의학석사 학위논문

Inhibition of allergic response by intranasal topical selective NF-κB inhibitor in murine model of allergic rhinitis

알레르기비염 마우스 모델에서 비강 내 국소 NF-κB 차단제 투여를 통한 알레르기 반응의 억제에 대한 연구

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College of Medicine

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ABSTRACT

Inhibition of allergic response by intranasal topical selective NF-κB inhibitor in murine model of allergic rhinitis

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Background: Study of murine asthma model revealed that specific local nuclear factor-kappa B (NF-kB) inhibition achieved using NF-kB decoy oligodeoxynucleotides (ODNs) had therapeutic potential in the control of pulmonary allergy. However, the questions of whether local inhibiting NF-kB could have therapeutic value in the treatment of allergic rhinitis (AR). To address these issues, we have assessed the effect of selective NF-κB inhibition using NF-κB decoy ODNs for local treatment of AR in ovalbumin (OVA)-sensitized wild-type mice.

Materials and methods: BALB/c mice were sensitized with OVA and alum and then challenged intranasally with OVA. NF-kB inhibitor, NF-kB decoy ODNs was given intranasally to the treatment group and NF-kB scrambled ODNs to the sham treatment group. Allergic symptom scores, eosinophil infiltration, the levels of cytokines in the nasal mucosa, nasal lavage fluid, and spleen cell culture, serum total and OVA-specific immunoglobulins, and intercellular adhesion molecure-1 (ICAM-1) in the nasal mucosa were analyzed.

Results: NF-κB decoy ODNs reduced allergic symptoms and eosinophil infiltration in the nasal mucosa. It also suppressed serum total IgE level and

OVA-specific IgG1. The levels of inflammatory cytokine including IL-1β, IL-6 and TNF-α decreased and the expression of ICAM-1 was suppressed in the nasal mucosa of the treatment group when compared to the positive control and sham treatment groups. Furthermore, NF-κB decoy ODNs significantly reduced expression of systemic Th2 cytokine, IL-4 and IL-5 in the spleen cell culture.

Conclusions: This study demonstrates for the first time that local NF-κB inhibition using NF-κB decoy ODNs has a therapeutic potential of in the control of AR.

Key words: Allergic rhinitis, Anti-allergic agents, NF-kappaB, NF-kappaB decoy, Oligodeoxyribonucleotides, Immunoglobulin E.

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LIST OF ABBREVIATIONS

NF-κB: Nuclear factor-kappa B

ODNs: Oligodeoxynucleotides

AR: Allergic rhinitis

OVA: Ovalbumin

PBS: Phosphate-buffered saline

mRNA: messenger RNA

cDNA: complementary DNA

PCR: Polymerase chain reaction

IL: Interleukin

TNF-α: Tumor necrosis factor-α

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase

ELISA: Enzyme-linked immunosorbent assay

FBS: Fetal bovine serum

IFN-γ: Interferon-gamma

Igs: Immunoglobulins

mAb: Monoclonal antibody

PBST: Phosphate-buffered saline containing 0.05% Tween-20

BSA: Bovine serum albumin

HRP: Horseradish peroxidase

TMB: Tetramethylbenzidine

OD: Optical density

ICAM-1: Intercellular adhesion molecule-1

SEM: standard error mean

INTRODUCTION

Nuclear factor-kappa B (NF-κB) is a multicellular transcription factor, and it plays an important role in inflammatory and immune responses by regulating immune and inflammation-related cytokine and inflammatory mediator genes (1). In addition, the NF-κB pathway is involved in proliferation and pathogenesis of tumors by regulation of cell proliferation, control of apoptosis, promotion of angiogenesis, and stimulation of invasion/metastasis (2). Because of its pivotal role in inflammation and cell proliferation, several studies have been reported to get therapeutic approaches by inhibiting NF-κB in several human diseases.

Transfection of decoy oligodeoxynucleotides (ODNs) has been reported to be a therapeutic strategy by inhibition of NF-κB activation (3). NF-κB decoy ODNs bind the transcription factor and thereby interfere with binding of NF-κB to promotor regions in genes and reduce expression of its target genes (4). Topical application of NF-κB decoy ODNs induces antigen-specific peripheral tolerance in delayed-type hypersensitivity to OVA (5).

Study of murine asthma model revealed that specific local NF-κB inhibition achieved using NF-κB decoy ODNs is associated with strong attenuation of allergic lung inflammation, airway hyper-responsiveness, and local production of mucus, and cytokine expression and had therapeutic potential in the control of pulmonary allergy (6). However, information regarding the association of local NF-κB inhibition with allergic inflammation in AR is limited, although it has been demonstrated in humans and animal models that NF-κB is up-regulated in the nasal mucosa of allergic rhinitis (AR) (7-9).

The present study aimed to evaluate the effect of selective NF-κB inhibition using NF-κB decoy ODNs for local treatment of allergic rhinitis in OVA-sensitized wild-type mice.

MATERIALS & METHODS

Animals

Four-week-old female BALB/c mice were used as the experimental animals. Each mouse weighted 20 to 30g and was maintained under specific pathogen-free conditions. All animal experiments in the present study followed the guidelines and ethics of the Institutional Animal Care and Use Committee of the Biomedical Research Institute of Seoul National University Hospital, and was approved (IACUC number: 12-0098 & 13-0088).

NF-κB decoy and scrambled ODNs

Double-stranded NF-κB decoy and scrambled ODNs were synthesized by Cosmo GENETECH (Seoul, Korea). Double-stranded NF-κB decoy ODNs containing the consensual NF-κB binding site (GGGATTTCCC) were generated using equimolar amounts of single-stranded sense and antisense phosphorothioate-modified ODNs (sense strand: 5'-CCT TGA AGG GAT TTC CCT CC-3'). Double-stranded NF-κB scrambled ODNs were used as controls (sense strand: 5'-TTG CCG TAC CTG ACT TAG CC-3').

Induction of the murine model of allergic rhinitis and treatment

Mice were divided into 4 groups (Group A: negative control group, Group B: positive control group, Group C: NF-κB decoy ODN treatment group, Group D: NF-κB scrambled ODN sham treatment group). Allergen sensitization, challenge for the development of the AR murine model and treatment were summarized in Fig. 1.

Briefly, the mice were sensitized by intraperitoneal injection with 25 μg of ovalbumin (OVA; grade V; Sigma, St. Louis, MO) complexed with 2 mg of aluminum hydroxide (alum) on days 0, 7, and 14. The mice were then subjected to an intranasal challenge with 100 μg of OVA on 7 consecutive days from day 20 to day 26. The negative control mice were intraperitoneally injected and intranasally challenged with phosphate-buffered saline (PBS) instead of OVA on the same schedule. NF-κB decoy and scrambled ONDs were given by intranasal instillation (15 nmol in 30 μl of TE buffer/mouse) on days 20, 22, 24 and 26 (6 hour before intranasal OVA challenge) to mice of group C and D respectively.

