

Oral Immunotherapy for Allergic Diseases Using Transgenic Rice Seeds: Current State and Future Prospects

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Key Words

Allergic disease · Asthma · Oral immunotherapy · Rhinitis · Transgenic rice

Abstract

Allergen-specific immunotherapy (IT) has been shown to provide clinical benefit for patients with allergic diseases. At present, subcutaneous and sublingual ITs are mainly authorized for clinical treatment. Oral administration of allergens seems to be the easiest way to achieve IT, though it has yet to be translated to the clinical setting, mainly due to the requirement of a large amount of allergens. Plants, especially rice seeds, have recently been recognized as superior allergen carriers for oral administration, because of their high productivity, stability and safety. Therefore, in order to establish clinically applicable oral IT, we have been developing transgenic rice seeds (Tg rice), in which major epitopes of cedar pollen allergens or house-dust mites (HDM) are expressed. The efficacy of this orally administered Tg rice was confirmed in murine models of allergic rhinitis and bronchial asthma. In the safety study of the Tg rice, no adverse effects on cynomolgus macaques were observed. In this review, we summarized the current state and future prospects of allergen-specific IT, focusing particularly on oral IT with allergen-expressing Tg rice.

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Introduction

The number of patients with allergic diseases has continuously increased over the last few decades. For example, the prevalence of allergic respiratory disorders including allergic rhinitis and bronchial asthma in 10 European countries has already reached 24% [1].

Since a century ago, when Noon [2] described that allergen-specific immunotherapy (IT) desensitized patients with grass pollen allergy, this therapy has been recognized as an effective means to treat allergic diseases. Several possible mechanisms of allergen-specific IT, including the production of IgE-blocking antibody, the expansion of regulatory T cells and the induction of T cell anergy and apoptosis, have been demonstrated [3–9]. Most importantly, IT has the potential to cure allergic diseases, in contrast to other medical treatments with relievers, preventers and symptom controllers [10]. Currently, the clinical use of subcutaneous IT (SCIT), sublingual IT (SLIT) and nasal IT are established. However, in order to improve the efficacy and safety of IT, several trials to modify allergens, adjuvants and routes of administration are still in progress [11].

In addition to an increase in adult patients, the prevalence of bronchial asthma, allergic rhinitis, eczema and food hypersensitivity in 3- to 6-year-old children is al-

ready over 10, 3, 20 and 20%, respectively, in several European countries [12]. For these younger children, subcutaneous injections cause much discomfort and having to keep drugs under the tongue is challenging. Therefore, the development of a new IT in which allergens can be administered via an oral route is desired. Due to several problems, oral IT has yet to be clinically applied, but we have been developing a new concept employing transgenic rice seeds (Tg rice) in which specific allergen epitopes are expressed. In this review, the potential of Tg rice-based oral IT as an easier, safer as well as effective treatment of allergic diseases is discussed by comparison with and summarizing of other types of ITs.

Allergen-Specific IT

Allergen-specific IT provides meaningful clinical benefits for patients with allergic diseases induced by a variety of allergens such as grass, weeds, tree pollens, house-dust mites (HDM) and cat and dog epithelia [13, 14]. SCIT injection is the most popular procedure to administer allergens and this has been demonstrated to reduce the IgE/IgG4 ratio, an augmentation of allergen-specific IgA, and increase interleukin (IL)-10 production [3–5]. As SCIT requires repeated allergen injections and involves the risk of severe side effects including anaphylactic shock, safer, painless and noninvasive IT with oral, sublingual or nasal administration procedures have been explored as alternatives (table 1) [15]. SLIT, in particular, is an improvement on the safety profile of SCIT, and has therefore been applied for the treatment of allergic rhinitis [15]. The clinical efficacy and safety of SLIT in patients with other allergic diseases, such as bronchial asthma, food allergy and atopic dermatitis are currently being confirmed clinically or preclinically [15]. Nasal IT, on the other hand, though effective for the treatment of allergic rhinitis [16], has not been widely used probably due to the complicated instructions.

Oral IT for Allergic Diseases

The gut is the largest immune organ responsible for surveillance of ingested materials. Oral tolerance is the state of local and systemic immune unresponsiveness induced by oral allergen administration, and it has been proposed as the strategy to establish new therapeutic disciplines, including oral IT.

