

Immunomodulatory effect of cytosine-phosphate-guanosine (CpG)–oligonucleotides in nonasthmatic chronic rhinosinusitis: An explant model

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ABSTRACT

Background: The use of cytosine-phosphate-guanosine–oligodeoxynucleotides (CpG-ODNs) or immunostimulatory sequences (ISSs) in the treatment of airway diseases is gaining interest. Binding of the CpG-ODN ligand to Toll-like receptor 9 (TLR9) triggers a shift from a Th2- to a Th1-type response in the target tissue. In this study, we explored the potential use of CpG-ODN to dampen the predominantly Th2-driven chronic inflammatory state in our cohort of patients.

Methods: An in vitro explant model comprising of sinonasal tissue from patients with asthma ($n = 12$) and without asthma ($n = 11$) were stimulated with CpG-ODN or *Staphylococcus aureus* enterotoxin B (SEB) or CpG-ODN in combination with SEB for 48 hours. Ten of the 12 asthma patients had nasal polyps. RNA was extracted for multiplex real-time reverse transcription polymerase chain reaction analysis and the $2^{-\Delta\Delta C_T}$ method used to determine interleukin (IL)-5, p35 IL-12, interferon (IFN) gamma, and TLR9 expression levels.

Results: CpG-ODN significantly reduced IL-5 mRNA expression in patients without asthma ($p = 0.0379$) but not in the asthma-associated group. SEB alone caused an increase in IL-5 levels that could be dampened when CpG-ODN was added in combination with SEB. Significant differences in mean IL-5 expression levels between the asthmatic and nonasthmatic categories were detected (Welch t -test; $**p = 0.0041$). Asthmatic and nonasthmatic patients present as two distinct categories as reflected by significant differences in their IL-5 response to CpG-ODN ($F = 11.93$; $***p = 0.0008$), SEB ($F = 41.34$; $*p = 0.0476$) and CpG-ODN with SEB ($F = 13.2$; $*p = 0.0114$). In contrast, no significant differences were observed in the expression levels of IL-12, IFN-gamma, and TLR9.

Conclusion: Localized application of CpG-ODN on its own or in combination with SEB may potentially reduce the expression of the proinflammatory cytokine IL-5 in nonasthmatic patients and may be further developed as an immunotherapeutic agent.

(Am J Rhinol Allergy 23, 123–129, 2009; doi: 10.2500/ajra.2009.23.3279)

Key words: Asthma, CpG-ODN, eosinophilic mucus chronic rhinosinusitis, IL-5, immunostimulatory sequences (ISS), immunotherapy, toll-like receptor 9

Conventional medical and surgical treatments for chronic rhinosinusitis (CRS) have been met with varying degrees of success. It is now recognized that this disease encompasses a heterogeneous group of disorders as evidenced by recent efforts in formulating more precise classification for rhinosinusitis based on histopathological and clinical presentation.¹ Eosinophilic mucus is a major distinguishing feature that broadly divides rhinosinusitis into two categories, *viz.*, CRS and eosinophilic mucus CRS.² CRS is defined as an absence of eosinophilic mucus while the latter have pronounced eosinophil associated mucus.

Asthma is defined as a chronic inflammatory disorder of the airways in which mast cells, eosinophils, T lymphocytes, macrophages, neutrophils, and epithelial cells play prominent roles. Similarities in the pathophysiology of asthma, allergic rhinitis, and rhinosinusitis have led researchers to promulgate

the “one airway disease” hypothesis.^{3,4} Certainly, histopathological features such as epithelial damage, thickening of the basement membrane, and eosinophilic inflammation of sinonasal tissue are common features of asthmatic and eosinophilic mucus CRS patients.⁵ The characteristic eosinophilia is associated with the release of interleukin (IL)-5, a major proinflammatory cytokine that significantly contributes to the chronic inflammatory state of the sinus mucosa and stimulates eosinophil chemotaxis and activation.

There is increasing interest in adopting novel immunotherapies that exploit Toll-like receptor (TLR) signaling pathways to attenuate the chronic inflammatory response.⁶ One such novel approach is localized immunotherapy where putative therapeutic agents such as synthetic oligodeoxynucleotides (ODNs) are applied to the local area of inflammation.^{6,7} This form of therapy that specifically uses the binding of TLR9 to its ODN ligand has the downstream effect of reducing a Th2-type inflammatory response.

