NEW METHODS OF IMMUNOTHERAPY IN ASTHMA AND ALLERGIC RHINITIS

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ABSTRACT

Allergen immunotherapy, first introduced in the early part of the twentieth century, is widely practiced despite several limitations. Considerable effort has been devoted to developing new anti-allergic therapeutic vaccines that, compared with conventional allergen immunotherapy, improve efficacy, decrease the time required to achieve effect, reduce inconvenience, and enhance safety. Increased understanding of the molecular biology of allergic respiratory inflammation has led to the development of therapeutic vaccines that potentially suppress or arrest the disease process in asthma or allergic rhinitis. This paper addresses a specific DNA vaccine approach in which highly active immunostimulatory phosphorothioate oligodeoxyribonucleotide moieties (ie, immunostimulatory DNA) are conjugated to the principal allergenic moiety of a relevant aeroallergen (ie, ragweed Amb a 1). A study group at Johns Hopkins University has recently completed the first human safety study of AIC, a new therapeutic vaccine composed of a conjugate of Amb a 1 and 1018 ISS, in patients with allergic rhinitis. The results demonstrate that AIC was several hundred-fold less reactive than licensed ragweed when evaluated by quantitative intradermal skin titration methodology. Furthermore, AIC reduced histamine release from basophils. The DNA vaccine induced IgE antibody production in patients with allergic rhinitis. AIC compared with licensed ragweed exhibited fewer local reactions on skin testing, a finding that suggests that AIC may offer the potential for an improved safety profile for immunotherapy. Additional trials to further evaluate the safety, immunologic effect, and therapeutic efficacy of AIC for ragweed-induced allergic rhinitis and asthma are ongoing.

INTRODUCTION

Increased understanding of the molecular biology of allergic respiratory inflammation in the patient suffering from allergic rhinitis and/or asthma has led to the development of specific therapeutic vaccines that afford the potential to suppress, if not altogether arrest, the disease process. These new molecular approaches include:

a) Specific antibody-directed therapy—anti-immunoglobulin (IgE);

b) Anticytokine agents—soluble interleukin-4 (IL-4)-receptor antagonists, anti-interleukin-5 (IL-5) antibody;

c) DNA vaccination with allergen incorporated into a plasmid; and

d) Specific T-cell-directed therapies such as

(i) Peptide immunotherapy with T-cell active epitopes; and

(ii) Adjuvant immunotherapy with immunostimulatory oligonucleotides (eg, CpG) linked to purified allergen.

IgE antibody is a critical determinant in the initiation of the allergic diathesis. It is found circulating freely and bound to tissue-resident mast cells and circulating basophils. In the context of the IgE-mediated allergic reaction, the mast cell is regarded as the pivotal cell in the ensuing allergic response. The mast cell elaborates a variety of inflammatory mediators (eg, histamine/prostaglandin D$_2$/leukotrienes) and specific cytokines (eg, IL-4/tumor necrosis factor) capable of inducing the subsequent allergic inflammatory cascade. Tangential to this direct pathway is the observation that antigen is also presented, independent of IgE, to the T cell. Within this context, it is recognized that the T cell is the principal orchestrator of the chronic inflammatory cascade as the T cell elaborates specific cytokines (eg, IL-3/4/5) and chemokines responsible
for recruitment and/or activation of a variety of secondary inflammatory cells including basophils, eosinophils, neutrophils, epithelial cells, and endothelial cells.\textsuperscript{5-7} Hence, therapies directed at manipulating the T cell, especially within the framework of the TH2:TH1 paradigm, may more effectively abrogate, or even arrest, the TH2-driven diathesis.\textsuperscript{8-10}

Conventional immunotherapy is a well-recognized method that aims to modulate an allergic patient’s immune response through administration of increasing doses of an extract, comprised of the aeroallergens to which the patient has been demonstrated to be allergic, and thereby to attenuate or eliminate the patient’s symptoms. Allergen immunotherapy was first introduced in the early part of the 20th century and has been widely practiced. Controlled clinical trials have demonstrated the therapeutic efficacy and detailed the favorable immunologic changes associated with allergen immunotherapy for the treatment of allergic rhinitis, asthma, and venom sensitivity.\textsuperscript{11-15}

