

Modulation of allergic response in nasal mucosa by antisense oligodeoxynucleotides for IL-4

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Background: The cytokine IL-4 is highly involved in T_H2 inflammation, such as that seen in allergic rhinitis. IL-4 can induce IgE synthesis and eotaxin. Recent studies have shown that stimulation of allergic nasal tissue with ragweed allergen can induce local IL-4 mRNA production.

Objective: We set out to determine whether IL-4 antisense phosphorothioate-modified oligodeoxynucleotides (PS-ODNs) could inhibit IL-4 production and downstream events of IL-4.

Methods: Nasal mucosa biopsy specimens were obtained from patients with seasonal ragweed allergic rhinitis out of season, incubated *ex vivo* with or without PS-ODNs, and challenged with ragweed. FITC-labeled oligonucleotides were used to determine tissue uptake. By using immunocytochemistry, IL-4-, IL-13-, eotaxin 1-, and IFN- γ -producing cells were enumerated, and by using *in situ* hybridization, ϵ germline transcript RNA- and IL-4 mRNA-positive cells were examined. **Results:** The antisense PS-ODN was taken up by the tissue, and preincubation of the tissue with the IL-4 antisense PS-ODN caused a decrease in allergen-induced IL-4 mRNA and a decrease in the amount of IL-4 immunoreactivity ($n = 7$, $P < .001$). PS-ODNs had inhibitory effects on allergen-induced ϵ germline transcript RNA expression ($n = 7$, $P < .001$) and mucosa eotaxin 1 immunoreactivity ($n = 7$, $P < .05$). In contrast, the PS-ODNs increased the amount of IFN- γ immunoreactivity ($n = 7$, $P < .05$), suggesting a nonspecific mechanism for reduced synthesis of IgE and eotaxin.

Conclusions: Our results show that the IL-4 antisense PS-ODN effectively inhibits IL-4, IgE synthesis, and eotaxin, principal mediators of allergic inflammation, suggesting that PS-ODNs might offer a possible topical treatment for allergic rhinitis. (J Allergy Clin Immunol 2003;111:580-6.)

Key words: Allergy, nasal mucosa, cytokines, antisense oligonucleotides, immunomodulation

Abbreviations used

BCM: Buffered culture media

I ϵ C ϵ : Germline transcript

PS-ODN: Phosphorothioate-modified oligodeoxynucleotide

SSC: Standard saline citrate

It is well established that the T_H2 cytokines IL-4, IL-5, IL-9, and IL-13 can directly and indirectly account for many of the pathophysiologic manifestations of allergies. Allergic diseases are correlated to the number of IL-4-producing T cells and serum IgE levels. IL-4 is essential in promoting the commitment of T_H cell precursors to produce the T_H2 subset of cytokines and is important for the development of humoral-mediated immunity.¹ In addition to promoting T_H2 differentiation, IL-4 promotes T-cell proliferation. IL-4 activates IgE isotype switching of B cells. IL-4 can contribute indirectly to inflammation by promoting tissue eosinophilia at the site of allergen contact. IL-4 can regulate production of eotaxin, a potent eosinophil chemoattractant, by fibroblasts, endothelial cells, and epithelial cells.²⁻⁴

The involvement of IL-4 in many processes of instigating and maintaining allergic inflammation makes it a key target for treating allergic disease. Numerous strategies have previously been attempted in specifically targeting IL-4 in T_H2-mediated inflammation. Anti-IL-4 mAbs were effective in suppressing antigen-induced IgE synthesis in murine models of inflammation.⁵ Phase 1 and 2 trials on soluble IL-4 receptor proved that it was safe and effective at improving FEV₁ and decreasing inflammation in atopic asthma.⁶ Similarly, late airway responses in rats induced by aerosolized ovalbumin were inhibited by antisense oligodeoxynucleotides specific for IL-4.⁷

We have shown that culture of explanted human nasal mucosa for 24 hours with ragweed allergen causes increases in IL-4 and IL-13 mRNA-positive cells, as well as in IgE ϵ germline transcript RNA (I ϵ C ϵ RNA) and the IgE heavy chain constant region RNA (C ϵ RNA).⁸ This model isolates the nasal mucosa from the circulation, enabling the separation of local immune reactions from those mediated by the influence of cell recruitment. Using this model, we have described local eosinophilic differentiation,⁹ local upregulation of the T_H2 transcription factor GATA-3,¹⁰ and increases in local I ϵ C ϵ RNA expression.⁸