There are at least 11 TLR members that are important signal transducers in the mammalian antifungal and antibacterial defense.⁸ TLRs consist of an extracellular domain with leucine-rich repeats and an intracellular signaling domain known as the Toll/IL-1 receptor domain. TLR ligands include bacterial lipopolysaccharide, lipoteichoic acid, peptidoglycans, and bacterial-derived cytosine-phosphate-guanosine ODNs (CpG-ODNs). Receptor to ligand binding allows the Toll/IL-1 receptor domain to complex with the cytoplasmic

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Presented at 6th European Congress of Oto-Rhino-Laryngology Head and Neck Surgery, Vienna, Austria, June 30–July 4, 2007

Supported by grants from the Garnett Passe and Rodney Williams Memorial Foundation, Victoria, Australia

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adaptor protein myeloid differentiation factor 88, thereby triggering a signaling cascade *via* the NF- κ B and mitogen-activated protein kinase pathways. In monocytes, natural killer cells and macrophages binding of the strongly immunogenic CpG-ODNs to TLR9 stimulated IL-12 and interferon (IFN) γ secretion that promotes a Th1 response.⁹

Using an allergic rhinosinusitis model of ovalbumin-sensitized mice, Broide *et al.* reported that i.p. injection of CpG-ODNs prevented the development of Th2-mediated eosinophilic inflammation and symptoms of allergic rhinosinusitis. When coadministered with ovalbumin, it resulted in the induction of a rapid Th2- to a Th1-type reaction.¹⁰ This effect was further enhanced when CpG-ODNs were covalently linked to ovalbumin, which resulted in a 100-fold reduction in airway eosinophilia in mice when compared with the use of unconjugated CpG-ODNs coadministered with ovalbumin.¹¹ Mo *et al.* reported a significant antiallergic effect of CpG-ODN conjugated to *Dermatophagoides farinae* (Der f) in sensitized C57BL/6 mice.¹² Specifically, they detected decreased IL-5 and a corresponding increase in IFN- γ in the nasal lavages of these mice.

Recent experimental studies have explored the use of alternative administration routes such as subcutaneous or sublingual delivery of CpG-ODN linked allergens to increase the efficiency of the Th2 to Th1 shift.^{13–16} Targeting the sinonasal mucosa as an optimal entry point is rapidly gaining serious consideration in light of the study of Takabayashi *et al.* that intranasal rather than intradermal delivery of immunostimulatory ODNs could better induce tolerance to allergens in Th2-sensitized mice.¹⁷ Further impetus was provided by Ramanathan *et al.* who showed that functional TLR9 protein could be detected in sinonasal epithelial cells in culture and the expression of TLR9 mRNA in sinus mucosa was lower in CRS patients with polyps compared with healthy individuals. This prompted us to investigate the sinomucosa as an alternative delivery route of CpG-ODN to attenuate the primarily Th2-driven inflammatory reaction in our patient cohort.¹⁸

The primary aim of this study was to determine whether stimulation of TLR9 in human sinonasal tissue with CpG-ODN ligand attenuated the Th2-type response in asthmatic and nonasthmatic patients. Second, we explored whether TLR9 expression could be induced in these patients. An *in vitro* explant model was used to measure IL-5, p35 IL-12, IFN- γ , and TLR9 transcription by real-time reverse transcription polymerase chain reaction when treated with CpG-ODN and coadministered with *Staphylococcus aureus* enterotoxin B (SEB), as representative of the staphylococcal superantigen family that is widely postulated to be a major contributor to nasal polyposis by stimulating Th2 cytokine expression.¹⁹

MATERIALS AND METHODS

Patient Classifications

The institutional review committee granted ethics approval for this study. The patients with eosinophilic mucus were noted as asthmatic or nonasthmatic patients. Their age, polyp status, symptom scores, and number of surgeries were noted.

Symptom scores were graded (0–5) according to severity of the following five symptoms: nasal obstruction, rhinorrhea, postnasal drip, headache/facial pain, and loss of smell.²⁰ Median IgE levels were recorded from patient blood serum.

Cytosine-Phosphate-Guanosine Deoxynucleotides (2006)

Phosphothiorate-linked CpG-ODN 2006 (5'-TCG TCG TTT TGT CGT TTT GTC GTT-3') was purchased from GeneWorks (Adelaide Australia).