Multiple mechanisms of oral tolerance are implicated. Upon oral administration, low-dose allergens favor the expansion of regulatory T cells while high-dose allergens favor the induction of clonal anergy/deletion of allergen-specific T cells [6–9].

Successful induction of tolerance and desensitization by oral IT has been evaluated in animal models but also in patients with a food allergy to milk, egg and peanuts, and those with autoimmune diseases [17–19]. Even though it has also been reported that oral IT is effective for the treatment of allergic rhinitis and bronchial asthma in murine models [20, 21], this therapy has yet to be used clinically. One of the major reasons for prohibiting the clinical application of this therapy is the requirement of a large amount of allergens to exhibit therapeutic efficacy. For example, in the report of Taudorf et al. [22], the amount of birch extracts required for the downregulation of nasal symptoms in birch pollinosis patients by daily oral administration was about 200 times larger than that used in conventional SCIT. In addition, oral IT still causes a variety of side effects, even though these are usually less severe than those evoked by SCIT. For example, several patients with food allergy suffer from gastrointestinal symptoms such as vomiting after oral administration of allergens [18, 19]. These side effects may be avoided by using heat-denatured proteins, mutant allergens or T cell-epitope allergen peptides, though they cannot overcome the issues regarding the requirement of massive allergens. Further studies are needed to establish effective and safe oral IT.

Advantages of Rice Seed-Based Oral IT

To solve the current problems attending oral IT, plants have recently been recognized as superior allergen carriers for administration [23]. Compared to other protein expression systems based on bacteria and mammalian cell cultures, plants involve a much lower risk of human pathogen contamination [24–26]. Cereal crop seeds have the capacity to produce a relatively large amount of recombinant products [27]. It was shown that products in such seeds were stable for more than 6 months at room temperature [26]. Furthermore, the edible parts of plants such as the fruit and the crop seeds can be administered to people of all ages without purification. For these reasons, it has been considered that the seeds of cereal crops, especially rice which is the major staple diets in Asian countries, can be used in oral IT (table 1).

Table 1. Characteristics of allergen-specific ITs

	SCIT	SLIT	Nasal IT	Oral IT	
				rapid	slow
Stage	clinically applied	clinically applied	clinically applied		
Target diseases	allergic rhinitis bronchial asthma clinical trials are ongoing venom allergy	allergic rhinitis clinical trials are ongoing bronchial asthma food allergy	allergic rhinitis	clinical trials are ongoing food allergy autoimmune diseases preclinical stage allergic rhinitis bronchial asthma	preclinical stage allergic rhinitis bronchial asthma
Efficacy	effective	effective	effective	still to be confirmed in large-scale clinical studies	still to be confirmed in clinical studies
Administration					
Routes	subcutaneous	sublingual	nasal	oral	oral
Difficulty	difficult	easy for adults difficult for younger children	techniques required	easy	easy
Places	in hospital	at home	at home	in hospital	at home
Comfort	painful, administered by a doctor	painless, simple and convenient	painless	painless, simple and convenient	painless
Duration	long	long	6 months to 1 year	short	long
Side effects	rare but serious anaphylaxis respiratory symptoms cardiovascular symptoms skin symptoms	fewer and less severe than with SCIT oropharyngeal symptoms gastrointestinal symptoms asthma, urticaria and abdominal pain (quite rare)	rare and local	moderate gastrointestinal symptoms skin symptoms respiratory symptoms	rare in macaques unknown for humans
Allergen storage	2–8 °C	2–8 °C	stable at room temperature	depends on allergen	stable at room temperature

Effect of Tg Rice in a Murine Model of Allergic Rhinitis

Japanese cedar pollinosis has become a serious social problem in recent years, because as many as 20% of the Japanese population is suffering from this disease [28]. Two major allergenic proteins, Cry j 1 and Cry j 2, have been identified in cedar pollen allergens [29, 30]. We developed Tg rice, accumulating a fusion protein of mouse T cell-epitope peptides in Cry j 1 and Cry j 2, and a soybean storage protein, glycinin A1aB1b (Cry J Tg rice) [31]. Oral administration of Cry j Tg rice to BALB/c mice inhibited cedar pollen allergen-induced IgE and IgG responses and T helper 2 (Th2) cytokine synthesis (IL-4, IL-5 and IL-13). Accordingly, allergen-induced sneezing response as well as histamine release was attenuated [31]. These findings suggest that oral IT with Cry j Tg rice is useful to treat cedar pollen-mediated allergic rhinitis.