However, this approach is saddled with a number of encumbrances including the need for frequent dosing over years—which impacts upon patient compliance; the need to administer a relatively large dose of the immunizing agent to achieve control of symptoms; and the potential for clinically significant allergic reactions to the treatment. Therefore, considerable effort has been devoted to developing improved therapeutic vaccines for treatment of allergic diseases to a) improve efficacy; b) decrease the time required to achieve effect; c) reduce the inconvenience and hence improve compliance with immunization regimens; and d) enhance safety.

Allergen dose is limited by systemic reactions to the respective allergens. Thus, efforts have largely been directed at decreasing the “allergenicity” of the antigens (ie, their potential for inducing an allergic reaction) while maintaining or heightening their immunogenicity (ie, their ability to induce a beneficial immunologic response). Although various chemical modifications of allergens have been attempted, the end-result has been that allergenicity and immunogenicity have either decreased or increased in tandem. Certainly, an allergenic vaccine with reduced allergenicity, but maintained immunogenicity, that could be given in a few doses would have important therapeutic implications. Millions of patients with poorly controlled allergic rhinitis and asthma who would be candidates for such a form of immunomodulation.

Other papers in this monograph focus on anti-IgE and cytokine antagonists. This paper addresses a specific DNA vaccine approach in which highly active immunostimulatory phosphorothioate oligodeoxyribonucleotide moieties (immunostimulatory DNA) are conjugated to the principal allergenic moiety of a relevant aeroallergen (eg, ragweed Amb a 1).\textsuperscript{16,17} This adjuvant approach may prove to be highly effective at directing the immune response toward upregulation of a more favorable TH1 phenotypic expression to counter-balance the untoward TH2-driven pro-inflammatory allergic process.

**Mechanisms of Immunotherapy**

To improve materials for immunotherapy, it is important to understand the pathophysiology of the disease process. Upon allergen exposure in a susceptible individual, there is an acute allergic response reflective of IgE-dependent mast cell activation resulting in release of histamine, leukotrienes, and other mediators. In addition, allergen is processed by antigen-presenting cells that display allergen in association with class 2 human leucocyte antigen molecules. If appropriate costimulatory signals are also induced, the result is T-lymphocyte activation with induction of TH2 cells that produce “proinflammatory” cytokines (eg, IL-4, IL-5, IL-6, IL-10). These cytokines induce recruitment of inflammatory cells such as eosinophils and basophils with the resultant development of airway inflammation.\textsuperscript{18-20}

The allergy group at Johns Hopkins took an early lead in examining the mechanisms by which allergen immunotherapy effects clinical improvement. As increasing doses of extract are injected, there is an initial elevation in the levels of both IgG- and IgE-specific antibodies. With continuing therapy, IgG levels further increase and plateau whereas antigen-specific IgE titers gradually decline toward pretreatment levels and are not boosted by subsequent environmental exposure to the allergen. Induction of IgG antibody is a predictor of clinical success albeit clinical benefit is not likely to be achieved until doses are large enough to risk anaphylaxis.\textsuperscript{21-24}

Nasal allergen challenge is a useful in vivo tool to evaluate the effect of immunotherapy. This provocation procedure causes release of mediators such as histamine, prostaglandins, and leukotrienes paralleled by acute clinical manifestations including sneezing, rhinorrhea, and mucosal swelling, and—in many allergic
patients—a late phase of inflammatory reactivity.\textsuperscript{25,26} Immunized patients demonstrate less immediate mediator release in their nasal secretions and less late-phase eosinophil migration.\textsuperscript{27-30}