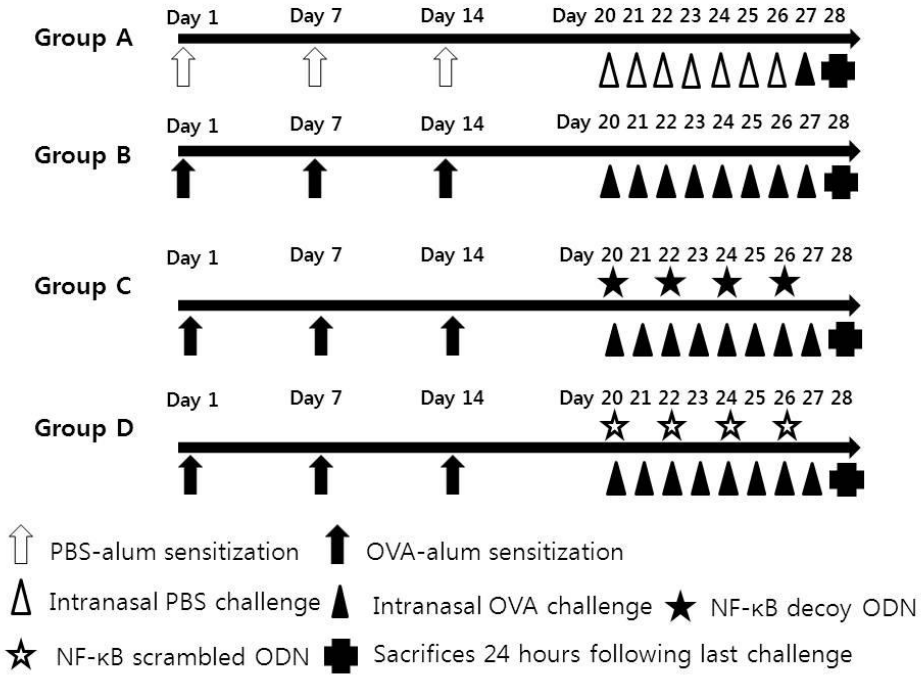


Figure 1. Experimental protocol. BALB/c mice were sensitized with intraperitoneal injection of ovalbumine (OVA) and alum on days 0, 7, 14. Intranasal challenges were administered via daily OVA instillation from days 20 to 26. The control mice

(group A) were intraperitoneally injected and intranasally challenged with phosphate-buffered saline (PBS) instead of OVA on the same schedule. In addition to sensitization and challenge, mice of group C were treated with nasal instillation of the NF-κB decoy oligodeoxynucleotides (ODNs) and that of group D were sham treated with NF-κB scrambled ODNs on days 20, 22, 24, 26 (6 hour before nasal challenge). On day 27, after intranasal allergen provocation with 100 μg of OVA, the frequencies of sneezing and nasal rubbing behaviors were recorded during 15 minutes. Mice were killed 24 hours after the final OVA challenge.

Symptom scores

On day 27, after intranasal allergen provocation with 100 μg of OVA, the frequencies of sneezing and nasal rubbing behaviors were recorded during a 15-minute period to evaluate early allergic responses by blinded observers. Mice were then killed 24 hours after the last OVA challenge, and nasal tissues/cells and nasal lavage fluid were obtained for analysis.

Evaluation of histologic findings in the nasal mucosa

For evaluation of nasal histology, heads of mice were fixed with 10% formaldehyde solution. The nasal tissues were decalcified with hydrochloric acid, embedded in paraffin, sectioned coronally into 4 μm slices, and stained with hematoxylin-eosin for inflammatory cells and Sirius red for eosinophils. Under a light microscope (x400 magnification), infiltrating eosinophils were counted in 4 fields of the nasal septal mucosa by a single blinded observer. Eosinophils were defined morphologically by the existence of eosinophilic granules stained by Sirius red in the cytoplasm and the presence of a 2-lobed nucleus.

Messenger RNA (mRNA) for cytokines in the nasal mucosa

After exposing the nasal cavity out of the head of mice, the nasal mucosa was taken out meticulously using a curette. Total RNA was prepared from the nasal mucosa with TriZol reagent (Invitrogen, Carlsbad, CA). Complementary DNA (cDNA) was synthesized using with SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA) and oligo(dT) primers (Fermentas, Burlington, ON, Canada). The sequences of the specific polymerase chain reaction (PCR) primers (Table 1) used for reverse transcription PCR analysis of interleukin (IL)-1β, IL-6, tumor necrosis factor-α (TNF-α) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). PCR cycling conditions were as follows: 94℃ and 56℃ (IL-1β, IL-6, GAPDH) or 58℃ (TNF-α) followed by extension at 72℃ (30 cycles for IL-6 and TNF-α; 26 cycles for IL-1β, GAPDH). The PCR products were separated on 1.2% agarose gels and stained with ethidium bromide. Band intensity of PCR products was analyzed by densitometry using the TINA 2.0 software from the National Institutes of Health.

Primer	Sequence Size	Size, bp
IL-1β	5’-ATA ACC TGC TGG TGT GTG AC-3’ 5’-TGC AGA CTC AAA CTC CAC TT-3’	365
IL-6	5’-TTG TGC AAT GGC AAT TCT-3’ 5’-AGA GCA TTG GAA ATT GGG-3’	296
TNF-α	5’-ACA CCG TCA GCC GAT TTG C-3’ 5’-CCC TGA GCC ATA ATC CCC TT-3’	373
GAPDH	5’-ACC ACA GTC CAT GCC ATC AC-3’ 5’-TCC ACC ACC CTG TTG CTG TA-3’	451

Table 1. Sequence of primers for reverse transcription–polymerase chain reaction.

Measurement of cytokines in nasal lavage fluid

A 24-gauge catheter was inserted into the nasopharynx through the tracheal opening at the time of killing. The nasal passages were gently perfused twice with 200μl of PBS from the choana to the nostril, and nasal lavage fluid was collected. The lavage fluid was centrifuged and supernatants were frozen at -70℃ until analysis. The expression of IL-6 was measured in the nasal lavage fluid by enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN). The sensitivity of IL-6 concentration that was measurable through this method was 1.6 pg/mL.

Measurement of cytokines in the spleen cell culture

Spleen single-cell suspensions were plated in 24-well cell culture plates at a final concentration of 5 x 106 cells/mL using RPMI 1640 containing 10% fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 ug/ml streptomycin (Gibco, Grand Island, NY). The cells were incubated in a CO2 incubator at 37℃ for 72 h and stimulated with OVA for 72 hours. The culture supernatant was collected and stored at -70℃ until cytokines were measured. Cytokines were assayed in culture supernatant using a sandwich ELISA kit (R&D Systems, Minneapolis, MN), according to the manufacturers' protocol. After measuring the OD at 450 nm, the concentrations of IL-4, IL-5, IL-10, and interferon-gamma (IFN-γ) were determined by interpolation from a standard curve, and all data were expressed in pg/ml.

