

Effects of Dexamethasone on the Expression of Transforming Growth Factor- β in the Mouse Model of Allergic Rhinitis

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Objectives/Hypothesis: This study aimed to evaluate the effect of dexamethasone on the expression of transforming growth factor (TGF)- β in the mouse model of allergic rhinitis.

Study Design: Female BALB/c mice were randomly assigned to four groups, including two control groups and two treatment groups.

Methods: General sensitization and local challenge were performed with ovalbumin (OVA). In the treatment groups, dexamethasone was injected intraperitoneally 3 hours before general sensitization or local challenge. Symptom score, eosinophil infiltration, and immunostaining for TGF- β 1 and CD4 in nasal mucosa, and TGF- β 1 and OVA-specific immunoglobulin E (IgE) in sera were analyzed.

Results: Dexamethasone administration before general sensitization reduced the symptom score, OVA-specific IgE, and eosinophil infiltration and increased the serum level of TGF- β 1 significantly. Dexamethasone administration before local challenge reduced only the eosinophil infiltration significantly. Immunoreactivity of TGF- β 1 and CD4 was lower in both treatment groups.

Conclusion: These results suggest that dexamethasone may play an important role in the regulation of allergic reactions by at least two mechanisms; one by suppressing allergic sensitization through decrease of CD4⁺ T cells and increase of TGF- β , and the other by suppressing late allergic reactions through the inhibition of proliferation and chemotaxis of inflammatory cells such as eosinophils.

Key Words: Allergy, dexamethasone, TGF- β , CD4⁺ T cell, regulatory T cell.

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INTRODUCTION

Although not life threatening, allergic rhinitis can deteriorate the quality of life and can be a major risk factor for paranasal sinusitis and bronchial asthma. Despite its clinical and socioeconomic impact, advances in its treatment still have a long way to go. Although immunotherapy has been achieving success, the mechanisms of immune modulation are not completely understood yet.

Transforming growth factor (TGF)- β is released by lymphocytes, eosinophils, fibroblasts, and respiratory epithelial cells. It has three isoforms that are functionally indistinguishable: TGF- β 1, TGF- β 2, and TGF- β 3. TGF- β elicits its effects through a cell-surface receptor which is a heterodimer of type I (T β RI) and type II (T β RII) receptors.¹ TGF- β is known to provide an important signal for fibroblast growth and activity in wound healing.² On the other hand, another important function is the inhibition of inflammatory and immune responses through its antiproliferative effects on CD4⁺ T cells and macrophages.³

Corticosteroids, the most potent therapeutic agents used for allergic rhinitis, profoundly inhibit the activity of T cells largely through the inhibition of expression of various cytokines. It is also reported to act indirectly by inducing TGF- β expression.⁴ Corticosteroids are reported to increase TGF- β expression in patients with nasal polyps,⁵ bronchial asthma,⁶ and leprosy type 1 reactions.⁷ However, there are only few reports regarding the effects of corticosteroids on TGF- β expression in allergic rhinitis.

In this study we investigated the effects of corticosteroids on allergic inflammation as well as TGF- β expression and analyzed these effects according to the time of administration in experimentally induced mouse model of allergic rhinitis.

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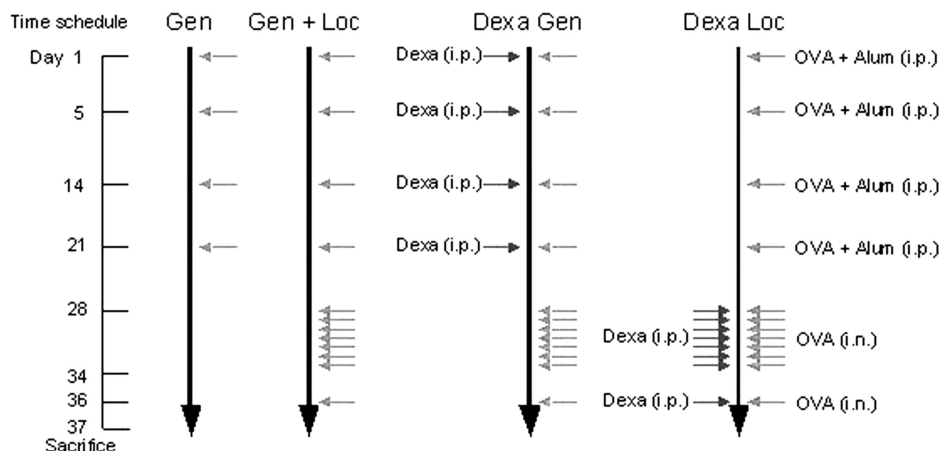


Fig. 1. Experimental protocol. BALB/c mice were sensitized with ovalbumin (OVA) and 1 mg of aluminum hydroxide gel (Alum) on days 1, 5, 14, and 21 (general sensitization). All groups except for general sensitization-only group (Gen) received intranasal OVA from day 28 through day 34 and on day 36. Along with the series of sensitization, the animals were treated with dexamethasone (Dexa) 3 hours before each general sensitization (Dexa Gen) or local challenge (Dexa Loc).