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We propose the use of antisense oligodeoxynucleotides specific for IL-4 to decrease human T_H2 -mediated inflammation. Using the explant system, we sought to determine whether local inflammatory events after allergen challenge, such as increases in IL-4, I ϵ C ϵ transcription, and eotaxin production, could be downregulated with the use of antisense phosphorothioate-modified oligodeoxynucleotides (PS-ODNs) for IL-4. Incubation of the tissue with IL-4 antisense oligodeoxynucleotide during ragweed exposure decreased IL-4 mRNA and protein levels. Additionally, allergen-induced I ϵ C ϵ RNA and eotaxin were decreased. IFN- γ (a T_H1 cytokine and inhibitor of T_H2 inflammation) levels were also increased nonspecifically. Taken together, the IL-4 PS-ODN offers a potent dual mechanism of action in inhibiting local human allergic reactions.

METHODS

Synthesis and design of PS-ODNs

Antisense PS-ODNs designed to inhibit IL-4 translation and the corresponding PS-ODN sense control were synthesized at the Sheldon Biotechnology Institute of McGill University (Montreal, Canada). The antisense oligodeoxynucleotides were phosphorothioate modified to increase their resistance to nuclease resistance while maintaining their polarity and charge. The sequence corresponded to 5'-GAAGCAGTTGGGAGGTGAGACC-3' for the IL-4 antisense oligodeoxynucleotide and 5'-GGTCTCACCTCCCACTGCTTC-3' for the IL-4 sense control oligodeoxynucleotide, which corresponds to the IL-4 translation initiation region of the mRNA (+4 to +25).

Culture of nasal mucosa explants and tissue preparation

Patients undergoing sinus surgery at the Nasal Clinic of the Jewish General Hospital and the Nôtre-Dame Hospital (Montreal, Canada) provided informed consent for use of their resected tissue in these experiments. The study was approved by the Ethics Committee of the McGill University Health Center and the Centre Hospitalier de l'Université de Montreal. Inferior turbinate biopsy specimens were obtained from 7 patients not taking topical steroids who had seasonal allergic rhinitis outside of the season and had positive skin test responses to ragweed allergen (>5-mm wheal). Specimens were removed with Gerritsma forceps just beyond the anterior tip of the turbinate at a time when the patients were asymptomatic. The specimen was serially sectioned into pieces (2 mm $\times$ 3 mm $\times$ 2 mm), each consisting of an epithelial and submucosal layer. Sections of tissue were each placed in 30-mm, filtered-well Millicell CM inserts (Milipore Corp) with a 0.4- μ m pore diameter within a 6-well culture plate (Corning Inc) containing 2 mL of fresh buffered culture media (BCM). Sections were cultured with or without PS-ODNs (10 μ g/mL) and with or without ragweed allergen (10,000 protein nitrogen units/mL, Hollister-Stier) in 5% CO₂/95% air for a period of 24 hours at 37°C. After incubation, the sections were fixed in 4% paraformaldehyde and washed in 15% sucrose-PBS, blocked in optimal cutting temperature medium, and snap-frozen in liquid nitrogen-cooled isopentane. Five- to 8- μ m cryostat sections were mounted onto poly-L-lysine (0.1%)–coated glass slides (Microm HM500, Microm International GmbH) and baked overnight at 37°C.

Tissue absorption of fluorescent oligodeoxynucleotide

3' FITC-tagged IL-4 antisense PS-ODNs were synthesized at l'Institut National de la Recherche Scientifique–Institut Armand-Frappier center of Microbiology and Biotechnology

(Laval, Canada). Tissue was cultured in a 6-well culture dish in 2 mL of BCM on a Millicell CM filter insert. Two hundred fifty microliters of FITC-tagged PS-ODN (100 pmol/ μ L) were added to the tissue, and the tissue was incubated for 24 hours at 37°C in 5% CO₂/95% air. Tissue was washed 3 times in BCM and fixed in 4% paraformaldehyde, washed with PBS-15% sucrose, blocked, and frozen in optimal cutting temperature medium. Seven-micrometer sections were taken and air-dried at 37°C for an hour. The sections were stained with 1 μ mol/L Hoechst 33342 (Molecular Probes) for 15 minutes at room temperature, washed with PBS, and mounted in PermaFluor Mounting Media (Shandon). The slides were visualized at 20 $\times$ by using a fluorescence microscope (Nikon Eclipse E600, Nikon Inc), with FITC and DAPI (4',6'-Diamidino-2-phenylindole) filters for blue and green channels, respectively. Superpositioning of the channels was performed with Adobe Photoshop (version 6.0, Adobe Systems Incorporated).