Determination of serum levels of total and OVA-specific immunoglobulins (Igs)

Serum samples from mice were obtained at the time of killing. Serum levels of

total IgE and OVA-specific IgE, IgG1, and IgG2a were measured by ELISA. For the analysis of total IgE, 96-well flat-bottom plates were coated overnight with anti-mouse IgE monoclonal antibody (mAb) (BD PharMingen, San Jose, CA) at 4℃. The plate was washed with PBST (PBS containing 0.05% Tween-20) 3 times and nonspecific antigen-antibody reactions were blocked with 300 μL of 3% bovine serum albumin (BSA) per well for 1 hour at room temperature. Serum samples added to the 96-well plates and purified mouse IgE isotype (BD PharMingen, San Jose, CA) used as a standard was added, and the plate was incubated for 3 hours at 4℃. For the analysis of OVA-specific IgE, 96-well, flat-bottom plates were coated with OVA (100 μg/mL in coating buffer (0.05M carbonate-bicarbonate) overnight at 4℃. The plate washed 3 times with PBST, and blocked with 3% BSA in PBS for 1 hour at 37℃. Serum samples were added to OVA-coated plates along with serial dilutions of a high titer OVA IgE standard and incubated for 2 hours at 37℃. After washing, 100 μL of biotin-conjugated rat anti–mouse IgE mAb (BD PharMingen, San Jose, CA) was added to each well and incubated for 1 hour at 37℃. For the analysis of OVA-specific IgG1 and IgG2a, 96-well plates were coated with 100 μg/mL OVA in coating buffer overnight at 4°C. Serially diluted serum samples were incubated with biotinylated rat anti-mouse IgG1 and IgG2a (BD PharMingen, San Jose, CA), respectively. After washing 3 times, the plates were then incubated with 100 μL of horseradish peroxidase (HRP)-conjugated secondary Ab (BD PharMingen, San Jose, CA) for 30 minutes at 37℃. The reactions were developed using 3,3’,5,5’-tetramethylbenzidine (TMB) (Moss Inc., Belfast, ME) and terminated by adding 2N H2SO4. Optical density (OD) was measured in a microplate reader at 450 nm.

Western blot for intercellular adhesion modecule-1 (ICAM-1) in the nasal mucosa

Protein was obtained from the nasal mucosa of each mouse 24 h after the final nasal challenge using commercial lysing buffer (TransAM kit, Active Motif, Carlsbad, CA). Protein concentrations were determined using BCA protein assay reagent (Thermo Fisher Scientific, Waltham, MA). Samples (40 ug protein per lane) were separated on Nupage 4%-12% Bis-tris mini gels (NOVEX, San Diego, CA) and transferred onto polyvinylidene fluoride membranes (Amersham Biosciences, Piscataway, NJ). ICAM-1 and β-actin were immunoblotted with a primary goat polyclonal anti-ICAM-1 Ab (R&D Systems, Minneapolis, MN) and anti- β-actin Ab (Cell signaling, Danvers, MA). The membrane was then immunoblotted with a secondary anti-goat IgG-HRP, or secondary anti-rabbit IgG-HRP (Santa Cruz Biotechnology, Santa Cruz, CA). The blots were visualized using SuperSignal West Pico Chemiluminescent Substrate (Pierce, Rockford, IL). Quantification of Western blots was performed using the TINA 2.0 software from the National Institutes of Health.

Statistical analysis

The data was presented as means ± standard error mean (SEM). A Mann-Whitney U-test was used to compare results between negative and positive controls, and treatment groups and positive control. A P-value < .05 was considered statistically significant. Statistical analysis was performed using SPSS 18.0 software (SPSS Inc, Chicago, IL).

RESULTS

Symptom scores (Fig. 2)

After the nasal challenge of OVA, mice of group B (positive control) sneezed (*P*=.002) and rubbed (*P*=.001) their noses significantly more frequently than mice of group A (negative control) did. The sneezing (*P*=.002) and nasal rubbing scores (*P*=.004) in the group C (NF-κB decoy ODN treatment group) was significantly lower when compared to the group B. Group D (NF-κB scrambled ODN sham treatment group) showed no significant difference in the sneezing (*P*=.207) and nasal rubbing scores (*P*=.526) compared to group B.

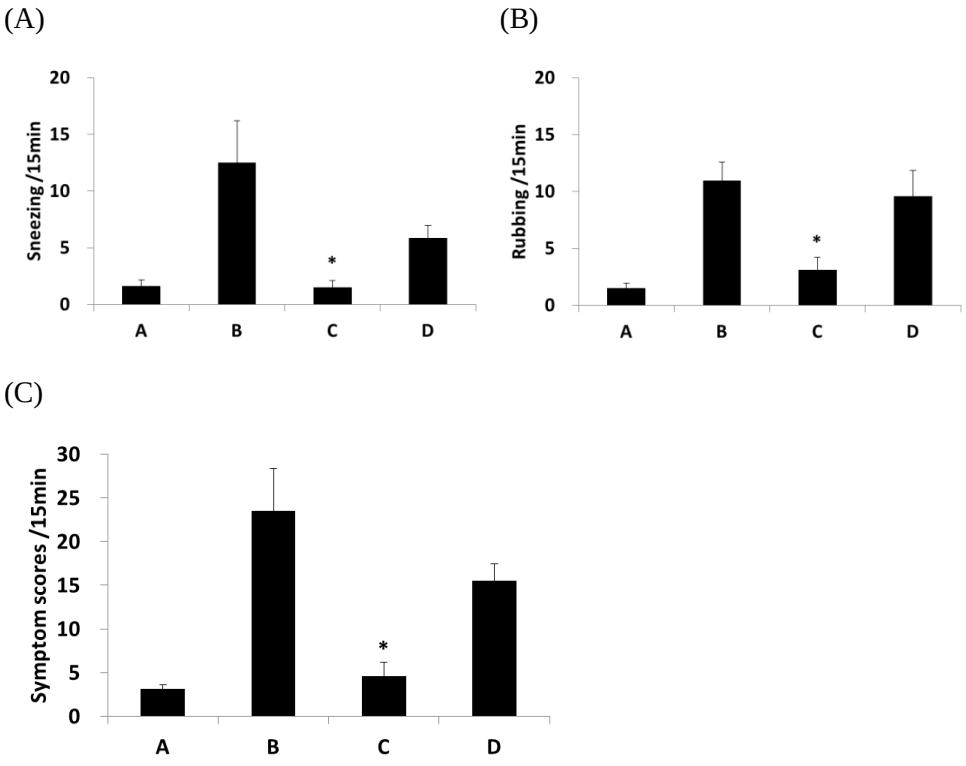
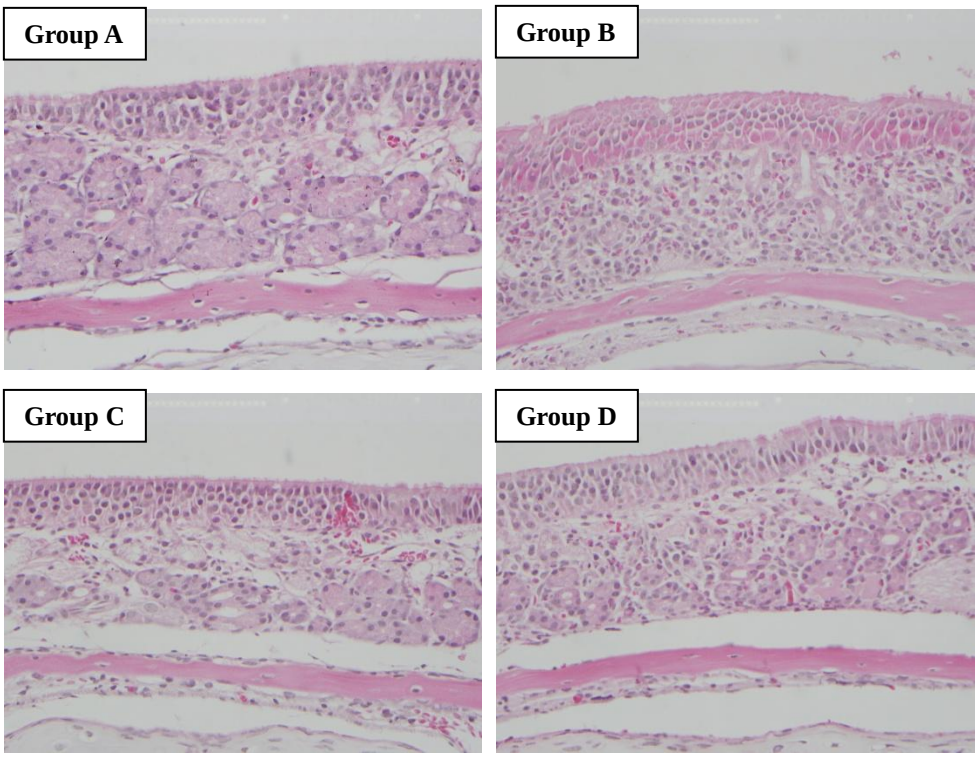