Staphylococcal Enterotoxin B

SEB was obtained from Sigma (Sydney, Australia).

Explant Model

We used an explant model previously developed in our laboratory^{21,22} and a pilot study was first performed where tissue explants were challenged with 0.5, 1.0, and 1.5 mM of CpG-ODN to determine the optimum concentration to be used for all additional experiments (data not shown). Briefly, sinus tissue was harvested from patients undergoing endoscopic sinus surgery and placed in airway medium at 37°C overnight or used immediately. Explant tissue was incubated in airway medium containing 1% bovine calf serum (control), or either 1 mM of CpG-ODN only or in combination with 0.1 mg/mL of SEB for 48 hours at 37°C. For ethical reasons nondiseased tissue could not be included in this study because the amounts of tissue required for treatments to be performed in duplicate limited the availability of tissue to only patients with extensive disease that required debridement.

Unless otherwise indicated, airway medium consists of Dulbecco's modified Eagle's medium nutrient mixture F-12 Hams (Sigma) containing 2 mM of L-glutamine (Sigma), 15 mM of HEPES, 10% bovine calf serum (JRH Biosciences, Melbourne Australia), 200,000 U/L of penicillin, and 200 mg/L of streptomycin (Sigma), 2.5 mg/L of amphotericin B, ITS + 1 Liquid Media Supplement, 10 mg/L of epidermal growth factor (Sigma), 25 mg of bovine pituitary extract (Invitrogen, Sydney, Australia), 0.02 mM of trans retinoic acid (Sigma), 0.5 $\times$ of serum replacement S-0638, and 15 mg/L of endothelial cell growth supplement (Sigma). Tissue was preserved in RNAlater (Ambion, Austin, TX) at 4°C overnight and then at -70°C.

Real-Time RTPCR

RNA Extraction. Up to 100 mg of tissue was homogenized in 1 mL of QIAzol reagent (Qiagen, Melbourne, Australia) using the TissueRuptor (Qiagen). RNA was extracted using the RNeasy Lipid Tissue mini kit according to the manufacturer's instructions (Qiagen). RNase Inhibitor (Qiagen) was added to prevent degradation and the RNA was stored at -70°C until required.

Complementary DNA Synthesis. Before cDNA synthesis, RNA was treated with RQ1 RNase-free DNase (Promega, Melbourne, Australia) according to the manufacturer's instructions. A total of 400 ng of RNA was used for cDNA synthesis using the iScript Select cDNA synthesis kit with oligo (dT)₂₀ primers (BioRad, Sydney, Australia). For IL-5 cDNA only, 400 ng of RNA was synthesized using a mixture of 0.33 μ L of oligo(dT)₂₀ primers and 1.66 μ L of random primers (BioRad).

Real-time PCR was performed for the following target genes: IL-5, p35IL12, IFN- γ , and TLR9 and the housekeeping gene human succinate dehydrogenase complex, subunit A

(SDHA). Taqman probes and primer pair sequences were designed using Beacon Designer .2 (Premier Biosoft International, San Diego, CA) and purchased from σ -Proligo (Sydney, Australia) and are as follows: SDHA sense 5'-CTGTTTC-CTACCAGGTCACACAC-3', SDHA antisense 5'-AGCCCTTCACGGTGTCGTAG-3' and SDHA probe 5'-(HEX) TGC-CACCTCCAGTTGTCCTCCTCCA (BHQ1)-3'; IL-5 sense 5'-CAATGAGACTCTGAGGATTCC-3', IL-5 antisense 5'-TTGAATAGTCTTTCCACAGTACC-3', and IL-5 probe 5'-(FAM) ACAGTTTGACTCTCCAGTGTGCCT (BHQ1)-3'; p35 IL-12 sense 5'-GTGATGAGCTATCTGAATGCTTCC; p35 IL-12 antisense 5'-TCCACATCCTATCAAAGTTTCCTC-3'; p35 IL-12 probe 5'-(Texas Red) AGCGAGGTCCCTCCAAACCGTTGT (BHQ2)-3'; IFN- γ sense 5' TTACTGCCAGGACCCATATG-3', IFN- γ antisense 5'-TCTTCAAAATGCCTAAGAAAAGAG-3' and IFN- γ probe 5'-(Texas Red) TCC-GCTACATCTGAATGACCTGCA (BHQ2)-3'; TLR9 sense 5'-CAATGTCACCAGCCTTTTCCTTG-3', TLR9 antisense 5'-TTCCACTTGAGGTTGAGATGCC-3', and TLR9 probe 5'-(Texas Red) CCTCCAACCGCATCCACCACCTCC (BHQ2)-3'.

