatory changes were observed in response to Sephadex beads in the airway lumen and walls that were not adjacent to granulomatous lesions (data not shown). Vitamin A inhibited granulomatous changes and infiltration of eosinophils and neutrophils into the periphery of the granulomatous lesion (Fig. 2B, C, E, and F). The

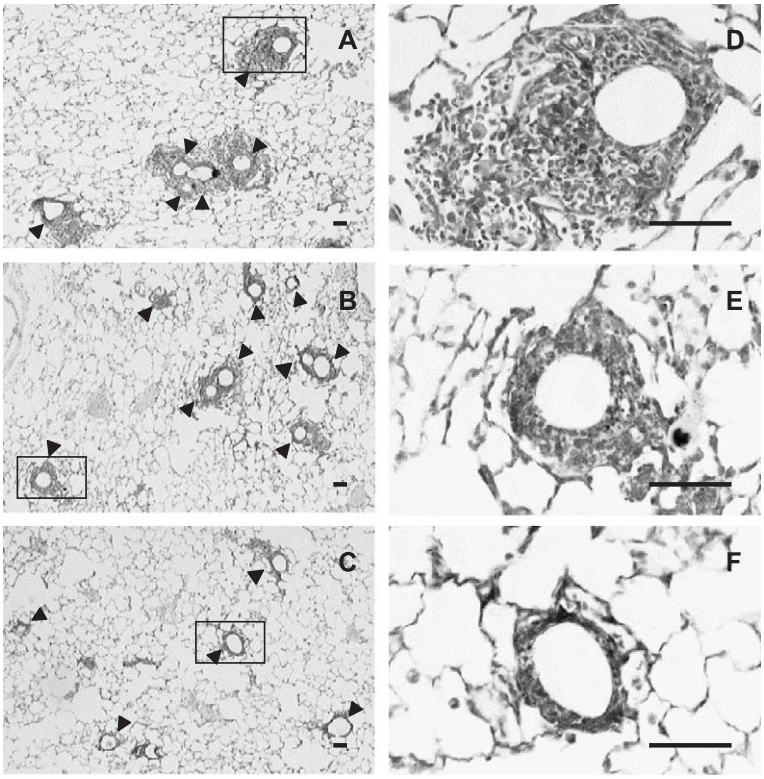

Fig. 2. Histopathological features of lung granulomatous inflammation induced by an intravenous injection of Sephadex beads (12.5×10^4 particles/kg) in three different treatment groups of Wistar rats. Rats were pretreated with a s.c. injection of vitamin A (10,000 or 30,000 IU/kg) or solvent (0.5 ml of 2.5% corn oil) once daily for 3 days. Lungs were removed 16 h after treatment with Sephadex beads, and paraffin-embedded sections (4 μm thick) were stained with May–Giemsa stain. (A and D) Lung sections from control groups; (B and E) lung sections from vitamin A (10,000 IU/kg)-treated rats; and (C and F) lung sections from vitamin A (30,000 IU/kg)-treated rats. A typical example of a granulomatous lesion induced by an embedded Sephadex particle is marked with arrows. Each rectangular lesion encompassing the granulomatous lesion (A–C) is shown at magnifications from ×40 to ×200 (D–F), ×40 (A–C), and ×200 (D–F). Bars indicate 100 μm.

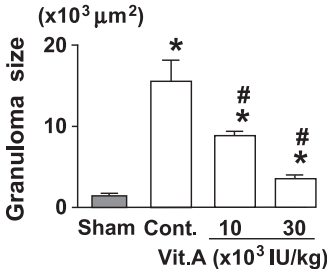

Fig. 3. Effects of different doses of vitamin A (10,000 or 30,000 IU/kg) on areas of each granulomatous lesion in lung induced by an intravenous injection of Sephadex beads (12.5×10^4 particles/kg) in rats. Areas of at least 10 granulomas with visible central Sephadex beads from different May–Giemsa-stained sections of each lung were measured quantitatively using the National Institutes Image program (National Institutes of Health). A microscope image (magnification ×200) was projected onto a charge-coupled device camera (HC-300Z/DL; Olympus). Lung samples from control and vitamin A-treated groups of rats were obtained 16 h after Sephadex bead treatment. On the other hand, a sham sample was obtained just after the treatment. Results are expressed as mean±S.E.M. (n=5). Statistical significance: *P<0.01 compared with the sham group, assessed by the unpaired Student's t test or Welch's test; $^{\#}P$<0.01 compared with controls, assessed with ANOVA and Fisher's test.

