

Immunostimulatory Sequences of DNA and Conjugates in the Treatment of Allergic Rhinitis

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Therapeutic strategies based on the use of immunostimulatory sequences of DNA containing a CpG motif (also known as ISS DNA, or CpG DNA) are focused on minimizing the allergenicity of immunotherapy while improving its effectiveness. Several ISS DNA-based methods of immunization (ISS DNA alone, ISS DNA conjugated to a protein allergen) have shown promise in animal models of asthma, and some of these ISS DNA-based therapies have entered clinical trials. These ongoing clinical trials will help to determine whether any of these ISS DNA-based therapies are safe and effective to use in subjects with allergic rhinitis and asthma.

Introduction

Immunostimulatory sequences of DNA (ISS DNA)-based vaccines (also known as CpG DNA) represent a novel therapeutic strategy in allergic inflammation, as they inhibit the Th2 cell from expressing several cytokines (interleukin [IL]-4, IL-5, IL-9, IL-13) important to the pathogenesis of allergic rhinitis and asthma. Recent studies in animal models of asthma, as well as preliminary studies in humans, have investigated the therapeutic potential of several ISS DNA-based vaccine strategies in allergic inflammation, including the use of ISS DNA alone, and ISS DNA conjugated to an allergen protein.

Immunostimulatory Sequences of DNA Inhibit Th2 Immune Responses

The immunostimulatory activity of ISS DNA is contained in a six base pair DNA sequence that follows the formula 5'-purine-purine-CpG-pyrimidine-pyrimidine-3' [1]. This sequence of DNA exerts its biologic function by binding to the Toll-receptor-9 (TLR-9), which is expressed on cells of the innate immune system [2]. Thus, ISS DNA acts indi-

rectly on T lymphocytes by inducing cells of the innate immune response (especially dendritic cells that express high levels of TLR-9) to express mediators that inhibit the Th2 response. ISS DNA inhibits expression of Th2 cytokines in the lung, including IL-4, IL-5, IL-9, and IL-13, while also reducing levels of expression of Th2 cytokine receptors such as the IL-4 receptor [3]. Recent studies have also demonstrated that ISS DNA induction of the enzyme indoleamine 2,3-dioxygenase could be an important mechanism by which ISS DNA inhibits Th2 responses [4]. In mouse models, pharmacologic inhibition of indoleamine 2,3-dioxygenase prevents ISS DNA from inhibiting Th2 responses in vivo, suggesting that ISS DNA induction of indoleamine 2,3-dioxygenase is important in mediating its anti-allergic effect [4]. The metabolites generated by indoleamine 2,3-dioxygenase that induce this beneficial inhibition of Th2 responses are currently unknown. The immune response to ISS DNA is also characterized by the expression of Th1 cytokines, including interferon (IFN)- γ , as well as IL-6, IL-10, IL-12, IL-18, IFN- α , and - β [3]. Although ISS DNA induces Th1 immune responses, this may not account for its ability to inhibit Th2 responses. This is suggested from studies in mice deficient in the Th1 cytokines IFN- γ or IL-12 in which ISS DNA remains effective in inhibiting Th2 immune responses [5].

Immunostimulatory Sequences of DNA in Mouse Models of Allergen-induced Asthma

ISS DNA can prevent Th2 cytokine responses, eosinophilic inflammation, and increased airway hyperreactivity to methacholine in mouse models of asthma when administered as preventive therapy prior to allergen inhalation challenge [6]. In addition, ISS DNA can be used to reverse an episode of established allergen-induced airway inflammation [7]. ISS DNA is effective in inhibiting eosinophilic airway inflammation when administered either systemically or mucosally to the airway; this effect lasts for at least 4 weeks after a single systemic dose. Interestingly, in preventive models of asthma in mice, a single dose of ISS DNA has been shown to reduce airway eosinophilia and airway hyperreactivity as effectively as systemic administration of corticosteroids administered for 7 days [6]. The

combination of ISS DNA and dexamethasone therapy is more effective than monotherapy with either agent alone in reducing airway hyperreactivity [7].

Immunostimulatory Sequences of DNA in Mouse Models of Viral-induced Asthma

Because respiratory syncytial virus (RSV) is one of the viral precipitants of asthma, the ability of ISS DNA to inhibit RSV-induced asthma has been examined in a mouse model [8]. Administration of ISS DNA to mice inhibits RSV replication within days of RSV infection *in vivo*, suggesting that the effect of ISS DNA is primarily due to activation of innate immunity as opposed to effects on adaptive immunity [8]. Activation of innate immunity by ISS DNA induces expression of the antiviral cytokines IFN- α , - β , and - γ in the lung, which is associated with significantly reduced RSV viral titers, peribronchial inflammation, and mucus secretion [8]. Thus, ISS DNA therapy not only prevents allergen-induced airway inflammation and airway hyperreactivity but could also have additional potential benefits in reducing the severity of RSV infections.