Figure 2. Symptom scores. Local treatment of NF-κB inhibitor suppressed allergic symptoms. (A) Sneezing symptom scores. (B) Nasal rubbing symptom scores. (C) Total symptom scores. * $P < .05$.

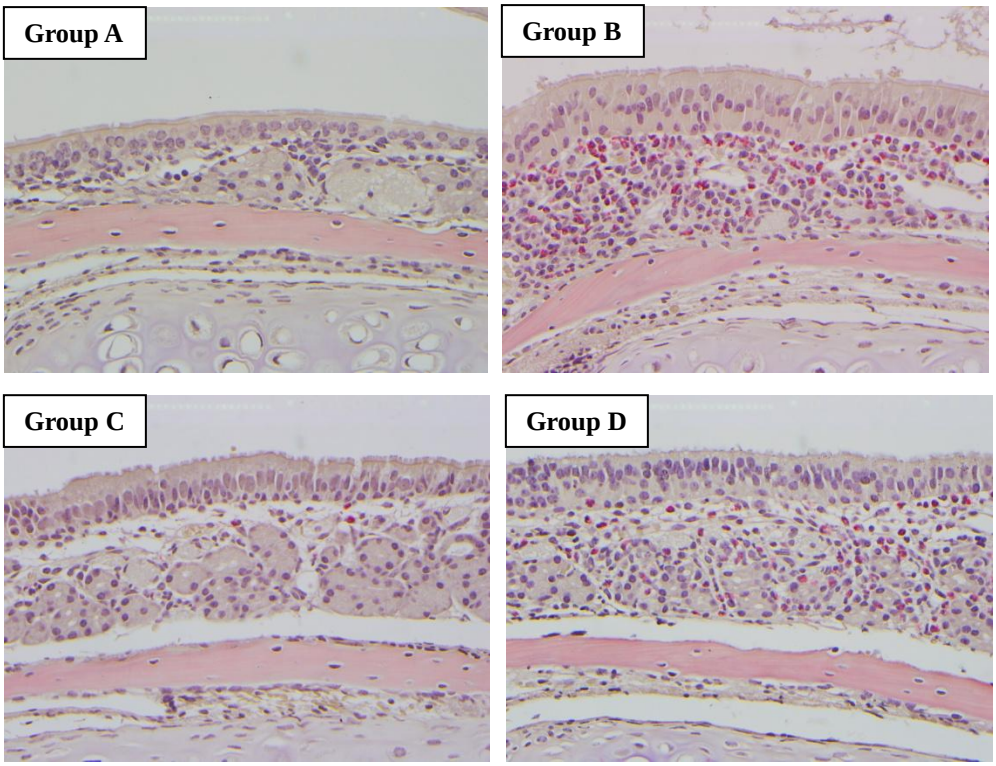
Histologic findings and eosinophil infiltration in nasal mucosa

Figure 3 shows histologic findings and eosinophil infiltration in nasal mucosa of each group. The mice of group B had more severe eosinophil infiltration than the mice of group A (*P*=.05). The numbers of eosinophil infiltrating the nasal mucosa per high-magnification field were lower in group C (*P*=.05), but not in group D (*P*=.127), when compared to the group B. These results show that the local NF-κB inhibition using NF-κB decoy ODN decreased eosinophil migration in the nasal mucosa. By contrast, treatment with scrambled ODNs had no significant effect on eosinophil influx.

(A)



(B)



(C)

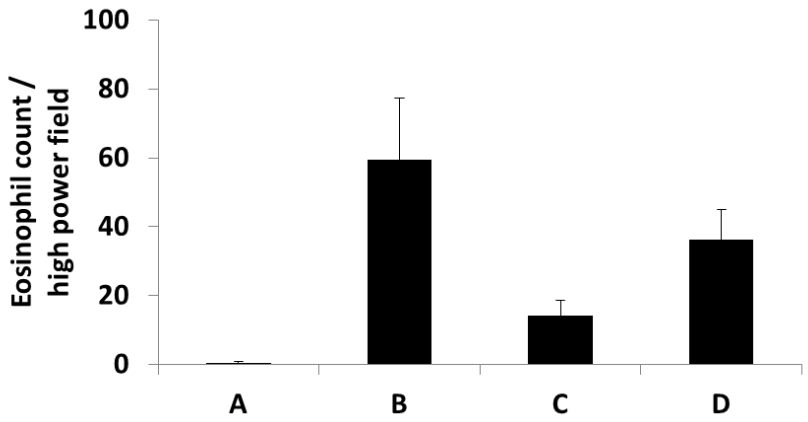
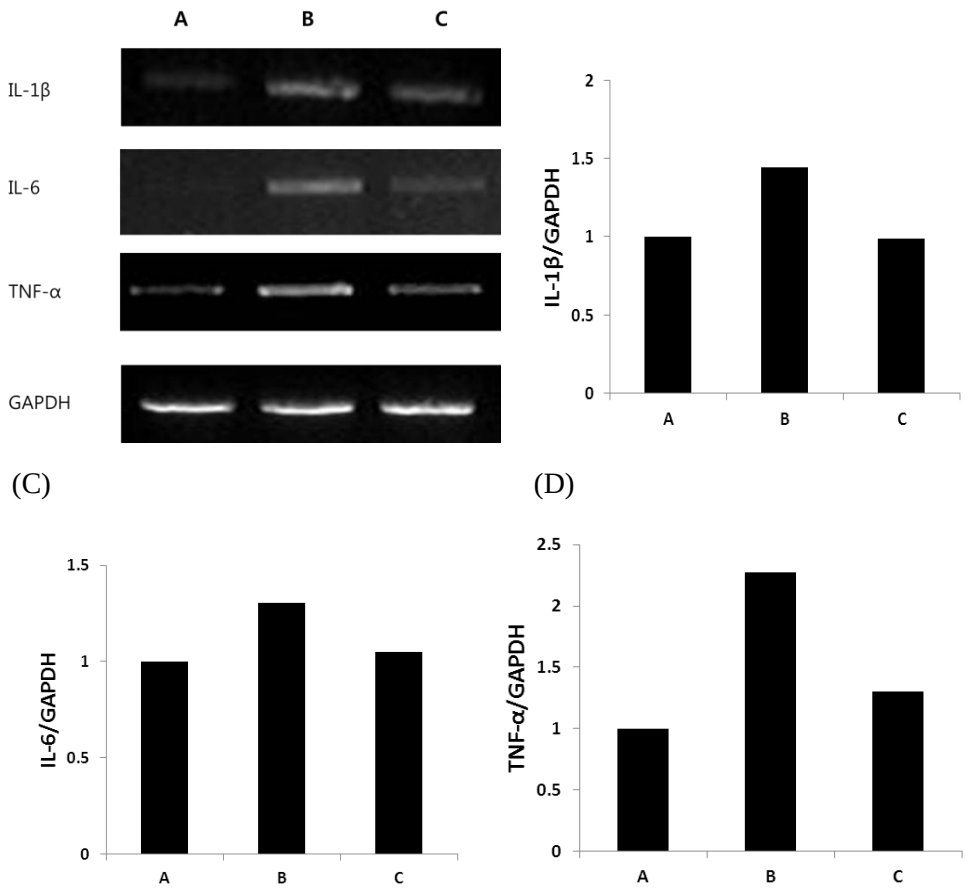