MATERIALS AND METHODS

Animals

Four-week-old female BALB/c mice were obtained from Korea Biolink Co. (Eumsung, Korea). All animal experiments conducted in this study followed the guidelines and ethics of Institutional Animal Care at the Clinical Research Institute of Seoul National University Hospital.

Allergen Sensitization and Challenge and Dexamethasone Administration

The procedure for allergen and dexamethasone administration is summarized in Figure 1. Mice were sensitized with an intraperitoneal injection of 25 μ g of ovalbumin (OVA; Grade V, Sigma, St. Louis, MO) and 1 mg of aluminum hydroxide gel on days 1, 5, 14, and 21. After general sensitization, mice were locally challenged with 500 μ g of OVA into their nostrils from day 28 through day 34 and on day 36. Along with the series of sensitization and challenge, selected groups of mice were treated with intraperitoneal injection of 100 μ g of dexamethasone 3 hours before OVA administration. Mice (10 per group) were classified into two control groups (general sensitization only or general sensitization with local challenge) and two treatment groups (dexamethasone administration before general sensitization or local challenge).

Symptom Score and Tissue Preparation

Fifteen minutes after final OVA challenge on day 36, the frequency of sneezing and nasal rubbing was recorded by a blinded observer over a 15-minute interval. Mice were then killed 24 hours after the last OVA challenge. After perfusion fixation with 4% paraformaldehyde, the head of five mice from each group was removed en block and then fixed in 4% paraformaldehyde. From the other five mice, nasal mucosa was obtained for reverse transcription-polymerase chain reaction (RT-PCR) and was immediately immersed in liquid nitrogen and stored at -70°C until use.

Eosinophils in Nasal Septal Mucosa

For evaluation of nasal histology, nasal tissue was decalcified, embedded in paraffin, and sectioned coronally (4 μ m thick) about 5 mm from the nasal vestibule. Each section was stained with hematoxylin and eosin and the number of eosinophils on both sides of the septal mucosa was counted.

Evaluation of Serum Level of OVA-Specific Immunoglobulin E

Serum level of OVA-specific immunoglobulin E (IgE) was measured by solid-phase enzyme-linked immunosorbent assay. Microplates were coated with 100 μ L of OVA (10 μ g/mL) and nonspecific antigen-antibody reaction was blocked with 3% bovine serum albumin. Serum samples diluted with PBS-T (5% Tween 20 in phosphate-buffered saline with 0.05% NaN_3 ; Sigma) (dilution 1:500) were added in a volume of 100 μ L and incubated for 2 hours. After washing with PBS-T, the plates were incubated with 100 μ L of biotinylated anti-mouse IgE (Pharmingen, San Diego, CA) (dilution 1:250) for 1 hour. The plates were then incubated with 100 μ L of avidin-horseradish peroxidase for 30 minutes. After incubation with *O*-phenylenediamine dihydrochloride (Sigma) and 30% H_2O_2 in a volume of 100 μ L, the reaction was stopped by addition of 50 μ L of 2.5 N H_2SO_4 and the optical density was measured at 490 nm.

Evaluation of Serum Level of TGF- β 1

Serum level of TGF- β 1 was measured with commercially available enzyme-linked immunosorbent assay kits (R&D Systems, Inc., Minneapolis, MN). After measuring the optical density at 450 nm, the concentration of TGF- β 1 was determined by interpolation from a standard curve and expressed as nanograms per milliliter.