In situ hybridization

In situ hybridization was performed as previously described.¹¹ Riboprobes were prepared for IL-4, and I ϵ C ϵ RNA was supplied as a generous gift from Dr H. Gould (London, United Kingdom). In vitro transcription from linearized pGEM vectors (Promega) was performed in the presence of ³⁵S-labeled uridine-5'-triphosphate and the appropriate RNA polymerases to generate antisense and sense probes. Explant section slides were permeabilized with 0.3% Triton X-100 (BDH Inc) and proteinase K solution (1 μ g/mL), prehybridized with 50% formamide (Sigma Chemical Co) in 2 $\times$ standard saline citrate (SSC; BDH Inc), and hybridized with 0.75 $\times$ 10⁶ cpm per section of ³⁵S uridine-5'-triphosphate-labeled riboprobes (either antisense or sense). Posthybridization washings were done with SSC solutions (4 $\times$ SSC and 0.1 $\times$ SSC), followed by ribonuclease A treatment to remove excess probe. Slides were dipped in emulsion (Amersham LM-2), stored at 4°C, and developed 2 weeks later in Kodak D19 solution (Eastman Kodak). Slides were counterstained with Gill II Haematoxylin, and cover slips were applied. Positive signal was identified as silver grains over the cells.

Immunocytochemistry

Alkaline phosphatase–antialkaline phosphatase immunocytochemistry was used to assess cytokine immunoreactivity with primary mAbs against IL-4 (1:50, DAKO Diagnostics), IL-13 (1:25), IFN- γ (1:50), and IL-12 (1:20, R&D Systems). Expression of eotaxin was studied by using the polyclonal primary goat anti-eotaxin antibody (1:50, Santa Cruz Biotechnology). Briefly, cryostat sections were incubated with universal blocking solution (DAKO Diagnostics), incubated with primary antibody overnight at 4°C, washed in Tris-buffered saline (pH 7.6), incubated with rabbit anti-mouse secondary antibody (1:60, DAKO Diagnostics), washed again, and incubated with mouse alkaline phosphatase–antialkaline phosphatase (1:60, DAKO Diagnostics). For eotaxin, the secondary antibody used was biotinylated swine anti-goat immunoglobulin (1:300, DAKO Diagnostics), followed by streptavidin-AP (1:200, DAKO Diagnostics). Immunostaining was developed with Fast Red TR (Sigma). All slides were counterstained with Gill II Haematoxylin, and positive cells appeared red under bright field illumination. For negative controls, an isotype-matched control antibody replaced the primary antibody.

Quantification of immunocytochemistry and in situ hybridization

Two observers counted all specimens blindly by using an Olympus light microscope (Carson Group Inc) at 400 $\times$ magnification with an eyepiece graticule of 0.202 mm². In the subepithelium the number of positive cells was averaged from 6 to 8 random and

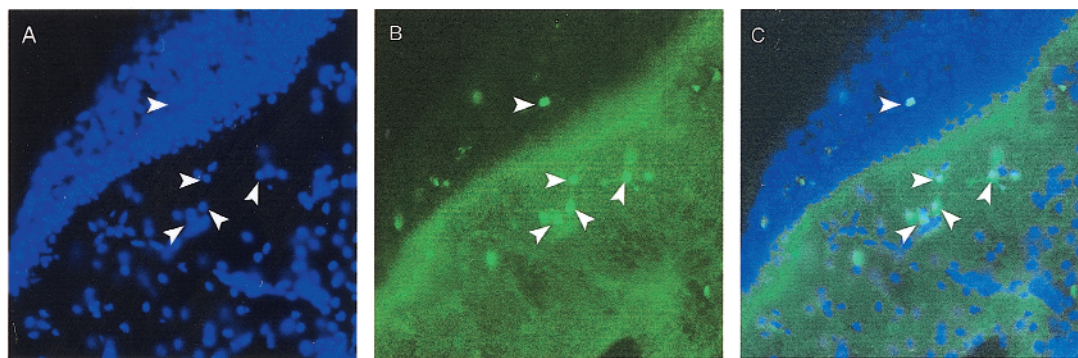


FIG 1. Representative fluorescence image of biopsy specimen incubated for 24 hours with FITC-tagged IL-4 antisense PS-ODN. Hoechst 33342 nuclear staining fluorescence (**A**), FITC-tagged PS-ODN fluorescence (**B**), and superpositioning of blue and green channels (**C**) are shown. Arrows denote representative cell-specific fluorescence.