Figure 3. Local treatment of NF-κB inhibitor suppressed eosinophil infiltration in the nasal mucosa. (A) H&E staining (x400 magnification), (B) Sirius red staining (x400 magnification), (C) Eosinophil count of the septal mucosa of each group.

Cytokines in nasal mucosa, nasal lavage fluid and spleen cell culture

Levels of inflammatory cytokine including IL-1β, IL-6 and TNF-α decreased in the nasal mucosa of treatment group compare to positive control group (Fig. 4). In addition, IL-6 significantly decreased in the nasal lavage fluid of treatment group (*P*=.027, Fig. 5). Systemic Th2 cytokines (IL-4, *P*=.014; IL-5, *P*=.049) significantly decreased in the treatment group C compared to the positive control group B (Fig. 6A, B). However, systemic regulatory cytokine IL-10 (*P*=.286) and Th1 cytokine IFN-γ (*P*=.394) and did not change in the treatment group (Fig. 6C, D).

(A) (B)



(C) (D)

Figure 4. Expression of IL-1β (B), IL-6 (C) and TNF-α (D) in the nasal mucosa by reverse transcription-PCR. Transcriptional levels of IL-1β, IL-6 and TNF-α decreased in the treatment group C compared to the positive control group B.

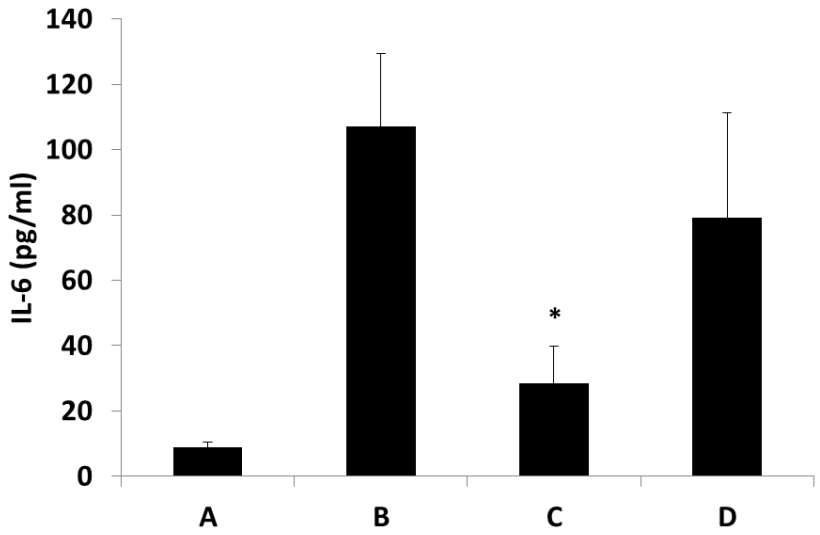
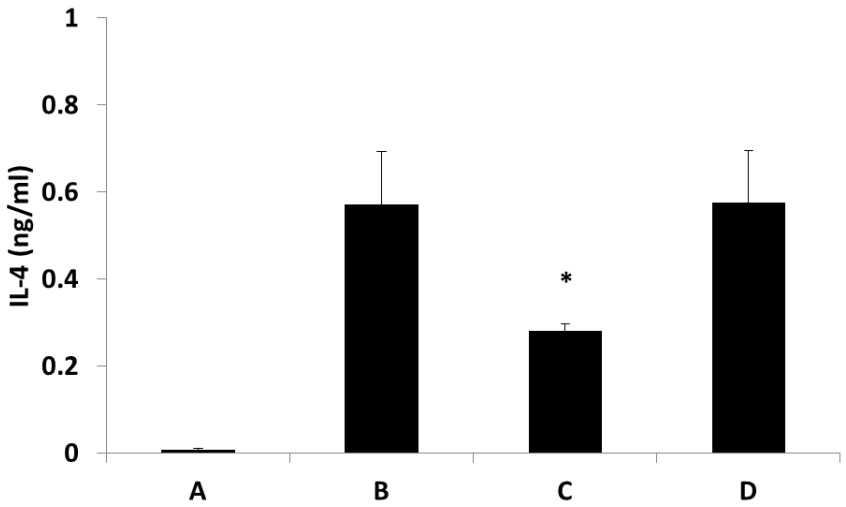
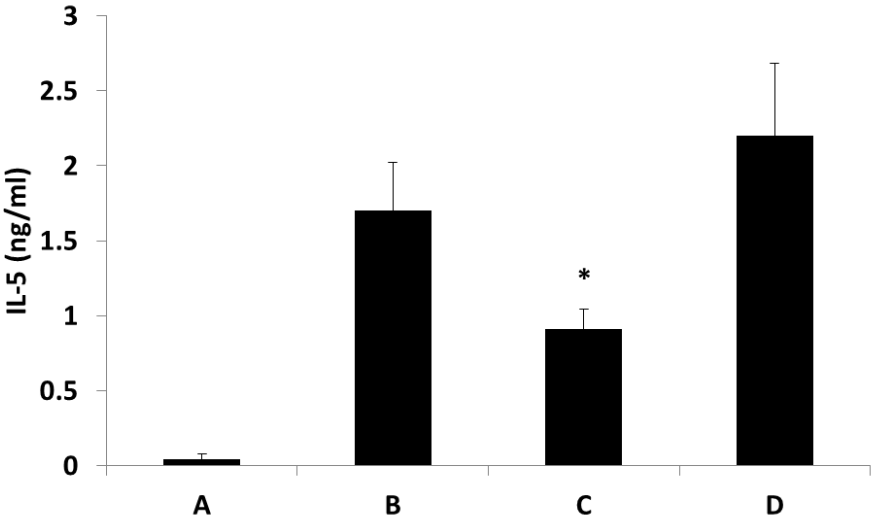


Figure 5. Concentration of cytokine IL-6 in nasal lavage fluid by ELISA. * $P < .05$.