RT-PCR Detection of TGF- β 1 mRNA in Nasal Septal Mucosa

Total RNA was extracted from the cells using the RNeasy mini-kit (Qiagen, Valencia, CA). For the synthesis of cDNA, a mixture of 1 μ g total RNA and 1 μ L Oligo dT 12 to 18 (0.5 μ g/ μ L) was incubated for 5 minutes at 70°C and placed on ice immediately. This mixture was transferred to an AccuPower RT Premix tube (Bioneer, Seoul, Korea) and left for 1 hour at 42°C for cDNA synthesis and 5 minutes at 94°C for RTase inactivation. PCR amplification was conducted using the AccuPower PCR Premix (Bioneer) reagent. PCR reaction conditions were 35 cycles of 94°C , 1 minute; 55°C , 1 minute; and 72°C , 1 minute in a Gene Amp PCR system 2400 (PerkinElmer, Norwalk, CT). The density of each band stained was standardized using glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The primer sequences of TGF- β 1 and GAPDH were as follows: TGF- β 1 sense primer, 5'-CACCATCCATGACATGAACC-3' and TGF- β 1 antisense primer, 5'-TCATGTTGGACAACCTGCTCC-3'; GAPDH sense primer, 5'-ACCACAGTCCATGCCATCAC-3' and GAPDH antisense primer, 5'-TCCACCACCCTGTTGCTGTA-3'.

Immunohistochemistry for TGF- β 1 and CD4

Nose specimens were decalcified for 5 days in 7% ethylene diamine tetra-acetic acid, embedded in paraffin, sectioned coronally (4 μ m thick) about 5 mm from the nasal vestibule, and deparaffinized in xylene. All sections were incubated in citrate buffer to activate the antigenicity and exposed to 3% H₂O₂ for 5 minutes to block endogenous peroxidase. The sections were incubated with rabbit polyclonal anti-TGF- β 1 (1:100, Santa Cruz Biotechnology, Santa Cruz, CA) for 1 hour and then incubated with anti-rabbit IgG and Envision (Dako Co., Copenhagen, Denmark). The staining was visualized using 3,3'-diaminobenzidine tetrahydrochloride for 5 minutes. After rinsing, the sections were incubated with rat polyclonal anti-CD4 (1:100, Santa Cruz Biotechnology) for 1 hour and then incubated with anti-rat IgG. CD4 was visualized with streptavidin-biotin peroxidase (Dako Co.) followed by incubation with Fast red (Dako Co.) for 20 minutes.

Statistical Analysis

Results are shown as mean $\pm$ SEM. The steroid treatment groups were compared with the control group, which received general sensitization and local challenge. Statistical analysis was performed using the Mann-Whitney *U* test. A *P* value less than .05 was considered statistically significant.

RESULTS

Allergic Symptom Score

Although there was no statistical significance, the allergic symptom score of control mice which received both general sensitization and local challenge was higher than in the control mice which received general sensitization only (24.1 ± 7.7 vs. 15.8 ± 2.7). The allergic symptom scores of both treatment groups were lower than those of control mice. However, only dexamethasone administration before general sensitization produced significant improvement in symptom score (13.3 ± 8.1 , $P < .01$, Fig. 2A) compared with the control.

Number of Eosinophils Infiltrated in Nasal Septal Mucosa

The number of eosinophils infiltrated in the nasal septum was significantly higher in control mice that received both general sensitization and local challenge than in control mice that received general sensitization only (248.4 ± 21.6 vs. 9.8 ± 3.5 , $P < .01$). Both treatment groups showed significantly lower number of eosinophils than the control mice (104.8 ± 17.3 , 144.2 ± 15.1 , respectively, $P < .01$, Fig. 2B).

Serum Levels of OVA-Specific IgE and TGF- β 1

There was no significant difference between serum levels of OVA-specific IgE between the two control groups (0.077 ± 0.001 vs. 0.079 ± 0.005). Dexamethasone administration prior to general sensitization reduced the serum level of OVA-specific IgE significantly compared with the control (0.063 ± 0.003 , $P < .01$, Fig. 2C). Serum level of TGF- β 1 was significantly lower in the mice that received both general sensitization and local challenge than in mice that received general sensitization only (35.9 ± 6.2 ng/mL vs. 54.8 ± 6.3 ng/mL, $P < .05$). Dexamethasone administration before general sensitization increased the

