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Recombinant house dust mite allergens

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ABSTRACT

House dust mites (HDM) are a globally important source of allergen responsible for the sensitization of more than 50% of allergic patients. Specific immunotherapy with HDM extracts is effective but allergen extracts cannot be fully standardized and severe side-effects can occur during the protracted course of treatment. The introduction of molecular biological techniques into allergy research allowed the indentification of more than 20 groups of HDM allergens. Recombinant HDM allergens can be produced in defined concentrations and consistent quality and allow the development of vaccines for HDM allergy with reduced allergenic activity and retained immunogenicity. The immunotherapy trials in pollen allergic patients with recombinant pollen allergens/hypoallergenic allergen derivatives have shown that this treatment is effective and indicated that recombinant HDM vaccines might improve immunotherapy of HDM allergic patients. Here we report the steps for the development of vaccines for HDM allergy. After selection of the most prevalent HDM species, the panel of allergens to be included into a therapeutic vaccine for HDM allergy needs to be determined. HDM allergens with high IgE-binding frequency and clinical relevance will be modified into hypoallergenic variants and evaluated for their allergenic activity and immunogenicity. Derivatives with reduced allergenic activity but with retained immunogenicity would be good candidates for a HDM vaccine for safe and efficient immunotherapy.

1. Immunotherapy for HDM allergy

The most frequent triggers for asthma attacks in subjects with house dust mite (HDM) allergy are thought to be non-allergenic nocuous insults acting on inflamed airway such as infections and irritants [1]. This differs from pollen-induced rhinitis, venom-induced anaphylaxis and cat allergy where the symptoms are most frequently caused by hypersensitivity reactions directly triggered by the allergen. It is therefore probable that the mechanism for optimal immunotherapy will be different. Although increasing attention is being paid to events that precede allergic sensitization in asthma it should nevertheless be appreciated that the probability of HDM-allergic children developing asthma is proportional to their anti-HDM IgE titre [2,3] and poor prognosis is associated with the early development of the IgE antibody and early sensitization for anti-HDM Th2 cytokine responses [4,5]. Interference with the sensitization either by HDM allergen immunotherapy or by the administration of anti-IgE monoclonal antibody omalizumab markedly ameliorates the disease for most people. Indeed omalizumab was found to be most effective for HDM-allergic children living in HDM infested homes and for cockroach-allergic children living in cockroach infested homes showing a specific anti-allergy action [6]. Subcutaneous injections of allergen extracts can ameliorate the three major diseases associated with HDM allergy, namely asthma, rhinitis and atopic dermatitis. To take examples, the double blind placebo controlled trial of Pifferi et al. [7] showed that the number of exacerbations of asthmatic children declined from 8 to 2 per year, β-blocker use dropped from 40 to 20 days per year and corticosteroid use from 20 to 5 days with very significant improvements in bronchial hyperreactivity to an average within the normal range. Similarly the double blind placebo controlled trial of Garcia-Robaina and colleagues [8] resulted in 50% reduction in symptom scores and medication use and bronchospasm induced by inhalation of HDM extract needing twice the dose of extract for a given drop in lung function. A similar degree of benefit for nasal symptoms was reported for patients with allergic rhinitis [9] and immunotherapy has been shown to decrease the symptoms of atopic dermatitis patients from moderate to mild with reductions in erythema, secondary skin infection, itch, sleep disturbance and lichenification [10,11].

Sublingual immunotherapy with HDM extract has also be examined and has the potential to reduce the anaphylactic side ef-

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fects produced by injected allergen and the need for the protracted course of injections. Several double blind placebo controlled trials have demonstrated that HDM sensitized subjects gain an improvement similar to found for subcutaneous immunotherapy [12–14] although there are reports to the contrary. Scrutiny of one these, which examined small treatment groups for 6 only months, did in fact show improvements of a similar magnitude to other studies but there was a large variation in the placebo [15] and another, not only examined rhinitis patients with few symptoms, but concomitantly instigated HDM avoidance and optimisation of corticosteroid treatment throughout the trial [16].

2. Allergen specificity

The objective of specific immunotherapy is to determine the allergen responsible for the sensitization of patient and then to administer it in a way that stimulates the patient's immune system to turn off or counter-act harmful response to the allergen. Evidence that the therapy used today, such as the injection of a succession of progressively increasing doses of allergen, is mediated by allergen-specific changes in the responses of allergen reactive antibodies and T cells is surprisingly scant. There are reports that describe specific immunological changes [17] but others show a lack of specificity [18]. There are also conflicting reports on the specificity of clinical efficacy. Successful ragweed immunotherapy for subjects with dual grass and ragweed sensitivity has been reported to only alleviate symptoms in the ragweed and not the earlier grass pollination season [19]. Conversely however a trial with sublingual immunotherapy of dual birch and grass pollen allergic patients found therapy with either allergen reduced symptoms during both the grass and birch pollen [20]. The treatment was however most effective for the homologous allergen and allergy combinations and a mixture of allergens was the most effective. Reports showing that children given immunotherapy with an extract do not develop new sensitizations to other sources of allergen also point to non-specific mechanisms [21,22]. Repeatedly administering allergens to sensitized people with the attendant cytokine and chemokine cascades could elicit all manner of specific and non-specific effects while the action of blocking antibody and allergen-specific regulatory cells would be specific. The clinical specificity of the different types of immunotherapy could reflect their mechanism of action and help identify immunological or inflammatory changes that could be monitored when developing new strategies.