nonoverlapping grids. Epithelial staining was assessed by using morphometry, and the results were expressed as the percentage of epithelium that was immunoreactive. The patients' data were pooled and expressed as the mean number of positive cells per square millimeter of mucosa $\pm$ SEM. Epithelial cell scoring was expressed as a percentage of total epithelium.

Statistical analysis

The difference in the number of immunopositive cells for a particular cell type or cytokine in the presence or absence of PS-ODN and with or without ragweed was analyzed by using ANOVA with Dunnett post hoc analysis for multiple comparisons (Systat version 10, SyStat Inc). *P* values of less than .05 were regarded as statistically significant.

RESULTS

IL-4 antisense PS-ODN decreases allergen-induced production of IL-4

FITC-labeled PS-ODNs were used to evaluate nasal mucosa absorption of the oligodeoxynucleotides from the media. The FITC-labeled ODN was colocalized with cells in the mucosa labeled with Hoechst 33342, but few were seen in the epithelium (Fig 1). With in situ hybridization and immunocytochemistry, a basal level of IL-4 mRNA-positive and IL-4 protein-positive cells was seen in the nasal mucosa specimens without allergen challenge ($n = 7$; 14.4 ± 2.4 cells/mm² [Fig 2, A] and 15.7 ± 4.1 cells/mm² [Fig 2, B], respectively). After 24-hour ragweed challenge, there was a significant increase in the numbers of IL-4 mRNA- and IL-4 protein-positive cells ($n = 7$; 48.21 ± 4.8 and 62.6 ± 7.9 cells/mm², respectively) when compared with numbers in the presence of medium alone ($n = 7$; $P < .001$; Fig 2, A and B). Incubation with the IL-4 antisense PS-ODN abolished the ragweed-induced increase in IL-4 mRNA-positive cells ($n = 7$; 18.3 ± 1.4 cells/mm², $P < .001$ [38%] vs ragweed alone and $P = 1.0$ vs medium; Fig 2, A). Similarly, IL-4 antisense PS-ODN eliminated IL-4 protein-positive cells induced by ragweed within tissue ($n = 7$; 16.4 ± 2.3 cells/mm², $P < .001$ [26%]; Fig 2, B). On the other hand, the sense control PS-ODN had no effect on the number of mRNA-positive cells compared with those present

after ragweed challenge ($n = 7$; 39.9 ± 4.0 cells/mm² [82%]; Fig 2, A). Unlike the hybridization results, the sense control PS-ODN caused a significant reduction in the number of IL-4 protein-positive cells in the tissue when compared with ragweed alone ($n = 7$; 39.8 ± 4.0 cells/mm², $P < .05$; Fig 2, B). The antisense PS-ODN effect on IL-4 protein positivity was greater than that of the sense control ($P < .05$; Fig 2, B).

Ragweed challenge of allergic nasal tissue can also increase levels of IL-13. IL-13 immunoreactivity was assessed to determine the specificity of our IL-4 antisense PS-ODN for IL-4. IL-13 immunoreactivity was detected at baseline ($n = 7$; 13.7 ± 4.2 cells/mm²). The number of IL-13-positive cells was increased on exposure to ragweed when compared with that after exposure to medium alone ($n = 7$; 42.9 ± 4.9 cells/mm², $P < .005$). IL-4 antisense and sense PS-ODNs had no significant effect on levels of ragweed-induced IL-13 ($n = 7$; 32.0 ± 5.2 and 36.1 ± 5.0 cells/mm², respectively; $P = .78$ and $P = 1.0$, respectively).