All reactions were performed in duplicate as a duplex reaction with SDHA. No template controls consisted of cDNA synthesized without reverse transcriptase to eliminate genomic DNA contamination. Blanks using MilliQ water were included to ensure that there was no contamination of the reagents. Real-time reverse transcription polymerase chain reaction was performed using 2X iQ Multiplex Powermix (BioRad) as per manufacturer's instructions. Each reaction was performed in a final volume of 50 μ L containing 100 ng of cDNA, and 300 nM of each primer and 200 nM of each probe (or 150 nM for IL-5) for both the target and the SDHA. Amplification was performed a four-channel iCycler iQ thermal cycler (Biorad) with an initial denaturation step of 95°C for 3' and then 50 cycles of 95°C for 30 seconds followed by 60°C for 1 minute (or 58°C for IL-5). Amplified bands were sequenced and subjected to online searches using BLAST software in the National Center for Biotechnology Information Database to verify their identity. Rather than calculating an absolute mRNA value, our data were analyzed by the comparative $2^{-\Delta\Delta C_T}$ method where $\Delta\Delta C_T = (C_{T, \text{target gene}} - C_{T, \text{housekeeping gene}})_{\text{treatment}} - (C_{T, \text{target gene}} - C_{T, \text{housekeeping gene}})_{\text{no treatment control}}$ as described by Livak and Schmittgen.²³ To use this method, we first validated the assumption that the efficiencies of target to reference gene (SDHA) were approximately equal. The comparative $2^{-\Delta\Delta C_T}$ method analyses the

relative change in expression of the target gene relative to an untreated control and expressed as the mean $\pm$ SE.

Statistical Analysis

Data analysis was performed by nonparametric Kruskal Wallis and a Dunn's *post hoc* multiple comparison test. The Welch's *t*-test (*F* statistic) for nonparametric, unpaired observations was used to determine if the classification of our patients into the two distinct groups of asthmatic and nonasthmatic patients based on their clinical characteristics were also reflected in their molecular and cellular responses to CpG-ODN, SEB, and CpG-ODN coadministered with SEB.

F test for unequal variances was used to determine unequal responses by to populations after a treatment (Graph Pad Prism v. 5a; GraphPad, San Diego, CA). Values of $p < 0.05$ were considered statistically significant.

RESULTS

Clinical, Demographical, and Laboratory Status

A total of 23 subjects with sinus disease were recruited for this study with patient details presented in Table 1. Asthmatic patients were more likely to require revision surgery (66.7%) compared with nonasthmatic patients (27.3%). Their median IgE levels were higher (495 KU/L) than nonasthmatic patients (218 KU/L). Asthmatic patients also had a greater propensity to develop polyps (83.3%) compared with nonasthmatic patients (45.5%).

CpG-ODN 2006 Reduces IL-5 Expression in Explants of Nonasthmatic Patients

A significant reduction in IL-5 expression below that of baseline untreated controls was detected in sinonasal tissue stimulated for 48 hours with CpG-ODN (0.71 ± 0.14 ; Kruskal Wallis, $p = 0.0379$; Fig. 1 A). As expected, SEB challenge induced an increase in the level of the proinflammatory IL-5 cytokine (1.21 ± 0.25). Coadministration of CpG-ODN with SEB was effective in further reducing IL-5 expression (0.49 ± 0.19). The Dunn's *post hoc* test was not significant. This was expected given the small data set examined. By contrast, mean expression levels $\pm$ SE in the asthmatic category trended toward increases in IL-5 expression across all treatments: CpG-ODN (2.2 ± 0.4), SEB (1.5 ± 0.3), and CpG-ODN with SEB (2.0 ± 0.6 ; Fig. 1 B).