3. Advantages from recombinant allergens

Broadly the advantages would be (1) the use of defined reproducible formulations, (2) the use of balanced formulations, (3) access to large amounts of allergen, (4) removal of non-allergen inflammatory stimuli and (5) entree to genetically modified allergens.

The current standardisation procedures for extracts measure their ability to induce skin test responses not their composition. A recent survey found that ratio of the two major allergens from *Dermatophagoides pteronyssinus* Der p 1 & 2 differed at the extreme by 16-fold between extracts and not uncommonly from 3–5-fold [23]. Only 4/13 extracts contained detectable quantities of all of the mid-tier allergens Der p 5, 7 and 21 (to be described below) and it was not uncommon to be unable to detect any of them [23]. The use of formulations with known and therapeutic doses of the important allergen would not only improve the prospect for efficacy but would allow reproducible investigations to made to establish optimal protocols. The poor balance of the concentrations of different allergens creates the inherent problem of trying to achieve therapeutic doses with an allergen present in low concentration when allergens at higher concentrations have the ability to cause anaphylactic side effects. The sometimes large discrepancy in the group 1 and 2 concentrations in *D. pteronyssinus* extracts, usually in favour of Der p 1, would not help achieve optimal desensitization to Der p 2 and some mid-tier allergens have been reported to be present in 100 fold less concentrations [24]. It should be noted that the concentration of the allergens in the extracts does not reflect the quantities made by the HDM, Der p 7 for example being made in similar quantity to Der p 2 [25]. In fact the culture conditions used to make HDM extracts are optimised to produce high amounts of the group 1 and 2 allergens and do not represent the growth conditions found in homes. Industrial scale recombinant technology has the ability to provide large quantities of allergen and this may have special application for modified allergens designed to be administered in large quantity without side effects and sublingual therapies where, as best studied for pollen allergy, about 10-fold more allergen than the current subcutaneous regimens is required. Extracts contain inflammatory molecules including enzymes such as kallikreins [26] and although not documented certainly ceramides and other immunomodulatory lipids as well as β-glucans [27] and the ever popular endotoxin [28]. Immunotherapy might be improved if predetermined and known immunomodulators were added to the allergens but the uncontrolled and varying presence of unknown modulators will not help establish a reproducible medicament. As will be discussed below recombinant allergens provide one the avenues to construct modified allergens for new types of immunotherapy and this might be the most important application.

4. The important allergens

The allergenic potential of different HDM allergens has been assessed by quantitative IgE binding with panels and purified and recombinant allergens [29–32] and by absorption of the IgE staining moieties on 2-D western blotting [33]. The IgE binding to Der p 1 and Der p 2 has been found to constitute 50–60% of the IgE binding to all of the HDM allergens for essentially all HDM allergic subjects with the summated titres to Der p 1 and 2 tightly correlating with the binding to extracts. The mid-tier allergens Der p 4, 5, 7 and 21, each of which only bind IgE in about 50% of patients, bind individually and collectively in proportion to the major allergens constituting over 30% of the total titre. This consistent proportional pattern provides an excellent platform for selecting recombinant allergens. Importantly the IgE binding pattern was the same in spectrum and proportion for children admitted to a hospital emergency department for asthma compared to children and adults with controlled and mild disease [30] and was the same for subjects with persistent and frequent disease compared intermittent asthmatics [34]. The formulation required for severe disease is therefore the same as that required for milder allergy. Results consistent with the IgE binding analyses have been obtained with skin test in several European countries [35].

The structures of these allergens are well defined [36]. The group 1 allergens are cysteine proteases similar to papain. The group 2 allergens are structurally the same as the myeloid differentiation (MD) antigen-like lipid binding proteins (ML domain proteins). Indeed there is evidence that Der p 2 can mimic the action of MD-2, which loads LPS unto toll-like receptor (TLR)-4 to activate an innate inflammatory cascade [37] and Der f 2 binds lipopolysaccharide (LPS) with high affinity in a manner similar to MD-2 [38]. The group 4 allergens are typical α-amylases [39] and the group 7 allergens are structurally related to the LPS binding bactericidal permeability increasing protein (LPB/BPI) family of proteins [40] that include the major horse allergen Equ c 3 and the cat allergen Fel d 8 [41]. The group 5 and 21 allergens are re-