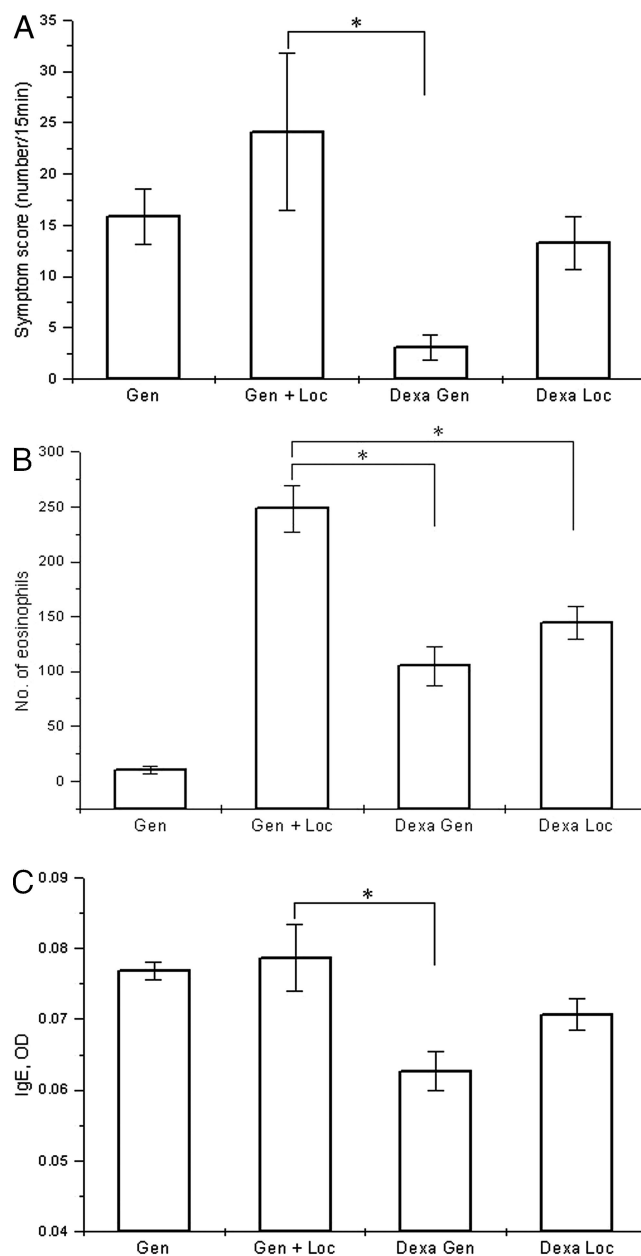


Fig. 2. Parameters of allergic responses. (A) Mice treated before general sensitization (Dexa Gen) show significant lower symptom score (A, $*P < .01$) and serum ovalbumin-specific IgE level (C, $*P < .05$) than that of control mice (Gen + Loc). Both treatment groups (Dexa Gen and Dexa Loc) show significant smaller number of eosinophils infiltrating nasal mucosa than that of control mice (Gen + Loc) (B, $*P < .01$). Data represent mean $\pm$ SEM.

serum level of TGF- β 1 significantly (53.6 ± 8.3 ng/mL, $P < .05$, Fig. 3A) compared with the control.

Expression of TGF- β 1 mRNA in Nasal Septal Mucosa

The density ratios of TGF- β 1 mRNA expression (TGF- β 1/GAPDH) from two control groups showed no significant difference (1.00 ± 0.10 vs. 0.97 ± 0.10). Dexamethasone administration before local challenge reduced