Challenge techniques have been used to demonstrate that immunotherapy shifts T\textsubscript{H}2 cell responses toward T\textsubscript{H}1 activation. Nasal biopsies of grass-immunized rhinitic patients after a grass pollen extract challenge show a significant increase in message for specific T\textsubscript{H}1 cytokines [interferon alpha (IFN-\(\alpha\)) and IL-12]. This is paralleled by a significant reduction in allergen-induced accumulation of total numbers of CD4+ T cells and eosinophils. Interestingly, these techniques showed little down-regulation of the expression of cytokines from T\textsubscript{H}2 cells (eg, IL-4, IL-5).\textsuperscript{31,32} Tangential to these findings, Secrist et al cultured peripheral blood mononuclear cells from allergic patients receiving maintenance grass immunotherapy and demonstrated a significant decrease in allergen-induced IL-4 synthesis when these cells were exposed to allergen in vitro. However, no demonstrable effect on IL-2 or IFN-\(\alpha\) synthesis was observed in these immunized patients.\textsuperscript{33}

These studies demonstrate that immunotherapy has the potential to down-regulate not only the immediate-phase allergic reaction, but also late-phase T-cell-mediated responses. The problem lies in administering a dose large enough to induce the desired changes without causing intolerable allergic side effects. Towards this goal, Dynavax Technologies Corporation has developed a novel product consisting of ragweed allergen (Amb a 1) linked to immunostimulatory phosphorothioate oligodeoxyribonucleotide. The Amb a 1-immunostimulatory oligonucleotide conjugate (AIC) induces an enhanced, ragweed-specific T\textsubscript{H}1-type response in mice in comparison to either Amb a 1 alone or Amb a 1 + alum.\textsuperscript{16,34}

**Background for Adjuvant Approaches**

Several molecules including lipopolysaccharides, aluminum hydroxide salts, and Freund’s adjuvant have long been observed to possess immunostimulatory properties with enhanced response to antigen. Attempts have been made to capitalize on this observation to improve a vaccine’s immunogenicity through an enhanced adjuvant effect. Tokunaga and his colleagues made the initial discovery of the adjuvant effect of bacterial DNA with their study of the active components of Freund’s adjuvant.\textsuperscript{29,36} Subsequent confirmation of the sequence specificity of the immuno-stimulatory DNA was made by Pisetsky et al\textsuperscript{37} and Klinman et al.\textsuperscript{38,39} Furthermore, Krieg et al\textsuperscript{40} recognized that specific oligonucleotides derived from bacterial DNA could induce B-cell activation. Subsequently, Raz, Carson, and colleagues\textsuperscript{41,42} made the observation of enhanced immunostimulatory properties if these specific immunostimulatory sequences were incorporated into a plasmid.

Knowledge of the basis for enhancement of T\textsubscript{H}1 response by immunostimulatory DNA sequences is derived from recent recognition that bacteria induce an immune response that is characterized by a potent IL-12 activation of T\textsubscript{H}1 cells to secrete IFN-\(\alpha\) and a much lower level of activation of T\textsubscript{H}2 cells secreting IL-4 and IL-5.\textsuperscript{43,44} For example, bacterial DNAs are now known to possess immunostimulatory properties that are absent in vertebrate DNA. These properties are related to the higher frequency of CpG motifs and to the absence of cytosine methylation in bacterial as opposed to vertebrate DNA— which otherwise would abolish the immunostimulatory activity.\textsuperscript{45} The effects of bacterial DNAs can be mimicked using synthetic oligonucleotides, thus allowing a more accurate definition of the bacterial DNA immunostimulatory sequences (ISS). Early research studies initially identified optimal ISS sequences containing palindromic hexamers based on the general formula of: 5’-purine-purine-CG-pyrimidine-pyrimidine-3’ (eg, 5’-GACGTC-3’, 5’-AGCGCT-3’ and 5’-AACGTT-3’). More recent work has extended these observations to include CpG-enriched nonpalindromic oligonucleotides with ISS activity.\textsuperscript{8,46} Bacterial DNA and synthetic oligonucleotides containing ISS have multiple effects on the immune system including induction of B-cell proliferation and immunoglobulin production; secretion of IFN-\(\alpha\), \(\beta\), IL-12, and IL-18 [interferon gamma inducing factor (IGIF)], by macrophages; and IFN-\(\alpha\) secretion from natural killer cells. Such cytokines are involved in the differentiation of T\textsubscript{H}0 to T\textsubscript{H}1-type cells upon encounter with specific antigen. Therefore, bacterial DNAs appear to stimulate the innate immune system to produce IFN-\(\alpha\) and inducers of IFN, (IFN-\(\alpha\), IFN-\(\beta\), IL-12, IL-18) and foster a cytokine milieu that drives the adaptive immune response to antigens toward a T\textsubscript{H}1 phenotype.\textsuperscript{8,10}

