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Prospects for CpG Based Immunotherapy in Asthma and Allergy

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Abstract

Although administration of protein allergens to subjects with allergic rhinitis and asthma is effective in modulating the immune response and reducing symptoms, alternative strategies to minimize the allergenicity of protein immunotherapy while improving the effectiveness are currently being investigated in animal models of asthma and in human subjects with allergic rhinitis. CpG DNA, and CpG DNA conjugated to a protein allergen have shown promise in animal models of asthma and have entered phase I/II clinical trials. Further, clinical trials are needed to determine whether any of these CpG DNA based therapies are safe and effective to use in subjects with allergic rhinitis and asthma.

Introduction

The immunostimulatory activity of CpG DNA is contained in a six base pair DNA sequence which follows the formula 5'-purine-purine-CpG-pyrimidine-pyrimidine-3' [1]. This sequence of DNA does not code for a protein, but rather exerts its biologic function by binding to Toll-like receptor 9 (TLR-9), which is expressed on cells of the innate immune system [2]. The ability of CpG DNA to inhibit Th2 cells, a key participant in allergic inflammation, is the rationale for investigating its effectiveness as a novel therapy in asthma. CpG DNA does not inhibit Th2 responses by binding to T cells (which do not express TLR-9), but rather acts indirectly on T lymphocytes by inducing cells of the innate immune response (especially dendritic cells which express high levels of TLR-9) to express cytokines, which inhibit the Th2 response. The immune response to CpG DNA is characterized by the expression of Th1 cytokines including IFN- γ , as well as IL-6, IL-10, IL-12, IL-18, IFN- α and - β [3]. In addition to inducing Th1 responses, CpG 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]. More recent studies have demonstrated that CpG DNA also induces indoleamine-2,3-dioxygenase

(IDO), an enzyme classically known for its role in the tryptophan degradation pathway [4]. Studies have also demonstrated induction of IDO expression by immunostimulatory sequences (ISS) of DNA containing a CpG motif in a mouse model of asthma [4]. Administration of ISS results in induction of both IDO and inhibition of allergic inflammation suggesting that IDO expression might play a role in limiting allergic inflammatory responses [4]. Administration of a pharmacologic inhibitor of IDO to mice treated with ISS inhibited IDO activity as assessed by inhibition of kynurenine levels in the lungs [4]. In parallel, the pharmacologic inhibitor of IDO inhibited the anti-allergic effect of ISS. These studies suggest that ISS mediates its anti-allergic effect through induction of IDO and its metabolites.

CpG DNA in mouse models of asthma and airway remodeling

In mouse models of asthma, CpG DNA can prevent eosinophilic inflammation and airway hyperreactivity when administered prior to allergen inhalation challenge [5], and can also reverse eosinophilic airway inflammation and airway hyperreactivity when first administered at a time point when there was already significant eosinophilic airway inflammation [6]. CpG DNA is effective in inhibiting eosinophilic airway inflammation when administered either systemically, or mucosally to the airway, and this effect lasts for at least 4 weeks after a single systemic dose.

Recent studies have demonstrated that CpG DNA can prevent [7] and reverse [8] airway remodeling in mice exposed to repetitive airway challenge with allergen. CpG DNA reduces the thickness of the peribronchial smooth muscle layer, accumulation of peribronchial myofibroblasts, and reduces peribronchial fibrosis (peribronchial collagen III and V deposition). In addition, CpG DNA significantly reduces bronchoalveolar lavage and lung levels of the pro-fibrotic cytokine TGF- β , suggesting that inhibition of TGF- β expression may account for the reduction in airway remodeling. Overall, these studies suggest that CpG DNA not only prevents Th2 mediated airway inflammation in response to acute allergen challenge, but also TGF- β expression and airway remodeling associated with chronic allergen challenge.

CpG DNA allergen protein conjugate based therapy in allergy and asthma

At present there are no published results of studies investigating the safety and efficacy of CpG DNA *in vivo* in human allergy and asthma. However, preliminary results from human studies with CpG DNA conjugated to a protein allergen in patients with ragweed allergy have been reported. The rationale for the conjugation of a protein allergen to CpG DNA is based on the observation that the conjugated CpG DNA protein antigen induces a Th1 immune response that is approximately 100-fold greater than that induced by a mixture of equivalent amounts of CpG DNA and protein antigen [9]. This is hypothesized to be due to the subcutaneously injected CpG DNA and conjugated protein antigen being engulfed by the same antigen presenting cell. In contrast, when they are not conjugated CpG DNA and the protein antigen will not always end up in the same antigen presenting cell. As dendritic

cells bind > 100-fold more CpG DNA conjugated to antigen compared to CpG DNA alone, the conjugate potentiates the ability of dendritic cells to promote Th1 cellular immune responses [10]. In a mouse model of asthma the CpG DNA allergen protein conjugate is a 100-fold more efficient than the unconjugated mixture in reducing airway eosinophilia and bronchial hyperreactivity [10,11].