Table 1 Demographical, clinical, and histopathology data of patients

	Asthmatic ($n = 12$)	Nonasthmatic ($n = 11$)
Age, yr (range)	51.5 (24–71)	60 (30–86)
Male/female ratio	9:3	6:5
Polyps (%)	10/12 (83.3%)	5/11 (45.5%)
Median symptom score (range)	19.5 (17–23) $n = 11$	19 (17–20) $n = 6$
Revision surgery (%)	8/12 (66.7%)	3/11 (27.3%)
No. of revisions per patient	1.6	0.6
Median IgE, KU/L (range)	495 (53–1313) $n = 5$	218 (7–498) $n = 5$

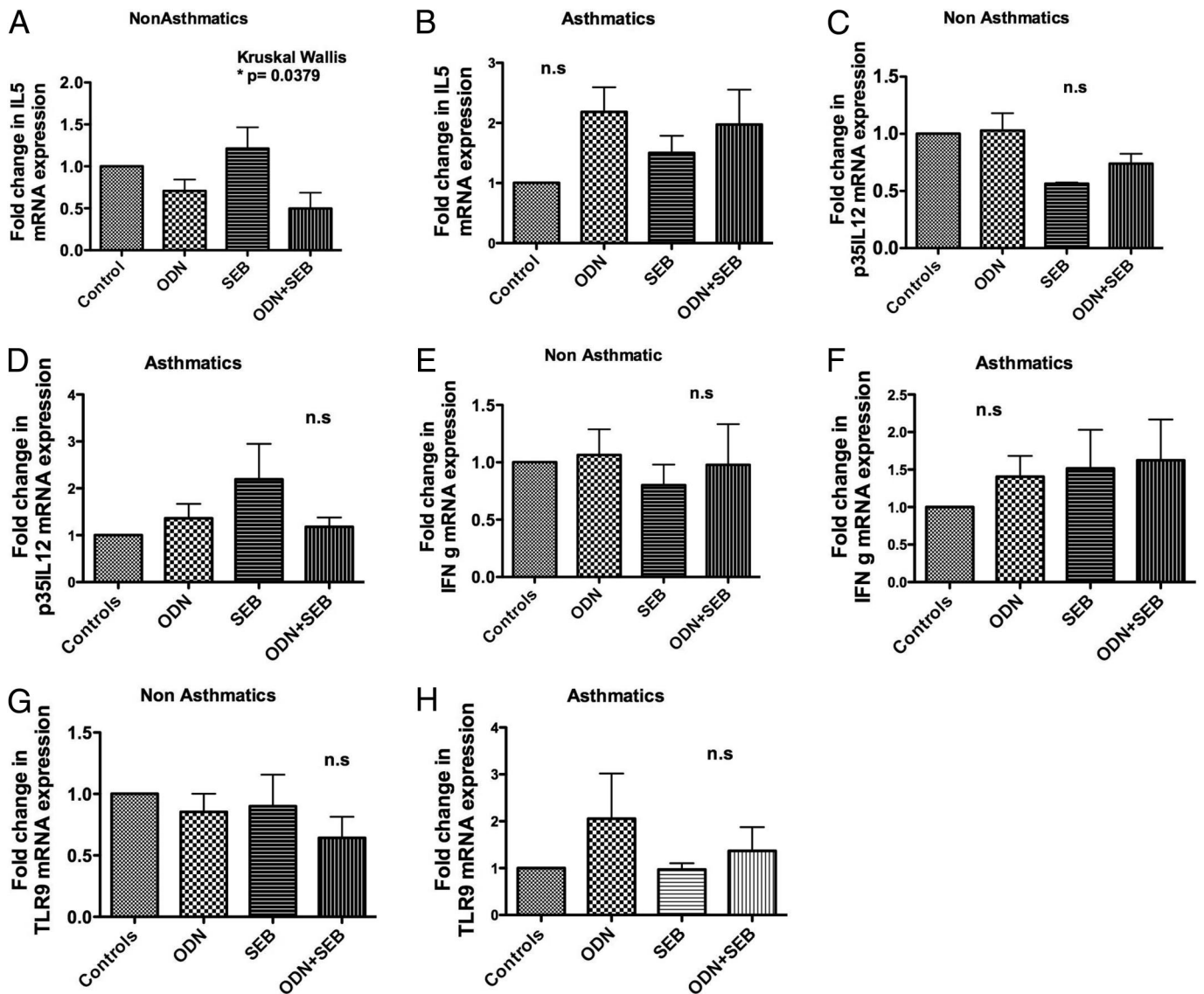


Figure 1. The relative increase or decrease in the expression of interleukin (IL) 5, p35 IL-12 subunit, interferon (IFN) γ , and TLR9 mRNA was determined using the $2^{-\Delta\Delta C_T}$ method according to Livak and Schmittgen.²³ where $\Delta\Delta C_T = (C_{T, \text{target gene}} - C_{T, \text{SDHA}}) \text{ treatment} - (C_{T, \text{target gene}} - C_{T, \text{SDHA}}) \text{ no treatment control}$. This is a more accurate method that compares fold change in mRNA of the target gene relative to a housekeeping gene that in this instance is the human succinate dehydrogenase complex, subunit A (SDHA). Statistical significance was determined at $p < 0.05$.

