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Allergen-specific immunotherapy with plant-based oral vaccines

“Allergen-specific immunotherapy based on mechanisms inducing immune tolerance against allergens for IgE-mediated type I allergy can be controlled more easily than immunotherapy using immune induction for vaccines against infectious diseases.”

Vaccines currently on the market are produced in *Escherichia coli*, yeast or insect cells. However, there is some evidence that plants could provide a more efficient production platform for vaccines than these. Plant-based vaccines are also advantageous in terms of their scalability, no cold chain required, stability, safety, cost-effectiveness and needle-free administration [1]. In particular, when antigen is expressed in seed, immunogenicity is not lost even if stocked at ambient temperature for several years [2]. Over the past two decades, many plant-made vaccines against infectious diseases have been produced [1,3]. Trials undertaken to feed these transgenic plants to animals and humans in clinical trials revealed their efficacy in protecting against infections through induction of target-specific systemic and mucosal immune responses. Furthermore, the feasibility of allergen-specific immunotherapy using plant-based vaccines has also been demonstrated recently.

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In industrial countries, 30–40% of the population suffers from some kind of allergic disease such as asthma, allergic rhinitis, conjunctivitis and atopic dermatitis. Treatment strategies for these allergic diseases generally involve pharmacotherapy using antihistamines, corticosteroids and other anti-inflammatories [4]. However, although these approaches reduce the clinical symptoms, they are not curative. It is well established that allergen-specific immunotherapy is the only way to modulate these immune reactions, with effects that persist for many years without further treatment. Conventional

allergen-specific immunotherapy has been practiced for almost a century. Success can be achieved by repeated subcutaneous injections of increasing doses of native allergen extracts over a period of at least 3–5 years. This treatment is sometimes accompanied by severe side effects, such as anaphylaxis, which is caused by capture of allergen together with specific anti-allergen IgE on the surface of mast cells and basophils. The development of safer, more effective and convenient allergen-specific immunotherapy has therefore long been desired. To achieve this, first, natural allergen extracts used for induction of immune tolerance (desensitization) need to be replaced by recombinant allergens with reduced allergenicity [5]. In order to develop such ideally hypoallergenic tolerogens, deletion, site-directed mutagenesis or molecular shuffling have all been introduced to alter the tertiary structure of the allergen and, thus, alter specific IgE binding [6]. Peptide immunotherapy using dominant T-cell epitopes derived from allergens represents another approach to developing safer treatment, because regions involved in allergenicity (i.e., responsible for reactivity with IgE) can be completely removed while retaining immunogenicity [7]. Second, the route of administration should preferably be changed from subcutaneous injection to mucosal application (oral, sublingual and nasal delivery). This would result in a safer and more convenient vaccine. Some modified recombinant allergens (Mal d 1, trimeric Bet v 1 and Bet v 1 fragment) have already been tested in animal models and evaluated as vaccines for subcutaneous and sublingual immunotherapy in allergic patients [7].

For practical use of plant-based allergy vaccines, high and consistent immunogen expression is critical for efficacy. To maximize the yield in a crop, the transferred genes encoding these tolerogens will need to be synthesized using codons optimized for expression in the selected

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target tissue [8]. Since the accumulation of recombinant proteins is also largely dependent on intracellular localization, it is important to target them to suitable locations by means of intracellular targeting signals [8]. In the case of seeds, a signal peptide leading to the secretory pathway is mandatory for stable accumulation of product. Strong tissue-specific promoters, rather than constitutive promoters, are preferable for high-level expression of antigens in the desired tissue. We have reported that seed is a good production platform for recombinant proteins because there is ample and stable storage space for foreign products. For example, for expression of antigens in transgenic rice seed, strong endosperm-specific promoters, such as those for the major storage protein glutelin, the 26-kDa globulin or 10-kDa and 16-kDa prolamin, have been utilized for expression in rice endosperm. Using these promoters, target gene products were highly accumulated as distinctly detectable proteins in CBB-stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels, accounting for 4–6% of total seed proteins (60–100 µg/grain of approximately 20 mg). Thus far, hybrid T-cell epitope peptides from Japanese cedar pollen allergens Cry j 1 and Cry j 2, subunit antigen from major mite allergen Der p 1 or modified mite allergen Der f 2, in which all the Cys residues were mutated to Ser (Ala), were all found to be highly accumulated in the endosperm of transgenic rice. Artificial hybrid T-cell epitope peptides, subunit Der p 1 or structurally altered mutagenized Der f 2 peptides were found to be deposited in endoplasmic reticulum-derived protein bodies or in specific compartments. These recombinant proteins are consistently inherited over several subsequent generations without silencing. Furthermore, these antigens remain stably present without loss of immunogenicity, even when the seed is stocked at ambient temperature for several years.