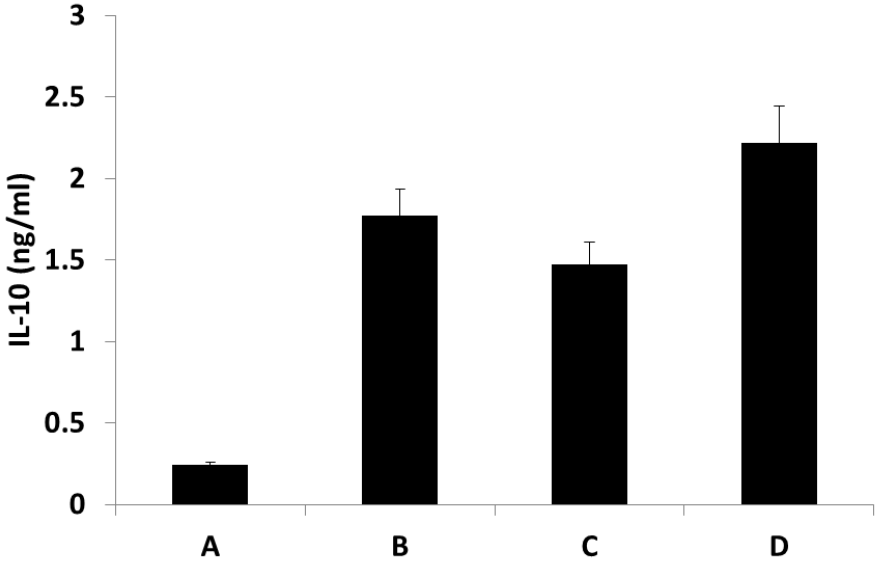
(A)



(B)



(C)



(D)

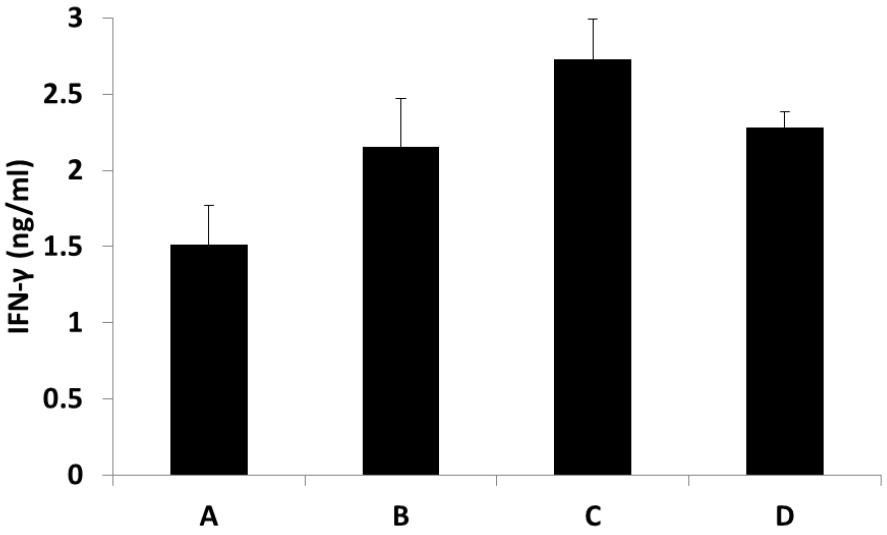
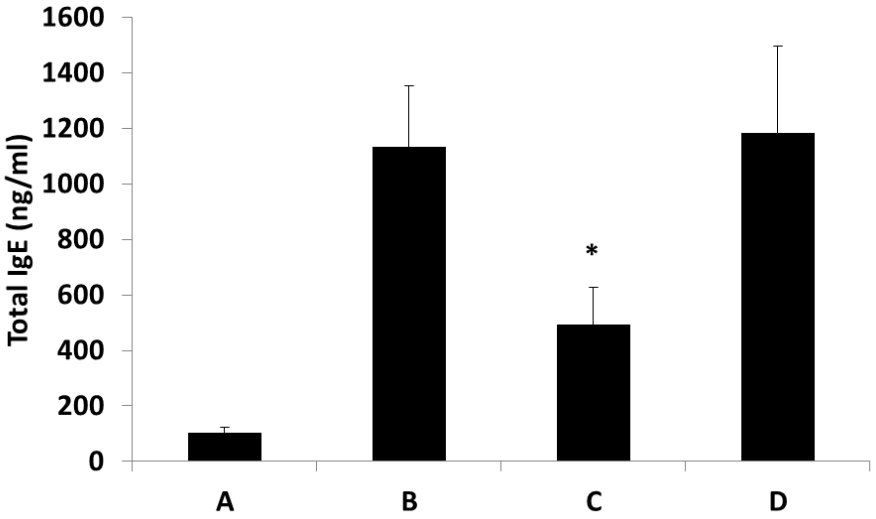


Figure 6. Concentration of cytokines in spleen cell culture by ELISA. (A) IL-4, (B) IL-5, (C) IL-10, (D) IFN-γ. Systemic Th2 cytokines (IL-4, IL-5) significantly decreased in the treatment group C compared to the positive control group B. However, systemic regulatory cytokine IL-10 and Th1 cytokine IFN-γ did not change in the treatment group. * $P < .05$

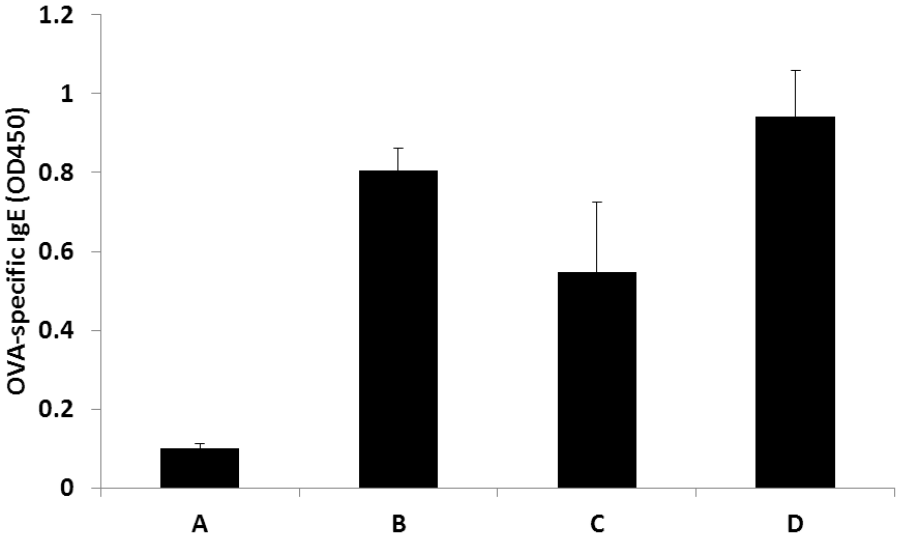
Serum total and OVA-specific Igs

The serum level of total IgE (P=.045, Fig. 7A) and OVA-specific IgG1, Th2-related Ig (P=.045, Fig. 7C) in group C significantly decreased when compared with group B and D. In addition, the level of serum OVA-specific IgE was lower in group C than in group B and D (Fig. 7B). The level of OVA-specific IgG2a, Th1-related Ig was higher in group C than in group B and D (Fig. 7D). However, these were not statistically significant.