**Effect of Immunostimulatory Oligonucleotide Sequences on Antibody Production**

The immunomodulatory effect of direct linkage or
conjugation of allergen with ISS is demonstrated in Figure 1. This graph shows that in a murine animal model immunization with a conjugate of Amb a 1 and 1018 ISS (AIC) promotes IgG2a antibody production that is significantly greater than that observed with either allergen mixed with ISS, allergen + alum, or allergen alone. In contrast, an inhibition of IgE production has been a corollary finding to this “protective” IgG antibody response. These observations bear particular relevance when it is recognized that in the murine model IgG2a is a marker of induction of a TH1 response; whereas IgE production is induced through TH2 mechanisms. Furthermore, these reciprocal IgG and IgE antibody responses are similarly reproducible in previously sensitized and not simply naive animals.16,47 This proffers the opportunity to not only use this approach as prophylactic intervention but also to employ this therapeutic construct in sensitized individuals that are in the midst of an allergic diathesis.

**EFFECT OF ISS ON CELLULAR PATHWAYS**

AIC results in the preferential induction of naive CD4+ T cells with differentiation toward a TH1 phenotypic profile. The corollary to this observation of a down-regulation in TH2-mediated responses to allergen (eg, IL-4, IL-5) is thought to be the result of macrophage or monocytic activation (IL-12, IL-18, IFN-α/β) with constituent effects on TH1 activation (increased IFN-α).16,17,47

Figure 2 demonstrates in a murine model the specific upregulation of IFN-α (TH1 profile) as a result of AIC immunization in contradistinction to the TH2 cytokine dysregulation that would otherwise be observed with allergen alone, allergen + alum, or allergen mixed with ISS but not linked to this specific adjuvant. Marshall et al17 have made similar observations by means of in vitro studies of peripheral blood mononuclear cells obtained from ragweed-allergic human subjects. Indeed this work demonstrates that cells exposed to AIC produced significant IFN-α (indicative of the TH1-response) with diminished production of IL-4/IL-5 (TH2-like profile).

**CLINICAL APPLICATIONS WITH DNA VACCINE**

Our study group at Johns Hopkins has recently completed the first human safety study of the use of immunostimulatory DNA in ragweed-allergic rhinitic
patients. This was a US FDA-defined clinical safety study that employed quantitative intradermal endpoint skin test titration to assess the relative potency of the AIC product to its comparator (standardized ragweed). The results of this study demonstrated that AIC was several hundred-fold less reactive than licensed ragweed when evaluated by this quantitative intradermal skin titration methodology.\(^4\) Furthermore, histamine release from basophils was similarly reduced in these ragweed-allergic subjects.\(^4\) Our first clinical trial with subcutaneous administration of AIC has demonstrated the ability of the DNA vaccine to induce IgG antibody production in ragweed-allergic patients. This is an important clinical observation since favorable clinical outcomes correlate with, and can be predicated by, induction of an IgG antibody titer.\(^4\)

Our observations that AIC exhibited fewer local reactions on skin testing, in comparison to licensed ragweed, suggests that this novel product may offer the potential for an improved safety profile for immunotherapy. We have initial observations that AIC has significantly reduced allergenicity as compared to a conventional ragweed extract, but additional clinical trials will be necessary to evaluate the immunogenicity and therapeutic efficacy of this product for treatment of ragweed-allergic disease. Indeed, we are currently proceeding with additional trials to further evaluate the safety, immunologic effect, and therapeutic efficacy of AIC as a treatment modality for ragweed-induced allergic rhinitis and asthma.

REFERENCES