Expression of p35 IL-12, IFN- γ , and TLR9

No significant differences in p35 IL-12 expression were detected in either the asthmatic (CpG-ODN, 1.4 ± 0.3 ; SEB, 2.2 ± 0.8 ; CpG-ODN + SEB, 1.2 ± 0.2) or the nonasthmatic (CpG-ODN, 1.0 ± 0.2 ; SEB, 0.6 ± 0.01 ; CpG-ODN + SEB, 0.7 ± 0.1) groups (Fig. 1, C and D). Similarly, IFN- γ expression in both asthmatic (CpG-ODN, 1.4 ± 0.3 ; SEB, 1.5 ± 0.5 ; CpG-ODN + SEB, 1.6 ± 0.5) and nonasthmatic (CpG-ODN, 1.1 ± 0.2 ; SEB, 0.8 ± 0.2 ; CpG-ODN + SEB, 0.9 ± 0.4) categories showed no significant differences in their responses (Fig. 1, E and F). Neither group showed significantly different TLR9 expression (Fig. 1, G and H).

Nonasthmatic Categories versus Asthmatic Categories Welch's *t*-Test

We examined the molecular responses of the asthmatic and nonasthmatic patients to CpG-ODN, SEB, and CpG-ODN co-administered with SEB.

CpG-ODN Stimulus. Differences in IL-5 expression between the two groups when exposed to CpG-ODN were statistically significant ($F = 11.93$; $***p = 0.008$). Rather than reduce the signal, CpG-ODN stimulated an increase in IL-5 expression in the asthmatic group (Fig. 2 A). No significant differences in p35 IL-12 (Fig. 2 D), IFN- γ (Fig. 2 G), and TLR9 (Fig. 2 J) between groups were detected.