IL-4 antisense oligonucleotide decreases allergen-induced I ϵ C ϵ RNA production

Allergen challenge can induce production of I ϵ C ϵ RNA in human allergic nasal mucosa. In situ hybridization was performed with a probe specific for the I ϵ C ϵ transcript. The number of I ϵ C ϵ RNA-positive cells within the nasal mucosa was 6.8 ± 2.6 cells/mm² ($n = 7$; Fig 3, A), and ragweed challenge significantly increased positivity ($n = 7$; 34.3 ± 2.8 cells/mm², $P < .001$ vs medium alone; Fig 3, A). IL-4 antisense PS-ODN effectively decreased this induction ($n = 7$; 9.3 ± 2.2 cells/mm², $P < .001$ vs ragweed alone; Fig 3, A), and the control sense had no effect ($n = 7$; 27.9 ± 6.1 cells/mm², $P = 1$ vs ragweed alone; Fig 3, A).

IL-4 antisense PS-ODN decreases allergen-induced eotaxin immunoreactivity

Immunocytochemistry for eotaxin showed baseline levels of eotaxin 1 (CCL1)-positive cells in the allergic mucosa ($n = 7$; 35.7 ± 6.9 cells/mm²; Fig 3, B), and ragweed challenge increased the number of positive cells in

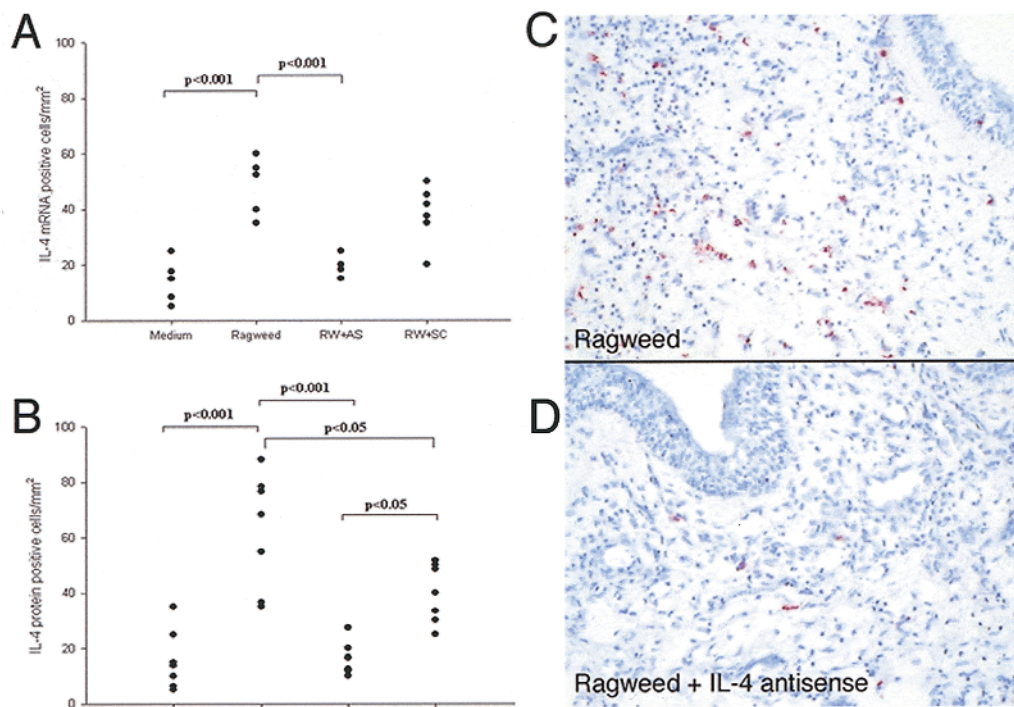


FIG 2. Amount of IL-4 mRNA-positive (n = 7; **A**) and IL-4 protein-positive (n = 7; **B**) cells in the nasal mucosa after stimulation with ragweed, ragweed with antisense to IL-4 (RW+AS), and ragweed with sense control (RW+SC). The antisense effect was specific at decreasing the ragweed-induced IL-4 positivity. Values are expressed as mean number of positive cells in the mucosa per square millimeter $\pm$ SEM. Representative alkaline phosphatase-antialkaline phosphatase immunocytochemistry showing ragweed-induced IL-4 production (**C**) and the ability of the IL-4 antisense PS-ODN to decrease the number of IL-4 protein-positive cells in the mucosa (**D**) is shown.