maximal dose of vitamin A (30,000 IU/kg, s.c.) markedly inhibited both lung responses (Fig. 2C and F).

Granulomas greatly developed in size 16 h after Sephadex bead treatment, reaching up to 16,535.7±2215.4 μm² from 1662.1±166.9 μm². Vitamin A significantly reduced the size of granulomas in a dose-dependent manner (Fig. 3), with the maximal dose of vitamin A strongly decreasing it by 83.4%.

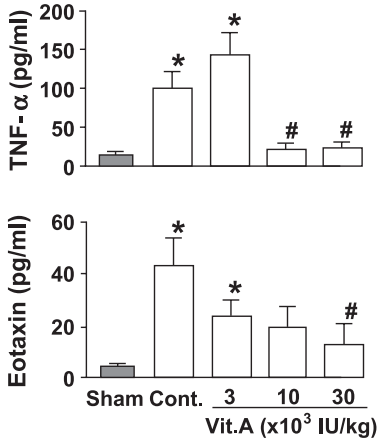

Fig. 4. Effect of different doses of vitamin A (3000, 10,000, or 30,000 IU/kg) on concentrations of TNF-α and eotaxin in BALF 16 h after administration of Sephadex beads to Wistar rats. Beads (12.5×10^4 particles/kg) were suspended in 1 ml of 0.9 % saline and administered via the tail vein. Pretreatment with a s.c. injection of vitamin A or solvent (0.5 ml of 2.5% corn oil) was done once daily for 3 days. A control group was pretreated with solvent and administered the beads. Sham-treated rats were pretreated with solvent and administered 0.9 % saline intravenously. Results are expressed as mean±S.E.M. (n=5). 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 controls, assessed with ANOVA and Fisher's test.

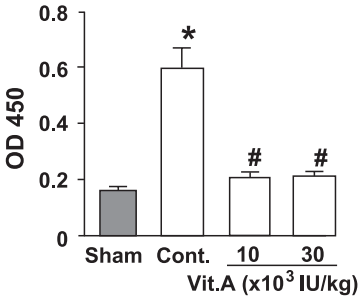

Fig. 5. Effects of different doses of vitamin A (10,000 or 30,000 IU/kg) in NF-κB DNA-binding activity of whole cell extract obtained from BALF 16 h after intravenous administration of Sephadex beads (12.5×10^4 particles/kg) in rats. Pretreatment with a s.c. injection of vitamin A or solvent (0.5 ml of 2.5% corn oil) was done once daily for 3 days. A control group of rats was pretreated with solvent and administered the beads. Sham-treated rats were pretreated with solvent and administered intravenously 0.9% saline alone. NF-κB activation was measured using an enzyme-linked immunosorbent assay with a commercially available kit (Trans-AM™ NF-κB kit; Active Motif North America). A sensitive colorimetric readout of the result was easily quantified by spectrophotometry at 450 nm. Results are expressed as mean±S.E.M. (n=6). Statistical significance: *P<0.01 compared with the sham group, assessed by the unpaired Student's t test or Welch's test; $^{\#}P$<0.01 compared with controls, assessed with ANOVA and Fisher's test.