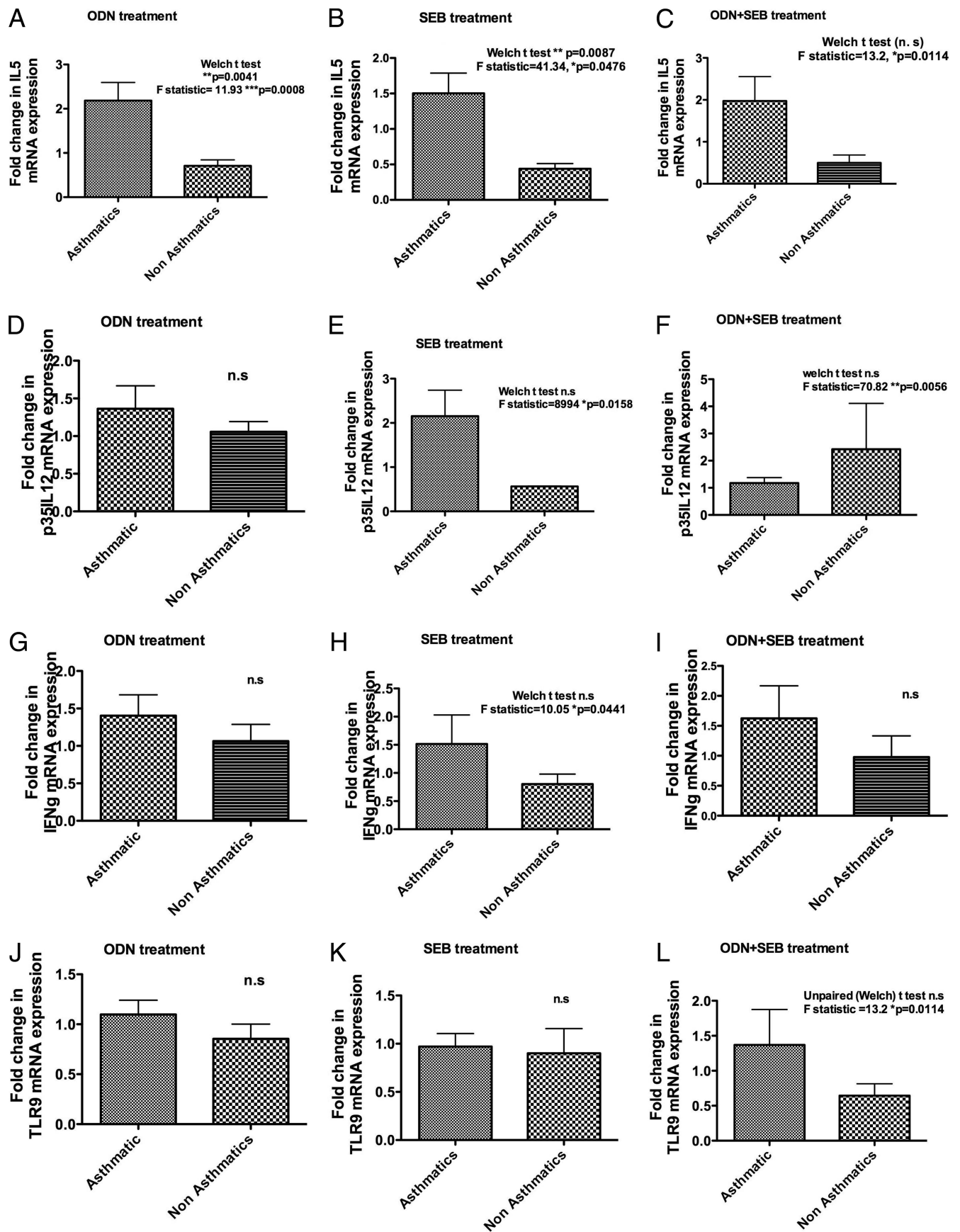


Figure 2. (A–L) Welch's t-test for asthmatic patients versus nonasthmatic patients. The statistically significant differences in the variances in the expression of the target genes to oligonucleotide (ODN), *Staphylococcus aureus* enterotoxin B (SEB), and ODN + SEB as shown by the large F-statistic value and $p < 0.05$ showed that the patients present as two distinct groups.

SEB Challenge. The asthmatic group was more sensitive to SEB challenge and recorded an increase in the expression of IL-5 ($F = 41.34$; $*p = 0.048$; Fig. 2 B), p35 IL-12 ($F = 8994$; $*p = 0.016$; Fig. 2 E) and IFN- γ ($F = 10.05$; $*p = 0.044$; Fig. 2 H) compared with the nonasthmatic category. No significant differences in TLR9 expression between groups were observed (Fig. 2 K).

CpG-ODN Coadministered with SEB. Nonasthmatic patients were responsive to the effect of CpG-ODN and SEB compared with asthmatic patients with regard to reduced IL-5 levels ($F = 13.2$; $*p = 0.011$; Fig. 2 C). Also, the higher p35 IL-12 expressed in nonasthmatic patients versus asthmatic patients were significant ($F = 70.82$; $**p = 0.006$; Fig. 2 F). Differences in IFN- γ between the two groups were not significant (Fig. 2 I). Asthmatic patients expressed higher TLR9 levels compared with nonasthmatic patients ($F = 13.2$; $*p = 0.011$; Fig. 2 L).

DISCUSSION

In this study the nonasthmatic group was able to be immunomodulated when treated with CpG-ODN. This was reflected in the reduced IL-5 expression, a known proinflammatory cytokine associated with exacerbating mucosal inflammation in CRS. Unexpectedly, we observed the opposite response to CpG-ODN in the asthmatic cohort of patients. We elected to use SEB as representative of the family of *Staphylococcus* superantigens known to be potent stimulators of proinflammatory cytokines such as IL-5. As anticipated, SEB challenge stimulated a rise in IL-5 expression in both subcategories. When CpG-ODN was coadministered with SEB, a significant dampening of the IL-5 stimulatory effect of SEB was seen in the nonasthmatic group but not in the asthmatic group of patients. Application of CpG-ODN was less effective in inducing any significant increases in p35 IL-12 and IFN- γ . We also found that CpG-ODN alone or when coadministered with SEB did not induce an increase in TLR9 expression in either asthmatic or nonasthmatic groups.