the tissue (62.9 ± 11.6 cells/mm², $P < .05$; Fig 3, **B**). IL-4 antisense PS-ODN decreased the levels of ragweed-induced eotaxin 1 (n = 7, 28.6 ± 6.9 cells/mm², $P < .05$ vs ragweed alone; Fig 3, **B**), whereas the sense control had no effect (n = 7, 54.3 ± 14.1 cells/mm²; Fig 3, **B**). The epithelium was scored for eotaxin production. The epithelial eotaxin 1 positivity was high without challenge ($20.8\% \pm 5.7\%$; Fig 3, **C**). Ragweed challenge increased epithelial cell production of eotaxin significantly ($53.6\% \pm 5.9\%$, $P < .01$; Fig 3, **C**). Incubation of IL-4 antisense or sense PS-ODNs did not significantly alter these levels (n = 7; $33.0\% \pm 6.5\%$ [$P = .10$ vs ragweed] and $45.8\% \pm 6.5\%$ [$P = .69$], respectively; Fig 3, **C**).

IL-4 antisense and sense control PS-ODNs increased IFN- γ levels

We wished to test the effect of the PS-ODN on T_H1 cytokine production. Levels of IL-12 (n = 7, 9.1 ± 4.2 cells/mm²) and IFN- γ (4.1 ± 2.2 cells/mm²; Fig 3, **D**) did not show high basal expression, and ragweed did not increase these levels significantly (n = 7; 18.7 ± 5.8 cells/mm² and 6.3 ± 1.4 cells/mm², respectively). IL-12 positivity did not increase compared with baseline values when the IL-4 antisense PS-ODN (n = 7, 30.7 ± 8.2 cells/mm²) or the sense control PS-ODN (n = 7, 31.7 ± 8.3 cells/mm²) was added to the tissue. Conversely, both the IL-4 antisense PS-ODN and the sense control PS-

ODN significantly increased the number of IFN- γ -positive cells (20.6 ± 6.0 and 22.9 ± 3.7 cells/mm², respectively; $P < .05$) when compared with that after medium alone (Fig 3, **D**).

DISCUSSION

Currently, many treatments exist for allergies, including symptomatic therapy and allergen immunotherapy, but these do not treat the underlying cause of the disease. We are the first to show that IL-4 antisense PS-ODNs are effective in inhibiting T_H2 inflammatory mediators ex vivo in the nasal mucosa of human patients with rhinitis. Furthermore, our experimental model illustrates the possibility of testing drugs on the tissue it is intended for before in vivo testing. The IL-4 PS-ODN specifically inhibited IL-4 mRNA and protein positivity in ragweed-challenged biopsy specimens. This would decrease levels of available IL-4 to upregulate T_H2 inflammatory mediators, such as IgE and eotaxin. Because the IL-4 antisense PS-ODN was effective at decreasing mediators of T_H2 inflammation, it might offer an alternative to these treatments in a growing list of immunomodulatory drugs.

In our experimental protocol the tissue is not submerged in the media; it is incubated on a filter disc, which floats on the media. This model allows us to mimic the nasal passages, where the epithelium is in con-

