

Vaccination with transgenic rice seed expressing mite allergen: a new option for asthma sufferers?

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Background

Immunotherapy for allergic diseases

Acute allergic inflammatory responses are mediated by provocation of allergen-specific CD4⁺ Th2 cells, together with class switching of B cells to generate IgE antibodies that bind to mast cells and basophils via their high affinity Fcε receptors. This process is known as allergic sensitization. Subsequent attachment of antigen to the Fab portion of cell-bound IgE results in the release of the contents of cytoplasmic granules (e.g., histamine) from mast cells and basophils as well as in the synthesis and secretion of biologically active arachidonic acid derivatives (e.g., leukotrienes). Thus, the induction of IgE responses is highly dependent on allergen-specific CD4⁺ T cells (Th2 cells) [1].

Conventional allergen-specific immunotherapy commonly consists of repeated subcutaneous injections of crude extracts for periods exceeding 3 years. T cells are key regulators of the pathogenesis of allergic disease. Clinical treatment of immunotherapy using short peptides containing T-cell epitopes (peptide immunotherapy) has been shown to induce unresponsiveness of T cells or changes in their cytokine production (e.g., IL-4), particularly by CD4⁺ T cells. Currently, very few patients (<5%) choose immunotherapy, as treatment takes several years and because subcutaneous

administration is associated with local and, in some cases, even systemic allergic side effects if allergen accidentally reaches the circulation. In order to resolve these problems, development of safer, more effective and more convenient allergen-specific immunotherapy is highly desirable. To achieve this, first, tolerogen used for desensitization to allergen needs to be changed from crude allergen extract to modified hypoallergenic tolerogen with reduced IgE binding or enzymatic activity, so that high doses can be administered with decreased side effects, such as anaphylaxis. Second, the route of administration should be changed from systemic to mucosal (oral, sublingual and nasal administration), resulting in increased convenience and relief of pain from needle stick. The WHO has determined that immunotherapy via subcutaneous, sublingual or nasal routes is effective [2]. On the other hand, the efficacy of oral vaccination has not yet been evaluated, although many long-term investigations are ongoing. For routine use of oral immunotherapy, it is critical to protect orally administered therapeutics from enzymatic digestion and inactivation in the GI tract by enhancing their bioavailability [3].

According to the most recent CDC documentation, the prevalence of asthma in 2001–2003 was at 6.7% in adults and 8.5% in children, and asthma burden

increased by more than 75% from 1980 to 1999 [4]. In most children, asthma develops before the 5 years of age, and, in more than half, before 3 years of age [4]. Repeated subcutaneous injections cause much discomfort for children. In addition, there are technical challenges for the practice of sublingual immunotherapy as an alternative for younger children under 3 years of age, because allergen extract usually has to be kept under the tongue for several minutes. Therefore, oral administration of subunit vaccines offers many advantages for babies and infants. Mucosal delivery of peptide/protein therapeutics in rice-based baby food via the oral route is a desirable strategy not only for children but also in adult immunotherapy.

Engineering of transgenic rice expressing mite allergens for oral vaccination

In designing an oral vaccine, mucosal antigens need to be made more effective in particulate form (encapsulated form) than soluble antigens (purified antigens) at both the site of response induction and the effector sites of the mucosal immune system. One reason is that particulate antigen complexes are more resistant to the harsh gastric environment, such as low pH, and the presence of digestive enzymes in the GI tract. Another reason is that particulate antigens are taken up by the specialized epithelial M cells with greater efficiency than soluble molecules, providing a higher antigen concentration at the M cells on the Peyer's patches of mucosal inductive sites, and acting as a depot for the slow release of antigens. Thus, transgenic rice seed containing mite allergen is an excellent approach for the development of oral immunotherapy.

Approximately 45–80% of patients with allergic asthma are sensitized to allergens from the house dust mite (HDM), especially *Dermatophagoides farinae* and *D. pteronyssinus* (Der p)-derived products, suggesting that HDM allergens are crucial for the development of bronchial asthma [5,6]. Most of the predominant Der p 1 T-cell epitopes for both human and murine T cells are located within the sequence from amino acids 45–145 of the mature Der p 1 protein [7]. Thus, an expression cassette of Der p 1 (45–145) was introduced into the rice genome by *Agrobacterium*-mediated transformation [7,8]. Der p 1 (45–145) containing immunodominant T-cell epitopes was specifically produced in endosperm cells and mainly deposited in a spherical endoplasmic reticulum-derived protein body (PB-I) [7,8]. Another study by our group showed in simulated gastric fluid that PB-I-localized T-cell epitopes exhibited higher resistance to enzymatic digestion than either chemically synthesized or irregular-shaped protein storage vacuole (PB-II)-localized forms [9]. Furthermore, the dose of T-cell epitopes required for suppression of allergen-specific IgE responses in mice was approximately 20-fold or sixfold less than chemically synthesized material when fed in PB-I or PB-II localized form [9]. As an addition to previous technology, this method might lead to the use of bio-encapsulation in PB-I for broad applications as a viable strategy to achieve more efficient mucosal delivery of oral peptide/protein therapeutics.

Great potential for oral immunotherapy with transgenic rice

To investigate the clinical efficacy of antigen-specific immunotherapy based on oral vaccination with plant products *in vivo*, we tested the effect of the consumption of transgenic rice seeds containing an immunodominant fragment of the Der p 1 (45–145) in a murine model of asthma. Depending on the suppression of airway inflammation and the level of serum antigen-specific IgE antibody, we found that production of Th2-type cytokines by peripheral CD4⁺ T cells *in vitro* was significantly reduced following oral vaccination with the transgenic rice in this murine asthma model, but not Th1-type cytokines [7,8]. Allergen-induced infiltration by eosinophils and neutrophils into the airways, and bronchial hyper-responsiveness, were also inhibited by oral vaccination in animal models [7].

“...immunization with particles of rice seed might represent an advanced system for induction of antigen-specific immune responses only...”

The central mechanisms of immunotherapy via the oral route are thought to be induced and maintained at the Th cell level for the suppression of immune responses, calling for ‘oral tolerance’. Clonal deletion and anergy, which can be elicited by large doses of antigen, are characterized not by only the absence of antigen-specific T-cell proliferation and decreased IL-2 production but also non-specific bystander suppression, resulting in the establishment of oral tolerance due to the induction of regulatory T cells [10]. However, our results demonstrated that immunotherapy based on oral vaccination with transgenic rice does not induce non-specific bystander suppression without regulatory T cells [7,11]. These findings suggested that immunization with particles of rice seed might represent an advanced system for induction of antigen-specific immune responses only, to avoid unexpected nonspecific immune responses (bystander suppression). The cellular mechanisms responsible for bystander suppression remain unclear. In the near future, it will be interesting to investigate the mechanisms of different immune responses observed in the induction of immune suppression by antigen deposited in PB-I of transgenic rice seed.

In conclusion, the novel approach of oral immunotherapy using this rice-based antigen delivery system to gut-associated lymphoid tissue is potentially applicable to bronchial asthma in humans.

Financial & competing interests disclosure

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