A significant contributor to the cause and maintenance of the chronic inflammatory state of the sinonasal mucosa is the release of Th2 cytokines such as IL-4 and IL-5 from the sinus epithelial cells in response to allergen stimulation.²⁴ These cytokines are also powerful chemoattractants of circulating polymorphonuclear cells as noted by the infiltration of eosinophils to the local site. This characteristic eosinophilia is responsible for further mucosal damage wrought by the action of major basic protein, a potent cytotoxic enzyme secreted by the activated eosinophils.^{25–27} We showed that IL-5 expression could be dampened in the nonasthmatic group by CpG-ODN treatment and in a clinical setting could be used as a means of treating chronic mucosal inflammation.

Unlike other researchers who have used murine models of asthma and allergic rhinitis, we have elected to use whole patient sinus tissue explants. The rationale being that we wish to approximate as closely as possible the human response to CpG-ODN treatment. We also considered an alternative *in vitro* wound healing model consisting of an epithelial respiratory monolayer that we established.²⁸ However, this would require pooling of epithelial cells from multiple patients that would have masked individual patient's response.

Notwithstanding the differences in models used, the cytokine responses detected in our sinonasal explant model mirrors some aspects of the study of Kline *et al.*²⁹ In their double

knockout eosinophilic airway asthma murine model where both IL-12 and IFN- γ genes had been disrupted, Kline *et al.* reported that after schistosome egg challenge, CpG-ODN treatment, albeit at higher doses, still conferred protection to these mice. Despite the absence of p35 IL-12 and IFN- γ cytokines, there was only a modest reduction in the protective effect of CpG-ODN in preventing airway eosinophilia and airway hyperreactivity. What was evident though was the suppressive effect of CpG-ODN on Th2 cytokine expression where neither IL-4 nor IL-5 was detected in the serum after exposure to allergen. They concluded that alternative pathways independent of IL-12 and IFN- γ were responsible for their observations. Similar redundancies in the regulation of immune response pathways may explain the cytokine response profile in our sinonasal explant model.

Our study was premised on TLR9 being expressed in human sinuses that was confirmed by Lane *et al.* who reported that TLR9 mRNA expression in the sinonasal mucosa. However, its expression was lower in CRS patients with recurrent nasal polyps compared with healthy controls.³⁰ This disparate pattern of expression was also evident at the protein level where the primary epithelial cells of CRS patients with polyps expressed a 50% lower level of TLR9 protein relative to healthy individuals.¹⁸ Also, our study showed that CpG-ODN did not stimulate significant increases in TLR9 expression in our patient cohort.

Differences in Cytokine Response between Asthmatic and Nonasthmatic Patients

The asthmatic and nonasthmatic patients behaved as two distinct groups based on their significantly different responses to CpG-ODN. A plausible explanation could be that asthmatic patients are genetically impaired in their immune response as alluded to by Wark *et al.* who described how primary respiratory epithelial cells isolated from asthmatic patients exhibited a defective Th1-type IFN response when challenged with rhinoviruses.³¹ Other innate immune signaling systems such as nucleotide oligomerization domain-like receptors although out of the scope of this study should also be considered.³² NOD-like receptors have the capacity to respond to a diverse range of molecules such as bacterial muramyl dipeptide to monosodium urate crystals by increasing proinflammatory cytokine expression. The unexpected increase in IL-5 responses to CpG-ODN in asthmatic patients may also be attributed to aberrant NOD-like receptor pathway signaling.

CONCLUSION

A fundamental challenge in the treatment of CRS is to reduce chronic inflammation of the mucosa thereby allowing the lining of the sinonasal cavity an opportunity to reepithelialize and recover functional ciliary activity. The high rates of disease recurrence in recalcitrant patients who are unresponsive to oral corticosteroids have been the impetus for exploring viable alternative treatments. In this context, the epithelium of the nasal mucosa and sinuses in the upper airways should no longer be regarded as just a mechanical barrier to inhaled aeroallergens and particulate matter but a biologically active interface amenable to interventions and that one pos-

sible option is to utilize allergens linked to CpG-ODNs in the treatment of nonasthmatic patients.

Our study showed that CpG-ODN has potential as an immunomodulatory molecule, especially when used in conjunction with a range of allergens. However, further *in vivo* studies using an animal model would be required before trailing the use of CpG-ODN as a localized therapy in the treatment of CRS.

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