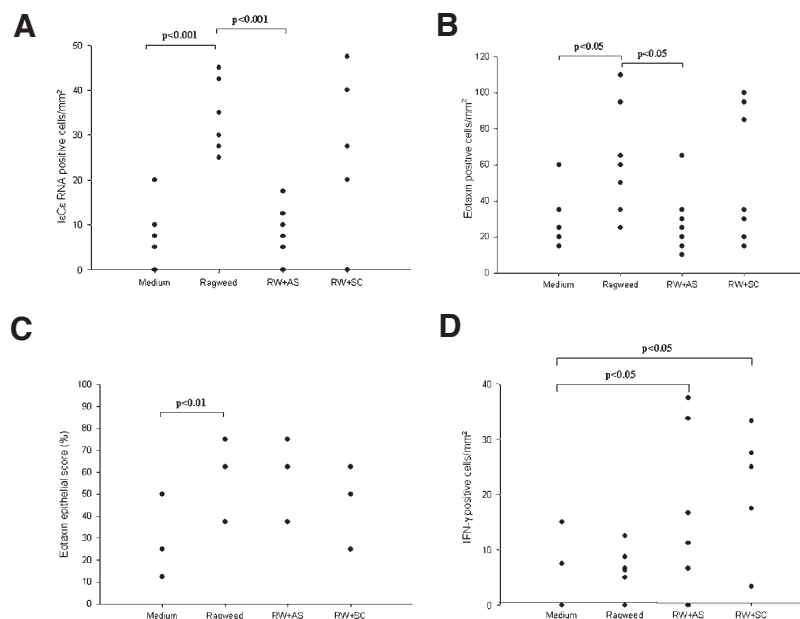


FIG 3. The number of lCe RNA-positive cells in the mucosa (**A**), eotaxin-positive cells in the mucosa (**B**), eotaxin scoring of the epithelium (**C**), and IFN- γ -positive cells in the mucosa after ragweed stimulation or stimulation with ragweed and antisense to IL-4 (RW+AS) or stimulation with ragweed and sense control (RW+SC; **D**) is shown. Antisense oligonucleotide decreased ragweed-induced lCe positivity. Antisense oligonucleotide decreased mucosa eotaxin production but not epithelial eotaxin production. Both the antisense and sense control oligonucleotides increased IFN- γ production. Values are expressed as mean number of positive cells in the mucosa per square millimeter $\pm$ SEM.

tact with air. Only the mucosa is in contact with the media, from which the allergen and the PS-ODN diffuse into the tissue. Penetration of the PS-ODN through the apical side of intact mucosa unaided will be significantly more challenging. The epithelium was largely unstained by the fluorescent PS-ODN, demonstrating poor diffusion into these cells. PS-ODNs are polyanionic at a physiologic pH, and this charge prevents their diffusion across the cell membrane. A delivery vector can be used to overcome this obstacle, such as polymerized lipid-soluble nanoparticle suspension, which entraps the PS-ODN and can cross the epithelium.¹² With FITC-tagged IL-4 antisense PS-ODNs, we showed that nasal mucosal tissue was able to effectively take up the PS-ODNs. Under basal conditions, the tissue displayed few IL-4-positive cells, as seen in a past report.⁸ The basal expression seen can be likely attributed to perennial allergies in some of the patients. Ragweed increased production of this cytokine, and IL-4 antisense oligonucleotide effectively decreased IL-4 mRNA and protein positivity in the mucosa. IL-4 is involved in many aspects of allergic inflammation, including IgE production and cellular influx; therefore we investigated the effects of the IL-4 antisense oligonucleotide on these 2 processes. The IL-4 antisense oligonucleotide effectively decreased lCe RNA involved in IgE isotype switching to IgE and the level of production of the chemokine eotaxin in the mucosa. Additionally, the antisense oligonucleotide increased the number of IFN- γ -producing cells, and there was a trend toward increase in the IL-12

protein-producing cells. This suggests that IL-4 antisense oligonucleotides decrease T_H2 responses to allergen while promoting T_H1 responses.

Antisense oligodeoxynucleotides function by means of base pairing and forming hydrogen bonds to their corresponding complementary strand (the sense strand) of mRNA. Once bound, the antisense oligodeoxynucleotide inhibits translation by blocking access of ribosome to the mRNA or by destabilizing the mRNA and making it more susceptible to ribonucleases.¹³ Our IL-4 antisense oligodeoxynucleotide specifically decreased IL-4 mRNA- and IL-4 protein-positive cells, suggesting a decrease in translation in the mucosa and degradation of the IL-4 transcript. This effectively decreased the ragweed-induced IL-4 available within the tissue, which would normally drive T_H2 inflammatory events.

Because both the antisense and sense oligodeoxynucleotides control nonspecifically induced IFN- γ and IL-12 production, this suggests a secondary mechanism of action. Oligodeoxynucleotides, particularly those that contain a CpG motif (purine-purine-CpG-pyrimidine-pyrimidine), can stimulate strong immune responses.¹⁴ The CpG oligodeoxynucleotides act mostly on antigen-presenting cells to stimulate priming of a T_H1 immune response.¹⁵ Toll-like receptor 9, a recently described innate immune pattern recognition receptor, has been determined to mediate the effects of CpG oligodeoxynucleotides.^{16,17} Despite inducing the T_H1 cytokine IFN- γ and a nonsignificant increase in IL-12, neither our antisense nor sense oligodeoxynucleotides do not contain a

CpG motif. However, it has been reported that PS-ODNs can elicit T_H1 cytokine production from human PBMCs, irrespective of the sequence used.¹⁸ Additionally, human monocytes have been shown to increase their chemotaxis if the stimulatory oligodeoxynucleotides used are phosphorothioate modified.¹⁹ Whether this effect in these systems, as well as in our system, is due to the toll-like receptor 9 or to an unidentified orphan receptor remains to be determined.