Studies in humans have thus far focused on conjugating Amb a 1, the major ragweed allergen, to CpG DNA. Similar to studies in mice, studies of human mononuclear cells demonstrated that a CpG DNA ragweed protein conjugate was more effective at enhancing Th1 immune responses and inhibiting Th2 immune responses compared to CpG DNA alone [12]. Preliminary studies suggest that a CpG DNA Amb a 1 conjugate is less allergenic than the allergen protein alone based on immediate hypersensitivity skin test reactivity [13]. This has allowed the development of an abbreviated six-injection up dosing regimen of the CpG DNA Amb a 1 conjugate to ragweed sensitized human subjects [13,14]. Studies of nasal mucosal biopsies in subjects with ragweed induced allergic rhinitis have demonstrated a significant decrease in nasal mucosal eosinophils and Th2 cytokines (i.e. IL-4), which was associated with a significant increase in Th1 cytokines (IFN- γ) in CpG DNA Amb a 1 conjugate-treated compared to placebo-treated subjects [15]. However, while these small-scale phase I/II studies have suggested the potential of this therapeutic approach, further large scale studies are needed to demonstrate whether this approach is safe and effective in the therapy of human allergy and asthma.

Conclusion

In pre-clinical studies in mouse models, CpG DNA and CpG DNA allergen protein conjugate strategies have demonstrated the potential to prevent or reverse allergen induced airway inflammation, Th2 cytokine responses, mucus production, and airway hyperreactivity. Further ongoing studies in human subjects will determine whether any of these strategies are safe and effective in the therapy of patients with asthma and allergic rhinitis.

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Discussion

Kalinke: Thank you for your very clear presentation. Right away I have a question. Various different CpG sequences behave differently in mouse and man and it is also known that TLR-9 expression patterns differ in mouse and man. So, how do you translate preclinical research into a development of therapeutics?

Broide: These are a few very important points. There are a variety of different immunostimulatory sequences in terms of the flanking regions and what might be the best sequence still needs to be investigated. As for human versus mouse, TLR-9 in the human system is predominantly expressed on a subset of plasmacytoid dendritic cells, which are the key cells responding in humans to the CpG. In the mouse system, however, many other cells in addition to dendritic cells, for example B cells and macrophages, can respond. So what you test in a mouse might not directly translate into a success or an effect in humans. Certainly, studying human cells is important as you move up, but getting some idea what is happening in the mouse system helps at least in terms of the AIC compound. With that DNA sequence you can get effects in the mouse and in the human that are similar.

Chapman: Has anyone looked at whether the AIC has immunomodulatory effects on other ragweed allergens when you have done these studies? And also are there any plans to go ahead with trials with any other allergens?

Broide: In terms of other ragweed allergens, other than Amb a 1, they have looked at total ragweed IgE and it tends to mirror the pattern of Amb a 1-specific IgE. But we have not looked at any other specific components of ragweed. In terms of conjugation to any other antigens, I think it would depend on whether this turned out to be successful or not. It is conceivable, but you first need to prove it a little more effectively that it works.

Bousquet: Congratulations for this excellent presentation. I have a small problem with ISS. There are five studies which have been performed. The Canadian study has been published it is vastly and entirely negative, because the very small effect on asthma in the second year is likely to be artifactual, because when you have a 0.3 risk on a 3.0 scale and you have 0.4 as a maximum level, then anything which is below might be very artifactual. The paper of Peter Creticos has not been published, even though it has been finished about two years ago. Then there are three big studies which have been performed, but we have no information on it. Do you not think that we need to ask the company to put all these studies either in the web or to publish these studies? Because then we will have a full evaluation of what ISS are currently doing. I am not saying that later they may show efficacy, but today I think we vastly need more information.

Broide: I could not agree more with you that we need more information. In terms of the studies in which I have participated and presented today, sponsored by the NIH and the Immune Tolerance Network, the company who makes this product is called Dynavax. I do not work with Dynavax and I am not a consultant to them. So I have no relation, financial or other, with them and cannot tell them what to do with their data. But I can tell you, because I am a co-author on the paper with Peter Creticos, that this is currently in a revised manuscript and we are hopeful that the revision will be accepted so that everyone can review the data and come to their own conclusions.

Drachenberg: I remember a report from Prof. Zinkernagel from Switzerland who reported horrendous toxicological problems with CpG motifs in mice.

Broide: If you used CpG at a high enough dose there could be potential toxicity with any therapy. When you administer CpG sequences to mice in doses that are three to five thousand times greater than what we are administering in humans you can get effects on spleen size and you can induce abortion. So there are effects that you can see, but they really occur at very high doses only that are not being administered in humans and you do not see that in the human studies. But again, more patients need to be studied. But certainly, if you used an excessively high dose that would be something to be concerned about. There are also studies with CpG alone, which has been investigated as an adjuvant for vaccines and in cancer studies. So beyond the allergy studies there is safety data from many other conditions other than just the allergy studies that I am talking about today.

Kalinke: Another comment to this: I think it is very well established that TLR-9 plays a role in certain autoimmune diseases, such as SLE and rheumatoid arthritis and so on. So I think it is an important issue to keep eyes open with respect to safety concerns.

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Seppälä: My question is a more technical one. How do you control the number of ISS conjugated to the allergen? How do you know that you have four, and not six or eight?

Broide: I am not a chemist, but the chemist can show you gels where one, two, three, four or how many ISS are conjugated and you can see that they run at different sizes on a gel. That is how they do the quality control.