Immunostimulatory Sequences of DNA Prevent and Reverse Airway Remodeling in Mice

Recent studies have demonstrated that ISS DNA can prevent [9] as well as reverse [10•] airway remodeling in mice exposed to repetitive airway challenge with allergen. ISS DNA reduces the thickness of the peribronchial smooth muscle layer, accumulation of peribronchial myofibroblasts, and peribronchial fibrosis (peribronchial collagen III and V deposition) [9,10•]. This effect of ISS DNA on airway remodeling is likely due to its ability to inhibit expression of the profibrotic cytokine transforming growth factor (TGF)- β by eosinophils and macrophages. Studies in IL-5-deficient mice have demonstrated an important role for eosinophil expression of TGF- β in airway remodeling [11]. Interestingly, studies in humans with asthma have also suggested an important role for eosinophil expression of TGF- β in airway remodeling [12]. In these human studies, asthmatics treated with anti-IL-5 had a significant reduction in airway remodeling that was paralleled by a significant reduction of bronchoalveolar lavage (BAL) eosinophils, BAL TGF- β , and eosinophil expression of TGF- β , suggesting that eosinophil expression of TGF- β played a significant role in remodeling [12]. Thus, the ability of ISS DNA to reduce levels of eosinophil TGF- β expression may be one key mechanism by which it reduces airway remodeling. Overall, these studies suggest that ISS DNA not only prevents Th2-mediated airway inflammation in response to acute allergen challenge, but also inhibits TGF- β expression and airway remodeling associated with chronic allergen challenge.

Immunostimulatory Sequences of DNA in Primate Models of Asthma

To determine whether ISS DNA prevent airway hyperresponsiveness and airway remodeling in primates, rhesus monkeys with experimentally induced allergic airway disease were treated with inhaled ISS DNA periodically for 8 months [13]. Airway hyperresponsiveness was reduced significantly (two-fold) in ISS DNA-treated compared with control allergic monkeys. Airways from ISS DNA-treated monkeys had fewer mucous cells, fewer eosinophils, and fewer mast cells than control allergic monkeys. In addition, the thickness of the reticular basement membrane was reduced in ISS DNA-treated monkeys, suggesting an effect on airway remodeling. These studies suggest that ISS DNA inhibits Th2 responses and airway remodeling in primates as well as in mice.

In humans, as in mice, TLR-9 is the receptor for ISS DNA, and thus cell types that express TLR-9 (in particular plasmacytoid dendritic cells) respond to ISS DNA stimulation [2]. ISS DNA directly activates human plasmacytoid dendritic cells to express increased levels of co-stimulatory molecules and cytokines. Studies with human peripheral blood mononuclear cells have also demonstrated that ISS DNA inhibits Th2 responses [14]. Currently, there are no published results of studies investigating the safety and efficacy of ISS DNA *in vivo* in human allergy and asthma.

Conjugation of Immunostimulatory Sequences of DNA and Protein Allergen

Studies have investigated whether conjugation of ISS DNA to a protein allergen would enhance the Th1 biased immune response to the protein allergen. The rationale for conjugation of ISS DNA to a protein allergen is based on the hypothesis that the subcutaneously injected ISS DNA and conjugated protein allergen would be engulfed by the same antigen-presenting cell (APC), whereas when they are not conjugated and injected separately, the ISS DNA and the protein antigen will not always end up in the same APC. Support for this hypothesis is derived from studies in mice in which an ISS DNA protein conjugate was fluorescent-labeled, and its enhanced uptake to APCs tracked. Studies have demonstrated that dendritic cells bound more than 100-fold more ISS DNA conjugated to antigen compared with ISS DNA alone [15]. ISS DNA conjugated to antigen, therefore, potentiates the ability of dendritic cells to promote Th1 cellular immune responses [15,16]. The ability of ISS DNA conjugated to a protein antigen to induce a Th1-immune response is 100-fold higher than that induced by a mixture of equivalent amounts of ISS DNA and protein antigen [16]. In mouse models of asthma, ISS DNA conjugated to an allergen protein significantly reduces airway hyperreactivity to methacholine for at least 2 months [17]. The ISS DNA allergen protein conjugate is significantly more efficient than the unconjugated mixture in reducing airway eosinophilia and bronchial hyperreactivity [17].