After oral administration of transgenic rice seeds containing major T-cell epitopes from cedar pollen allergens, mice were challenged in a preclinical model. Compared to mice fed non-transgenic control rice, proliferative responses of allergen-specific CD4⁺ T-cells, specific IgE levels and production of histamine were all significantly downregulated [9]. Allergen-associated Th2 type cytokines, such as IL-4, -5 and -13, were also inhibited by rice-based T-cell epitope peptide feeding. Furthermore, allergy symptoms such as sneezing and nose scratching were alleviated after exposure to cedar

allergens. These results indicate that mucosal immunization with rice seeds containing T-cell epitopes efficiently induced immune tolerance.

Similar preventive effects against mite allergen exposure causing asthma were observed in mice fed with transgenic rice containing the Der p 1 (p45–p145) subunit, encompassing most human and mouse major T-cell allergenic epitopes. Oral administration of this transgenic rice clearly reduced the levels of antigen-specific serum IgE and IgG through suppression of IL-4 and IL-13. Furthermore, infiltration of eosinophils into the airways and development of bronchial hyper-responsiveness was also inhibited through suppression of IL-5.

For induction of mucosal immune tolerance, repeated administration of allergens through mucosal routes is required. In the case of allergens modified to increase safety, their administration at high concentration is possible, resulting in shortening of the treatment regimen. However, compared with subcutaneous injection (systemic administration), much higher doses of antigens (10–100-fold) are generally required to achieve the same level of efficacy via mucosal immunotherapy. When antigen is produced in transgenic plants and delivered orally, it is more resistant to gastrointestinal enzymes than purified vaccine. This is because seed-based antigen is bioencapsulated by double barriers characteristic of plant cells (protein body as a unique protein-storage organelle, and the cell wall). This protects the antigen from digestive enzymes in the gastrointestinal tract. Vaccine packed in protein bodies provides an especially excellent delivery system to gut-associated lymphoid tissue (GALT). Oral delivery of antigens has other benefits as well, because it rarely causes side effects. Furthermore, immune tolerance to specific antigen is inclined to be induced easily, as with food proteins.

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Seed-based oral immunotherapy can offer very cost-effective production of T-cell epitope peptide or recombinant hypoallergens. Large amounts of antigens can easily be produced in fields, depending on current levels of demand by patients suffering pollen, house dust mite or other allergies.

Vaccine antigens must be produced in conformity with the specific guidelines and regulations for pharmaceutical products, under conditions of good manufacturing practice (GMP) [10]. It may be necessary to develop processing steps (purification) for each product, because currently approved antigens have to be purified. The downstream processing stage of production is generally expensive, representing up to 80% of overall production costs. It is important to note that direct oral vaccination using products of transgenic crops without any further processing is highly advantageous in terms of efficient delivery to the GALT and stability. Crop seeds containing antigen act as vehicles for mucosal delivery (natural coated oral formulations). Validation to be observed in plant production systems is one other obstacle to commercialization as a pharmaceutical product. There are usually variations in accumulation of product owing to physiological differences in biological systems.

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Furthermore, there are concerns regarding outcrossing with wild-type crops in the case of open field cultivation. Pharmaceuticals should be produced under the control of specific regulations and guidelines. Closed molecular farming facilities (closed glasshouses) with strictly controlled environmental conditions would be ideal and are recommended in order to reduce

variation of product concentration in targeted tissue as well as to prevent outcrossing. However, in the case of allergy vaccines, very large quantities of seeds will be required for commercialization. For example, daily oral consumption of cooked transgenic rice (~50–100 g) for a minimum of 3 months, and continuing for up to a few years, will be required for desensitization against cedar pollen allergens; and such transgenic rice will have to be produced for approximately 20 million patients afflicted with Japanese cedar pollinosis. It would be quite impossible to cultivate the required amount of transgenic rice in closed glasshouses similarly to most plant-based pharmaceuticals. Allergen-specific immunotherapy based on mechanisms inducing immune tolerance against allergens for IgE-mediated type I allergy can be controlled more easily than immunotherapy using immune induction for vaccines against infectious diseases. Different production systems, such as cultivation in physically isolated fields (areas) and biological containment, will have to be considered and specific guidelines and regulations will have to be developed for this purpose. Needless to say, entry into the food chain during transportation and handling will need to be strictly avoided.

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