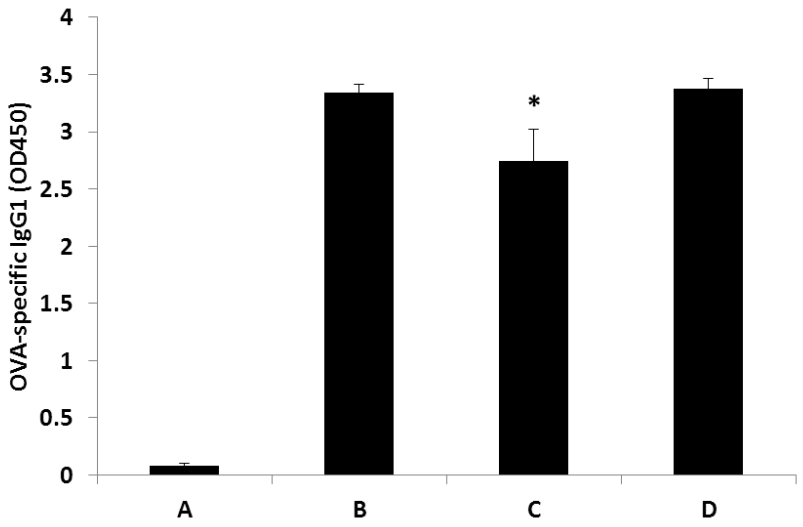
(A)


Total IgE (ng/ml)
1600
1400
1200
1000
800
600
400
200
0
*
A
B
C
D

(B)


OVA-specific IgE (OD450)
1.2
1
0.8
0.6
0.4
0.2
0
A
B
C
D

(C)



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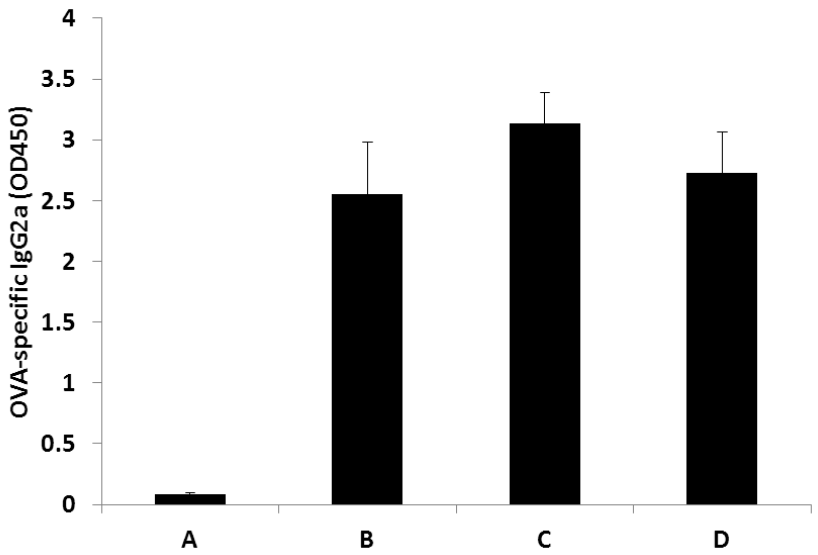
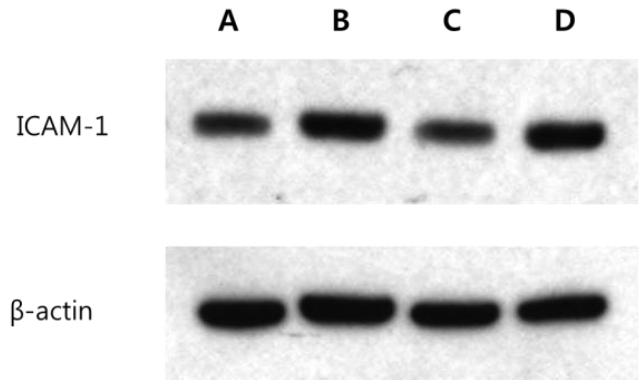


Figure 7. Serum immunoglobulin levels in each group. Local treatment with NF-κB decoy ODNs significantly suppressed serum total IgE and OVA-specific IgG1 levels (A, C). The serum level of OVA-specific IgE (B) was decreased and that of OVA-specific IgG2a (D) was increased, but it is not significant. * $P < .05$

ICAM-1 in the nasal mucosa

The expression level of ICAM-1 mRNA in nasal mucosa decreased in NF-κB decoy ODNs treatment group when compared to positive control group and was not decreased in sham treatment group (Fig. 8).

(A)



(B)

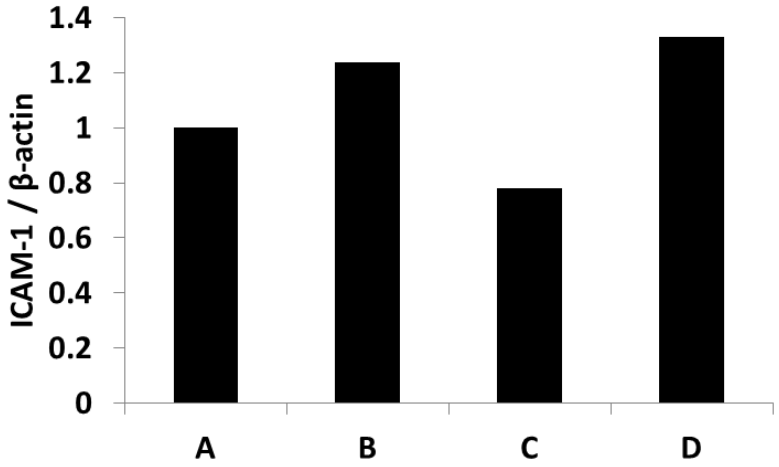


Figure 8. Local treatment with NF-κB decoy ODNs reduced the expression of ICAM-1 in the nasal mucosa by Western blot. (A) Immunoblot of ICAM-1 and β-actin (B) Quantitative expression level of ICAM-1 in the nasal mucosa.

DISCUSSION

The transcription factor NF-κB plays an important role in a wide range of inflammatory diseases (10). NF-κB not only induce the transcription of several cytokines [e.g. TNF-α, IL-1β and IL-6] and chemokines [e.g. IL-8, macrophage inflammatory protein-1a, methyl-accepting chemotaxis protein 1 and eotaxin], but also regulates the expression of adhesion molecules [e.g. E-selectin, vascular cell adhesion molecule-1 and ICAM-1] (11). In addition, a series of studies have indicated that NF-κB expression and activity are involved in proliferation and pathogenesis of tumors (12). Because of its important role in inflammation and cell proliferation, much effort has been put in the development of therapeutics achieved by inhibiting NF-κB and regulating the expression of the target genes.

Decoy ODN has been explored as tools for manipulating transcription factors. When a decoy ODN containing the consensus sequence of a specific transcription factor is introduced into the cells, the presence of high levels of the decoy ODN will compete with the endogenous gene targets for binding to the transcription factor, which will lead to alteration in transcription of the target genes (13). Several studies have been shown that NF-κB decoy ODNs suppress the transcriptional activity of NF-κB by specifically blocking its binding sites to the NF-κB sequence resulting in the inhibition of endogenous gene promoter. Fang et al. presented that NF-κB decoy ODNs suppressed proliferation and induced the apoptosis of androgen-independent prostate cancer by local NF-κB inhibition (14). Isomura et al. reported that NF-κB decoy ODNs suppressed OVA-induced delayed-type hypersensitivity by suppressing migration and maturation of dendritic cell, which is associated role of activated NF-κB (15).