Immunostimulatory Sequences of DNA Conjugated to Allergen Protein in Human Allergy

ISS DNA has been conjugated to the major ragweed protein Amb a I in a therapeutic product called AIC, which is undergoing human clinical trials in subjects with ragweed allergy. AIC inhibits Th2 and induces Th1-immune responses in peripheral blood mononuclear cells (PBMCs) derived from human patients with ragweed allergy [18]. ISS DNA conjugated to a ragweed protein was more effective than ISS DNA alone at enhancing Th1 (eg, IFN- γ production) and inhibiting Th2 (eg, IL-4 production) responses in studies of PBMC cultured in vitro with ISS DNA alone or cultured with ISS DNA conjugated to ragweed [18]. Human studies have also examined whether six injections of AIC administered to ragweed-allergic subjects out of season would redirect the immune response from a Th2 to a Th1 immune response [19]. In this study, recall immune responses of PBMC to ragweed, streptokinase, and phytohemagglutinin (PHA) were performed before and after the course of either AIC or placebo injections. AIC injections inhibited ragweed-specific Th2 responses by PBMC, as evident from decreases in the Th2 cytokine IL-5, and reductions in the Th2-dependent chemokines TARC (thymus and activation regulated chemokine, also known as CCL17) and MDC (macrophage-derived chemokine, also known as CCL22) [19]. In addition, AIC induced ragweed-specific Th1 immune responses at 2 and 16 weeks after the sixth injection, with significant increases in the Th1 cytokine IFN- γ , and increases in the Th1-dependent chemokines Mig (monokine induced by IFN- γ , also known as CXCL9) and IP-10 (IFN-inducible protein, 10 kDa, also known as CXCL10). Thus, AIC injected in concentrations that were approximately 40-fold lower than those used in most murine studies published to date led to a shift from Th2-immune responses to ragweed toward Th1-immune responses to ragweed. Cytokine and chemokine responses to the unrelated bacterial antigen streptokinase as well as the general ability to mount immune responses on polyclonal activation with PHA did not change. No clinically significant systemic or local allergic reactions were associated with AIC injections, and in these short-term studies during a 4-month period of follow-up, AIC appeared to be safe.

The ISS DNA conjugated to Amb a 1 protein induces significantly less basophil histamine release compared with ragweed alone [20]. Preliminary studies suggest that the ISS DNA conjugated to Amb a 1 protein is less allergenic than the allergen protein based on immediate hypersensitivity skin-test reactivity [20] and that a six-injection regimen of the ISS DNA Amb a 1 conjugate reduces symptoms of allergic rhinitis in ragweed-sensitized human subjects followed through the ragweed season in some but not

all studies [21,22•]. In a study in Montreal, Canada, patients with ragweed allergy and allergic rhinitis were treated with six escalating doses of AIC (0.06–12 μ g, $n = 28$) or placebo ($n = 29$) at weekly intervals immediately before the 2001 ragweed season [22•]. Symptom scores and medication use were recorded for the 2001 and 2002 ragweed seasons. Nasal biopsy specimens were taken on three occasions, once before AIC immunization (baseline) and on two occasions after immunization (pre- and post-first ragweed season). The post-AIC immunization nasal biopsy specimens (pre-season and post-season) were taken 24 hours after ragweed nasal allergen challenge, and the results of cytokine and inflammatory cell responses in these nasal biopsies were compared with the initial unchallenged nasal biopsy specimen using immunocytochemistry and in situ hybridization. These studies of nasal mucosal biopsies demonstrated a significant decrease in nasal mucosal eosinophils and Th2 cytokines (ie, IL-4), which was associated with a significant increase in Th1 cytokines (IFN- γ) in AIC-treated compared with placebo-treated subjects [22•]. However, during the first ragweed season, no difference between treatment groups was observed in rhinitis symptom scores or medication use. During the second ragweed season, there was a decrease in chest symptoms and a trend toward reduced nasal symptoms in the AIC-treated group. AIC was safe and well tolerated by all patients. This study suggests that short-course immunotherapy with AIC can modify the response of the nasal mucosa to allergen challenge by decreasing Th2 cytokine production and eosinophilia. This modification of the immune response in the nasal mucosa was not immediate but was observed 4 to 5 months after completion of AIC immunotherapy, and may account for the delay in evidence of any clinical efficacy to the second ragweed season. Further studies are therefore needed to address the issue of timing of AIC administration in relation to the ragweed season, as well as the need for booster AIC immunizations before each season.

Conclusions

A variety of DNA-based vaccine strategies (ISS DNA alone, ISS DNA conjugated to an allergen protein) have demonstrated in animal models the potential to prevent or reverse allergen-induced airway inflammation, Th2 cytokine responses, mucus production, airway remodeling, and airway hyperreactivity. Preliminary studies in humans have demonstrated the potential for immunotherapy with a conjugate of ISS DNA and ragweed protein to inhibit Th2 responses in nasal mucosa. Further ongoing studies in human subjects will determine whether any of these ISS DNA-based strategies are safe and effective in the therapy of patients with allergic rhinitis and asthma.

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