Inhibition of IL-4 by use of antisense oligodeoxynucleotides decreased downstream events of IL-4, such as local IeCe RNA production. It has been previously reported that production of IL-4 and IL-13, as well as IeCe RNA transcription, occurs in the nasal mucosa after antigen exposure.^{8,11} IeCe RNA is a sterile transcript encompassing the Ie exon, the IgE switch region, and the constant region of IgE (Ce). IeCe RNA transcription and splicing is essential for IgE isotype switching,^{20,21} the process in which intrachromosomal recombination of the immunoglobulin-constant regions of IgM (C μ), IgG (C γ) or IgA (C α) is replaced by that of IgE (Ce). Control of IeCe transcription depends on IL-4 or IL-13 with the aid of trimeric CD40 ligand (CD154).^{22,23} Concordant with these studies, we saw increases in IL-4, IL-13, and IeCe transcription after ragweed challenge of our tissue. Culture of nasal mucosa biopsy specimens with the IL-4 antisense oligodeoxynucleotide decreased the ragweed-induced IeCe RNA positivity. Because IL-13 protein positivity was not significantly affected, this suggests an intimate relationship between local IL-4 and increases in tissue IgE synthesis events. PS-ODN induction of IFN- γ might also play a part in inhibiting transcription of IeCe RNA because IFN- γ has been shown to inhibit the IL-4-controlled cis-controlling elements of the IeCe RNA promoter.²⁴

Eotaxin 1 (CCL11) is a chemokine and is produced both by leukocytes and structural cells. In this report we have shown, for the first time, that ragweed allergen is capable of increasing local eotaxin protein production in mucosal cells and the epithelium in in vitro cultured human nasal biopsy specimens. Similar to in vivo studies,²⁵ high baseline epithelial eotaxin positivity was observed before allergen challenge. Constitutive eotaxin expression has also been reported in the gastrointestinal tract, heart, and lungs.²⁶ Eotaxin production is regulated by a variety of cytokines. IL-4 can increase mucosal fibroblast production of eotaxin.^{3,27} Additionally, IL-13 induces eotaxin expression in epithelial cells of the lung, promoting lung eosinophilia in vivo.^{2,28} On the other hand, the T_H1 cytokine IFN- γ is a strong inhibitor of in vitro production of eotaxin.²⁹ IL-4 antisense PS-ODNs significantly decreased mucosa eotaxin-producing cells. This might be mediated by decreased production of IL-4 and increased production of IFN- γ . On the other hand, eotaxin positivity in the epithelia was not significantly decreased. This might be explained by the functional redundancy that exists between IL-4 and IL-13; the IL-4 antisense PS-ODN had no effect on IL-13 protein production, and therefore IL-13 could maintain epithelial

eotaxin positivity. With the IL-4 antisense PS-ODN decreasing mucosal eotaxin production, this could possibly decrease eosinophil and other CCR3-positive cell recruitment into the tissue. Unfortunately, in our model the tissue is separate from the circulatory system, preventing assessment of the potency of the IL-4 antisense oligonucleotide at decreasing eosinophil chemotaxis into the nasal mucosa.

Having mechanisms of action that specifically inhibit IL-4 and nonspecifically increase T_H1 cytokine production, we have shown that antisense IL-4 PS-ODNs effectively decreased downstream mediators of allergic disease. It decreases IL-4 synthesis without being globally immunosuppressive like other treatments of asthma and allergies. Because the antisense oligodeoxynucleotide increases T_H1 cytokines, it might help in bringing about balanced responses or even reverse the T_H2 bias to allergens. Taken together, this makes the antisense oligonucleotide a potential therapeutic agent for allergic rhinitis. Because oligonucleotides have strong immunomodulatory effects on the immune system, oligonucleotide treatment of allergy might be restricted to local and topical delivery with an appropriate delivery mechanism. Nevertheless, our oligonucleotide offers potential therapeutic benefit for allergic rhinitis.

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