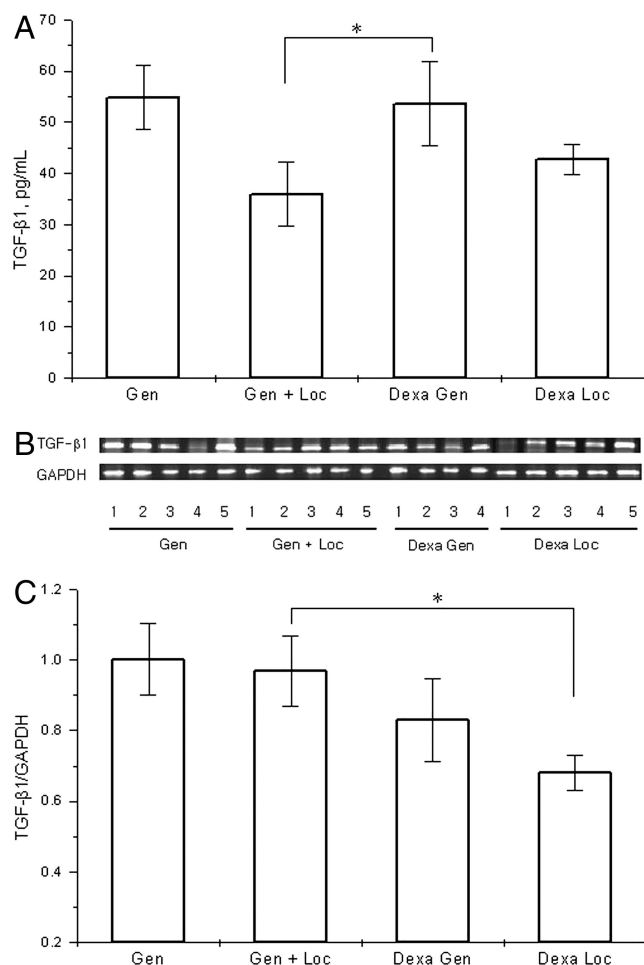


Fig. 3. Transforming growth factor (TGF)- β 1. Mice treated before general sensitization (Dexa Gen) show significant higher serum level of TGF- β 1 than that of control mice (Gen + Loc) (A, $*P < .05$). In reverse transcription-polymerase chain reaction analysis, mice treated before general sensitization (Dexa Gen) show significant lower TGF- β 1 mRNA expression in nasal mucosa than that of control mice (Gen + Loc) (B and C, $*P < .05$). Data represent mean $\pm$ SEM.

TGF- β 1 mRNA expression significantly (0.68 ± 0.05 , $P < .05$, Figs. 3B, C) compared with the control.

Immunohistochemistry for TGF- β 1 and CD4 in Nasal Septal Mucosa

TGF- β 1-positive cells and CD4-positive cells were found in the basal and the subepithelial layers, where infiltration of inflammatory cells was observed. The control group, which received general sensitization and local challenge, showed more prominent immunoreactivity for both TGF- β 1 and CD4 than the control group that received general sensitization only. In the treatment groups, immunoreactivity for TGF- β 1 and CD4 was lower than in the control groups. In particular, decreased immunoreactivity of TGF- β 1 was more prominent in mice treated with dexamethasone before local challenge (Fig. 4).

DISCUSSION

In this study, we designed two control groups; one group received only general sensitization and the other received general sensitization and local challenge. Although there was no significant difference of serum levels of OVA-specific IgE between the two control groups, allergic symptom score, eosinophil infiltration, and immunoreactivity for TGF- β 1 and CD4 in the nasal mucosa were significantly higher in the control group which received both general sensitization and local challenge. These results are compatible with the known pathophysiology of allergic rhinitis. Therefore the protocol and method of allergen administration used for the induction of allergic rhinitis in mice can be considered a suitable model for this study. For convenience, control mice which were compared with treatment groups indicate those which received both general sensitization and local challenge.

Allergic symptom score, serum level of OVA-specific IgE, and eosinophil infiltration in the nasal mucosa were significantly lower in mice treated with dexamethasone before general sensitization than in the control mice. These results suggest that dexamethasone, when administered before general sensitization, suppresses early and late allergic reactions. However, only eosinophil infiltration in the nasal mucosa decreased significantly in mice treated with dexamethasone before local challenge, suggesting that dexamethasone administration before local challenge in sensitized mice can only suppress late allergic reactions.

TGF- β plays an important role in immunomodulation mainly through suppression of differentiation and proliferation of T cells.⁸ In addition, TGF- β induces naive CD4⁺ T cells to differentiate into regulatory T cells.⁹ Oral administration of low-dose allergen is reported to up-regulate circulating levels of TGF- β and to improve autoimmune diseases.¹⁰

There have been rare in vivo studies concerning the effect of steroids on the expression of TGF- β . Topical application of steroids has been reported to increase the level of TGF- β in nasal polyps⁵ and bronchoalveolar lavage fluid.⁶ Contrary to these reports, in vitro studies using nasal polyps¹¹ and embryonal lung fibroblasts¹² demonstrated reduced expression of TGF- β with steroid administration and suggested its role in the treatment of airway remodeling seen in chronic inflammatory lung diseases. In this study we noted that the changes in the levels of TGF- β in the serum and in the nasal mucosa with dexamethasone treatment were in contrary directions to each other. When dexamethasone was administered, serum level of TGF- β increased and this increase was more prominent in mice treated before general sensitization. However, TGF- β expression in the nasal mucosa decreased and this was more prominent in mice treated before local challenge.

