A promising CpG adjuvant-loaded nanoparticle-based vaccine for treatment of dust mite allergies

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Keywords: allergy • asthma • biodegradable • CpG • Der p2 • house dust mite allergens • nanoparticles • PLGA

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We have recently reported on the development of a nanoparticle-based vaccine for protection and treatment against dust mite allergies [1]. The development of such a vaccine is important because in the USA, 84% of homes have measurable levels of house dust mite (HDM) allergens and a quarter of these houses have greater levels of allergens than the proposed limit for asthma [2]. Continuous exposure to HDM allergens can lead to lung inflammation as evidenced by lung eosinophilia and airway obstructions. This can lead to asthma attacks in sensitized individuals. Persistent exposure to HDM allergens stimulates dendritic cells (DCs) in lungs to initiate T-helper 2 (Th2) immune responses [3]. These Th2 responses promote eosinophil recruitment to lungs, inflammatory cytokines; and B cell production of immunoglobulin (Ig) E antibodies [4]. Existing strategies for treatment of the symptoms of dust mite allergies include the use of β_2 agonists and neutralizing antibodies such as Xolair® (omalizumab). Subcutaneous or sublingual immune-based therapies have been developed and tested to generate HDM-specific long-term tolerance, and have shown promising results in adults and children. However, a drawback to such approaches are that they require regular treatments of high doses of HDM allergens for at

least 3 years to show acceptable clinical efficacy. As such, there is a critical need for a long-term solution to HDM-induced asthma. The increased levels of HDM allergens present in households creates a need for the development of a vaccine that can switch the inflammatory immune response induced by HDM allergens to a protective immune response. This vaccine should ideally induce T-helper 1 (Th1) immune responses that result in production of IFN- γ and IgG2a antibodies [5]. The vaccine would also ideally reduce Th2-stimulated airway inflammation and initiate protective immunity by Th1 cells that would reduce symptoms associated with the allergy.

Dermatophagoides pteronyssinus (Der p) is one of the most common allergy-causing mites and produces Der p2, which is a strong immunogenic allergen [6]. Serum samples of 79% of patients suffering from asthma, rhinitis and/or wheezing test positive for titers of anti-Der p2 IgE antibodies, making it a strong potential allergen for development of vaccines against HMD allergies [7]. However, subcutaneous injections of Der p2 allergen alone to mice caused adverse increases in the production of IgE antibodies and Th2-biased immune responses [8]. This resulted in the need for a vaccine formulation that allowed for co-delivery of the allergen with adjuvants

that promote Th1-biased allergen specific immune responses to reduce lung damage linked with exposure to allergen [9]. Unmethylated cytosine-phosphate-guanine (CpG) oligonucleotides are used as a potent adjuvant for shifting immune responses to the Th1 type [10,11]. It is a Toll-like receptor-nine agonist which activates DCs and B cells to produce Th1-type cytokines and suppresses Th2-triggered allergic responses [9]. Co-administration and/or chemical conjugation of CpG with HDM allergen has been shown to decrease eosinophilia and IL-5 production while increasing the production of IFN- γ [12]. Peripheral DCs isolated from healthy patients vaccinated with ragweed allergen conjugated to CpG induced increased levels of Th1 cytokines and decreased levels of Th2 cytokines [13]. Chemical conjugation of CpG with allergen does have some limitations. It is expensive and can lead to structural alteration of fused molecules changing their immunostimulatory properties. Furthermore, *in vivo* cleavage of the bridge linking the allergen and adjuvant can prevent co-delivery of molecules to the same DCs.

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An alternative co-delivery method is to vaccinate with CpG and Der p2-loaded biodegradable poly(lactic-co-glycolic acid) (PLGA) polymer particles. In addition to co-delivering multiple molecules, PLGA particle-based vaccines can stimulate strong Th1 type responses as determined by measured levels of IgG2a antibodies. We have shown that PLGA particles loaded with antigen and CpG can stimulate potent antigen-specific immune responses when compared with vaccination of antigen and CpG in solution [14,15]. We have also shown that biodegradable particles loaded with CpG and antigen generate stronger antigen-specific IgG2a antibody responses than CpG-antigen conjugates at later time points. This is presumably because of the sustained release characteristics of the particle-based vaccine [16]. In addition, we have shown that the magnitude of immune response generated is correlated with the size of PLGA particles used for immunization [15]. While large particles loaded with antigen and CpG are known to produce high levels of total IgG1 antibodies, nanoparticles containing antigen with CpG have been shown to induce higher ratios of IgG2a to IgG1 antibodies. To develop a vaccine against allergy-associated lung disorders, stimulation of high IgG antibodies and Th1 type immune responses is necessary. Th1-based immunity would be expected to decrease the secretion of IgE antibody and inflammatory damage to lungs follow-

ing exposure to allergen. We have recently shown that PLGA nanoparticles that co-deliver CpG and Der p2 can generate increased Der p2 specific IgG2a antibody responses and decreased Der p2 specific IgE antibody responses. In these studies, we carefully characterized the effects of the size of PLGA particle vaccines and the influence of CpG in the overall immune response to Der p2-coated PLGA particle vaccines [1]. We showed that the size of the PLGA particle and the presence of CpG had a significant impact on the extent and type of immune response stimulated against Der p2 antigen. Three different sizes of CpG-loaded PLGA particles were prepared using a double emulsion solvent evaporation and differential centrifugation method. Mice were vaccinated with Der p2 and the different sizes of blank or CpG-loaded PLGA particles. The vaccinated mice were then exposed to intranasal instillation of Der p2 daily for 10 days. This was followed by euthanization to determine leukocyte accumulation in bronchoalveolar lavage (BAL) fluids, antibody response profiles and airway hyper-responsiveness. Vaccination with the PLGA particles resulted in a size-dependent decrease in the level of eosinophils found in BAL fluids. Mice vaccinated with nine micron-sized blank PLGA particles coated with Der p2 showed increased levels of IgE and IgG1 antibodies, as well as increased airway hyper-responsiveness. Loading CpG into PLGA particles of all sizes prevented airway hyper-responsiveness after Der p2 exposures. Inflammatory responses to Der p2 exposure were significantly reduced when smaller PLGA particles were used for vaccination. In addition, loading CpG in PLGA particles increased Der p2-specific IgG2a antibody levels. Our studies have shown that the size of PLGA particles used for vaccination plays a significant role in the prevention of HDM-induced allergy and that incorporation of CpG into the PLGA particles preferentially develops a Th1-type immune response. This biodegradable nanoparticle-based vaccination strategy has significant potential for treating HDM allergies and is ripe for further development. Future studies will need to evaluate the potential of the vaccine in a therapeutic model of pre-existing allergy and will need to identify the optimal dose concentrations, dose intervals and number of doses for generating maximum therapeutic efficacy. In addition to control over size [15], biodegradable nanoparticles are amenable to further manipulations such as changing surface chemistries [17], conjugating cell binding ligands, changing the polymer used [18] and packaging multiple synergistic adjuvants into the nanoparticles to further enhance the therapeutic response. The particles can also be used as a prime to alternative vaccination strategies or combined with small-molecule-based therapies to generate stronger therapeutic outcomes [19,20].

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