Effect of Tg Rice in a Murine Model of Bronchial Asthma

The exposure to HDM is strongly associated with the development of allergic diseases, such as bronchial asthma, allergic rhinitis and atopic dermatitis [32, 33]. *Dermatophagoides pteronyssinus* (Der p) and *D. farinae* are important sources of indoor allergens associated with bronchial asthma [32, 34]. We developed Tg rice expressing both human and mouse T-cell epitopes of Der p group 1 allergen (Der p 1), the major allergen in HDM (Der p 1 Tg rice) [35, 36]. Similar to the case of Cry j Tg rice, oral administration of Der p 1 Tg rice to BALB/c mice suppressed Der p 1-induced IgE and IgG responses and Th2 cytokine synthesis. In addition, allergen-induced inflammatory cell infiltration into the lungs and bronchial hyperreactivity were diminished [36]. These findings suggest that rice seed-based oral IT is useful for the treatment of not only allergic rhinitis but also bronchial asthma.

It is interesting that administration of a lower dose (approx. 5 g/kg/day) of Der p 1 Tg rice, compared with the dose employed in the above experiment (approx. 50 g/kg/day), also suppressed allergen-induced infiltration of inflammatory cells into the lungs, whereas allergen-specific IgE production was not affected [37]. Even though the reason for the difference in the effect on IgE production between high-dose and low-dose Der p 1 Tg rice is unclear, the effectiveness of oral IT with Tg rice in allergic diseases may be caused, at least in part, by mechanisms other than the downregulation of IgE synthesis. Importantly, in the

low-dose experiment, the efficacy for allergic inflammation was achieved by administering an amount of Tg rice that is reasonably consumable by human beings in everyday life.

Safety and Preclinical Study of Tg Rice Seeds

Seven predominant human T cell epitopes have been identified in Cry j 1 and Cry j 2 [38]. We demonstrated that peripheral blood mononuclear cells from 92% of patients with cedar pollinosis showed positive T-cell responses to a hybrid peptide in which these seven epitopes were linked together (7Crp) [39]. On the other hand, cedar pollen allergen-specific IgE detected in most of the patients was not reactive to the hybrid peptide. Therefore, 7Crp has the potential to modulate T-cell responses without inducing IgE-dependent side effects [39]. We developed Tg rice accumulating 7Crp (7Crp Tg rice) to be applied for clinical use [40]. Oral administration of 7Crp Tg rice to B10.S mice that recognize one of the 7 epitopes suppressed allergen-induced IgE and T-cell responses [40]. In addition, the effect of 7Crp Tg rice was almost unchanged even after boiling at 100°C for 20 min. This also points to the usefulness of this 'humanized' Tg rice in treating cedar pollen-mediated allergic diseases.

The biochemical safety profile of 7Crp Tg rice must be carefully examined prior to its clinical use. There was no significant difference between Tg and non-Tg rice, in the levels of nutrients such as amino acids, fatty acids, minerals and vitamins [41]. In the safety study with daily oral administration of high and low doses of steamed 7Crp Tg rice to cynomolgus macaques for 26 weeks, no serious adverse effects were observed [42]. In addition to further investigations into validation and quality control, another study for evaluating the clinical safety and efficacy of 7Crp Tg rice is currently being scheduled.

Conclusion

It appears that rice-based oral IT has the potential to improve the quality of the treatment of allergic diseases, in comparison with established drug therapies and other types of IT. We have demonstrated the usefulness and safety of this new therapeutic approach mainly via pre-clinical animal studies. Even though a couple of issues have still to be examined, such as the clinical dose, form of administration and efficacy in human diseases, oral IT with Tg rice may well become an effective means for the treatment of allergic diseases in the near future.

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Disclosure Statement

The authors declare that no financial or other conflict of interest exists in relation to the content of the article.

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