3.3. Cytokine levels in BALF

After the Sephadex bead injection, concentrations of TNF-α and eotaxin in BALF increased significantly from 22.3±1.4 to 103.2±28.9 pg/ml, and from 5.4±0.5 to 43.9±8.9 pg/ml, respectively. Vitamin A significantly inhibited the release of TNF-α and eotaxin into BALF in a dose-dependent manner (Fig. 4). Vitamin A at doses over 10,000 IU/kg completely inhibited TNF-α elevation, since there was no significant difference in the cytokine level between the group pretreated with 10,000 or 30,000 IU/kg vitamin A and the sham group (Fig. 4). Vitamin A at a dose of 30,000 IU/kg almost completely inhibited eotaxin elevation (Fig. 4).

3.4. NF-κB activity in whole cell extract obtained from BALF

After the Sephadex bead injection, NF-κB-binding activity in the whole cell extract obtained from BALF increased significantly from 0.160±0.01 to 0.585±0.08 (OD 450 nm). Vitamin A at doses more than 10,000 IU/kg completely inhibited NF-κB activity, since there was no significant difference in the cytokine level between the group pretreated with 10,000 or 30,000 IU/kg vitamin A and the sham group (Fig. 5).

4. Discussion

In the present study, Sephadex beads caused marked granulomatous lesions around the beads clogging the small

vessels in the lungs, with macrophages constituting the principal part of the lesions, although eosinophils, neutrophils, and lymphocytes also accumulated appreciably in the lesions and their surroundings. These results are consistent with previous reports (Walls and Beeson, 1972; Buyssens et al., 1995; Miyake et al., 2004). Examination of BALF also revealed significant increases in eosinophils and neutrophils, reflecting the inflammation of lung tissues. However, the number of macrophages observed in BALF did not necessarily increase. The effect of vitamin A on inflammatory changes in rat lungs exposed to Sephadex beads has not previously been investigated. Our results clearly demonstrated that vitamin A inhibits both inflammatory granuloma formation in the lungs and infiltration of eosinophils and neutrophils around the insult caused by Sephadex beads, and that it decreases the number of eosinophils and neutrophils in BALF.

The present study demonstrated that Sephadex beads promote DNA-binding activity of NF-κB of cellular components in BALF, and that vitamin A strongly inhibits this response. As shown in a previous study of ours (Ito et al., 2003), a complete inhibition of Sephadex-induced lung inflammation by glucocorticoids supports the involvement of increased transcriptional activity of NF-κB in this model, as glucocorticoids achieve their anti-inflammatory activity by inhibiting the transcriptional activity of NF-κB and AP-1 (Barnes, 1998).The inhibitory effect of vitamin A on NF-κB activation has also been demonstrated in previous in vivo and in vitro studies (Hisada et al., 1999; Chen et al., 2002). Additionally, we provided direct evidence here that treatment with Sephadex beads causes an increase in TNF-α and eotaxin production in lung tissues, and that this response can be markedly suppressed by vitamin A. The expression of the genes for TNF-α and eotaxin is increased mainly by NF-κB activation (Barnes and Karin, 1997); thus, vitamin A may decrease the transcription of these genes involved in the Sephadex-treated lung through inhibition of transcriptional activity of NF-κB. On the other hand, TNF-α promotes the transcriptional activity of NF-κB (Barnes and Karin, 1997), in turn causing the increased production of various cytokines, chemokines, adhesion molecules, and enzymes including TNF-α itself. Therefore, the positive regulatory interaction of TNF-α and NF-κB that may have amplified inflammatory responses in lung tissues after Sephadex bead treatment might have been blocked by vitamin A.