Regulatory T cells play an important role in protecting host from inadvertent immunologic reactions such as allergy and autoimmune diseases. These cells exert their immunosuppressive actions mainly through the release of TGF- β and/or interleukin-10.¹³ Regulatory T cells are reported to contribute to the establishment and

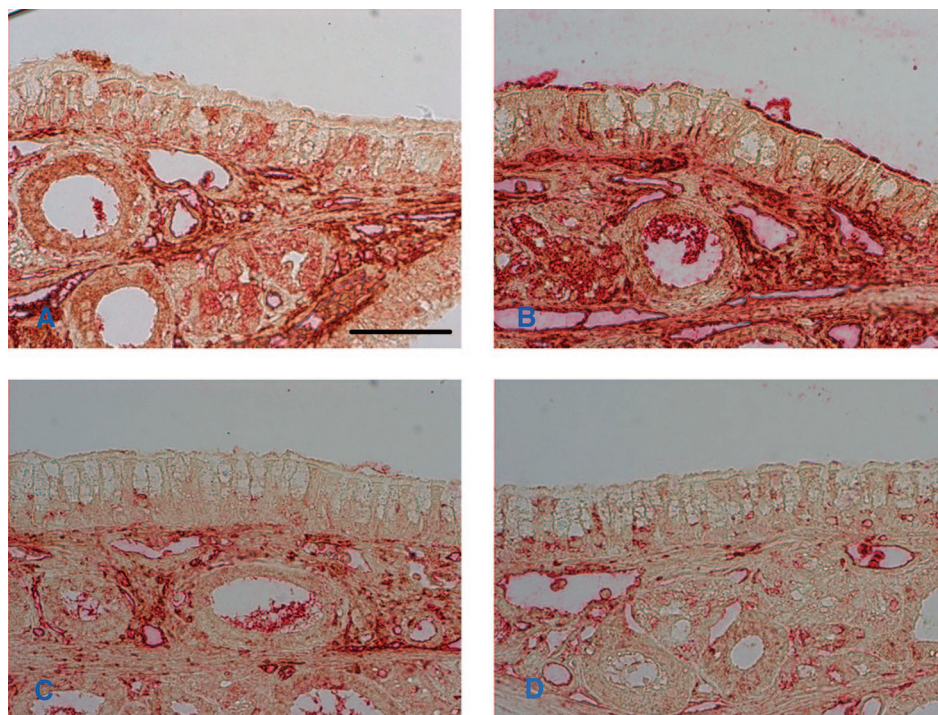


Fig. 4. Double immunohistochemistry of nasal mucosa for transforming growth factor (TGF)- β 1 and CD4. TGF- β 1-positive cells (brown) and CD4-positive cells (pink) are found in both basal and subepithelial layers, where inflammatory cells infiltrate. Immunoreactivity for both TGF- β 1 and CD4 is more prominent in the group which received general and local sensitization (B) than that which received general sensitization only (A). In dexamethasone-treated groups (C and D), immunoreactivity for both TGF- β 1 and CD4 was lower than that in control groups (A and B). Scale bar = 50 mm.

maintenance of tolerance through the action of TGF- β .¹⁴ Also CD4⁺ T cells are reported to play an important role in the induction of nasal allergy and occurrence of late allergic reaction.¹⁵ In this study we noted that immunoreactivity for CD4 in the nasal mucosa of mice treated with dexamethasone decreased compared with control mice and this was more prominent in mice treated before local challenge. Decreased immunoreactivity for CD4 in the nasal mucosa of mice treated with dexamethasone suggests that CD4⁺ T cells in the nasal mucosa may be mainly effector T cells rather than regulatory T cells.

This study suggests that dexamethasone may play an important role in the regulation of allergic reaction by the following mechanisms. When administered at the beginning of sensitization, dexamethasone can inhibit induction of nasal allergy through suppression of CD4⁺ T cells and activation of TGF- β . As regulatory T cells with eosinophils are the main source of TGF- β , increased serum level of TGF- β suggests that regulatory T cells may participate in the suppression of differentiation and proliferation of CD4⁺ T cells. When administered before local challenge in sensitized mice, dexamethasone can suppress late allergic reaction through suppression of CD4⁺ T cells and inhibition of proliferation and chemotaxis of eosinophils. Decreased levels of TGF- β in the nasal mucosa of dexamethasone treatment groups compared with the control group may be due to the suppression of proliferation and chemotaxis of eosinophils, which are also a main source of TGF- β .

CONCLUSION

This study suggests that dexamethasone can inhibit the induction of nasal allergy or late allergic reaction

according to the time of its administration. Also this study suggests that the mechanisms of its actions on nasal allergy may be direct or indirect and that its indirect action may be through activation of TGF- β and possibly regulatory T cells, and suppression of CD4⁺ T cells and eosinophils.

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