Allergic rhinitis and asthma are not only closely related in pathophysiology and

immunopathology, but have common treatment, showing that the upper and lower respiratory tract inflammatory response is consistent (16). Various therapeutic strategies targeted at the NF-κB pathway have been documented in experimental asthma models. Desmet et al. reported that intratracheal administration of NF-κB decoy ODNs leads to NF-κB inhibition, which has therapeutic potential in the treatment of asthma (6). Bao et al. documented a potential therapeutic value of andrographolide in the treatment of asthma by inhibiting the NF-κB inhibition (17). However, therapeutic challenge by NF-κB inhibition has not yet been established in AR. To our knowledge, the current study first showed the inhibition of allergic response by using local NF-κB inhibitor, NF-κB decoy ODNs, in the murine AR model.

NF-κB is widely found in the cytoplasm of a variety of cells in non-active state. NF-κB is activated by multiple stimuli including allergens and transferred into the nucleus, and then combined with the specific DNA sequences of certain related gene promoter or enhancer to regulate and control a variety of gene expression (12). The main activated form of NF-κB is a heterodimer, consisting of a p50 or p52 subunit and the trans-activating subunit p65. It had been reported that NF-κB is activated, resulting in the nucleus localization of NF-κB p65 and secretion of inflammatory cytokines, such as IL-1β, IL-6 and TNF-α, are increased (8, 18). Furthermore, several of these gene products, such as IL-1β and TNF-α, also directly activate the NF-κB pathway to amplify and increase the inflammatory response (11). In present study, mRNA expression of inflammatory cytokines including IL-1β, IL-6 and TNF-α decreased in the nasal mucosa of NF-κB decoy ODNs treatment group, suggesting decreased inflammatory cytokine expression by NF-κB inhibition as a key mechanism for its anti-allergic effect.

NF-κB decoy ODNs significantly decreased the allergic symptom scores and Th2

cytokine IL-4 and IL-5 in spleen cell culture in the treatment group. In addition, serum total and OVA-specific IgE were decreased. IL-4 is well known to be an important cytokine not only for the Th2 phenotype but also for IgE production (19). These findings are consistent with our results in NF-κB decoy ODNs inhibited IL-4 secretion, resulting in a decreased serum total and OVA-specific IgE. OVA-specific IgG1 has been used as a Th2 marker and has a correlation with total and OVA-specific IgE levels and (20). IFN-γ, a Th1 cytokine, enhances IgG2a secretion (21). OVA-specific IgG1 significantly decreased and OVA-specific IgG2a increased, but no significantly, in treatment group, demonstrating that local NF-κB inhibition decreased Th2 immunity and increased Th1 immunity.

The present study showed that the expression level of ICAM-1 mRNA and eosinophil infiltration in nasal mucosa decreased in NF-κB decoy ODNs treatment group when compared to positive control group. ICAM-1 plays an important role in local inflammation and eosinophil infiltration, and gene expression is regulated by NF-κB (22). Wang et al. showed the expression of NF-κB p50 and p65 corresponding to expression of ICAM-1 mRNA have a significant positive correlation and the NF-κB played an important role for ICAM-1 transcription in the nasal mucosa of AR patients (7). The observed decreased expression of ICAM-1 mRNA might suggest that local inhibition of NF-κB leads to the transcription decrease of ICAM-1 and prevents adhesion of eosinophils on endothelial cells, thus inhibiting eosinophil infiltration in the nasal mucosa.

In this study, intranasal instillation of NF-κB decoy ODNs suppressed both local and systemic allergic response. Morishita et al. showed that systemic suppression of NF-κB is likely to be harmful, given that knockout mice for various NF-κB signaling components suffer from immune deficiency or lack lymphocyte activation (23). In addition, Desmet et al. presented that local delivery of NF-κB decoy ODNs

inhibits NF-κB activity not totally but partially, as assessed by supershift assay (6). Therefore, we selected intranasal instillation instead of intraperitoneal injection as a treatment method for prevention of systemic adverse effects. We reported that local NF-κB inhibition is associated with strong attenuation of allergic inflammation such as cytokine expression and eosinophilic inflammation. However, for development of new therapeutic agents, additional studies about systemic side effects need to be clarified further.

CONCLUSIONS

We demonstrated that local NF-κB inhibition using NF-κB decoy ODNs suppressed the allergic response in murine AR model. To the best of our knowledge, this is the first report showing a therapeutic potential of local NF-κB inhibition in the control of AR.

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초 록

알레르기비염 마우스 모델에서 비강 내 국소 NF-κB 차단제 투여를 통한 알레르기 반응의 억제에 대한 연구

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서론: 천식 마우스 모델에서 국소 NF-κB 유인체 올리고디옥시핵산 투여를 통해 NF-κB를 차단함으로써 알레르기 반응을 조절함에 있어 치료적 가능성에 대해 제시된 경우는 많다. 하지만 알레르기비염 치료에서 국소 NF-κB 차단제가 치료적 효과가 있을지에 대해서는 알려져 있지 않다. 이에 본 연구에서는 난알부민으로 유도된 알레르기비염 마우스 모델에서 국소 NF-κB 유인체 올리고디옥시핵산 투여를 통한 NF-κB 차단의 알레르기비염에 대한 치료 효과에 대해 알아보고자 하였다.

방법: BALB/c 마우스에서 난알부민으로 감작된 알레르기비염 모델을 제작한 후 치료군에는 NF-κB 차단제인 NF-κB 유인체 올리고디옥시핵산을 비강내 점적하였고, 치료 대조군에는 NF-κB 스크램블된 올리고디옥시핵산을 점적하였다. 알레르기비염 증상, 비강

조직 내 호산구 침윤 정도, 비강 점막, 비관류액과 비장세포에서의 Th1/Th2 사이토카인 발현, 혈청 면역글로불린, 비강 점막에서의 세포간 접합분자의 발현 등을 비교 분석하였다.

결과: NF-κB 국소 차단제 치료군에서 알레르기비염 증상과 비강 조직 내 호산구 침윤을 감소시킬 뿐만 아니라, 혈청 전체 면역글로불린 E와 Th2 면역글로불린 G1의 생성이 유의하게 감소하였다. 비강 점막에서 염증 사이토카인인 IL-1β, IL-6과 TNF-α의 발현도 감소되었으며, 세포간 접합분자의 발현 또한 억제되었다. 비장세포에서의 전신 Th2 사이토카인인 IL-4, IL-5의 발현이 유의하게 억제되었다.

결론: 비강 내 NF-κB 유인체 올리고디옥시핵산을 NF-κB 차단제로 투여하였을 때 알레르기비염 증상이 완화되었으며, Th2 면역반응이 억제되고, 비강 점막에서의 세포간 접합분자의 발현을 감소시켜 알레르기 반응이 억제되었다. 본 연구에서는 처음으로 국소 NF-κB 차단제의 알레르기비염 치료제로서의 가능성에 대해 보여주었다.

주요어: 알레르기비염, 항알레르기제, NF-κB, NF-κB 유인체, 올리고디옥시핵산, 면역글로불린E.

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