TNF-α is produced by a variety of cells, but the principal source is macrophages (Barnes et al., 1998), thus indicating that vitamin A may potently inhibit TNF-α release from alveolar macrophages stimulated by Sephadex bead treatment. Our results are consistent with previous reports showing that vitamin A inhibits TNF-α release from rat macrophages stimulated by lipopolysaccharide (Mehta et al., 1994; Motomura et al., 2001). Renzetti et al. (1996) showed that Ro 45-2081, a soluble receptor composed of human p55 TNF receptor and human heavy-chain immunoglobulin G, inhibits eosinophilic and neutrophilic infiltration into BALF following antigen exposure in sensitized rats. The same doses of Ro 45-2081 used in the study by Renzetti et al. (1996) inhibited neutrophil recovery from BALF following administration of Sephadex beads, but eosinophil recovery was not inhibited (Gater et al., 1996). This indicates that in the Sephadex model, TNF-α is related to neutrophilic infiltration, but that eosinophilic infiltration is related to factors other than TNF-α. However, this conclusion contradicts the hypothesis that TNF-α promotes the transcriptional activity of NF-κB and upregulates the gene and protein expression of various mediators related to tissue infiltration of eosinophils, as noted above. Our present study likewise provided no direct evidence to determine whether TNF-α contributes to the development of eosinophilic infiltration in Sephadex-treated rats. From our data, despite the fact that production of TNF-α by Sephadex bead treatment was completely inhibited by vitamin A, neutrophilic infiltration into the lungs was only partly inhibited. TNF-α and other mediators therefore seem likely to make concurrent contributions to the responses of neutrophils to Sephadex beads.

In a previous study (Ito et al., 2003), we showed that Sephadex beads upregulate ICAM-1 expression in rat lungs. CD11a/CD18 and CD11b/CD18, classified into the β2 integrin subfamily, act as ligands of ICAM-1 on endothelial cells (Carlos and Harlan, 1994; Malik and Lo, 1996). ICAM-1-mediated mechanisms are involved in lung eosinophilic infiltration and neutrophilic infiltration in the Sephadex-treated model (Das et al., 1995). As well, ICAM-1-mediated mechanisms seem to play an important role in granuloma formation in the Sephadex-treated model, which may be primarily mediated by mononuclear cells because CD11a/CD18 and CD11b/CD18 are densely distributed on macrophages and lymphocytes (Carlos and Harlan, 1994; Malik and Lo, 1996). TNF-α significantly augments expression of ICAM-1 on various cell types such as endothelial cells, an effect that is likely important in the development and maintenance of granulomas (Pober et al., 1986; Lo et al., 1992; Carlos and Harlan, 1994; Malik and Lo, 1996). Inhibition of TNF-α production by vitamin A, as shown in the present study, may therefore result in suppression of granuloma formation induced by Sephadex beads.

Sephadex beads have been shown to evoke lung eosinophilic infiltration associated with increased gene and protein expression of the Th2 cytokines interleukin-4, interleukin-5, and interleukin-13, and eotaxin in lung tissues of rats (Das et al., 1995; Guo et al., 1999; Harrington et al., 1999; Haddad et al., 2002). Antibody blocking eotaxin suppresses eosinophilic infiltration into BALF 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 eosinophilic infiltration into lungs (Guo et al., 1999; Harrington et al., 1999; Haddad et al., 2002). These studies have revealed the important role played by eotaxin for

eosinophil recruitment in lung tissue. Our results suggest that vitamin A may thus inhibit lung eosinophilic infiltration, at least partly, through the prevention of upregulated eotaxin expression.

Interleukin-5 is a cytokine known to cause lung eosinophilic infiltration in Sephadex-treated lung (Das et al., 1995). Although we did not measure this cytokine in lung tissues, vitamin A is reported to inhibit interleukin-5 receptor expression of progenitor stem cells in human bone marrow and selectively block eosinophil differentiation (Upham et al., 2002). Such inhibition of eosinophil production may therefore represent one possible mechanism for the inhibited recruitment of eosinophils in our study.

The present study demonstrated that vitamin A inhibits inflammatory granuloma formation accompanied by eosinophilic and neutrophilic infiltration into lung tissues in Sephadex-treated rats. Vitamin A may inhibit the lung inflammatory response, at least in part, by its suppression of TNF-α and eotaxin production and NF-κB activation. These results indicate the possibility that vitamin A can mitigate various kinds of pulmonary inflammation, including eosinophilic inflammation as observed in bronchial asthma.

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