

Transgenic rice for allergy immunotherapy

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Allergic reactions represent exaggerated or inappropriate adaptive responses to a wide variety of environmental triggers. These hypersensitivity reactions typically involve the skin, respiratory tract, and gut. Allergy is a significant health problem; it currently affects >15% of children and adults worldwide and is rising in incidence (1). Allergy appears to be a major factor in the pathogenesis of most asthma and rhinitis, especially in children and young adults (2). Allergies can adversely affect the quality of life, and reactions to inhaled or ingested allergens can be severe and can lead to death. Although a number of immune factors participate in allergy, interaction of IgE antibody with mast cells that express high-affinity IgE receptors (FcεR1) is central to the most severe and immediate form of hypersensitivity (type I). Allergen-specific IgE attachment predisposes mast cells to activation, because contact with multivalent allergen crosslinks surface-bound IgE and leads to the release of inflammatory autocooids, including histamine (3). Current therapies such as corticosteroids and bronchodilators are nonspecific and expensive and are associated with adverse side effects (4). For these reasons, there has been an urgent search for more effective and specific therapies, including neutralization of IgE with monoclonal antibody (omalizumab) (5) and targeting of inflammatory cytokines and chemokine receptors (6). Although these strategies hold promise, they focus primarily on inhibiting effector phases of allergy, whereas prevention of allergy would likely be more effective. The article by Takagi *et al.* (7) in this issue of PNAS is timely, because it presents an approach using transgenic rice to express and orally deliver specific peptide epitopes of tree pollen allergens to attenuate the development of allergic immune responses and inhibit allergen-specific IgE production. Because this approach is specific, is unlikely to be harmful, is cost-effective, and might be applied to other immune-mediated immune diseases, it holds great appeal as well as questions (Fig. 1).

Oral administration of soluble antigens has been recognized for some time as a method to suppress cellular and humoral immune responses. Mechanisms include T cell deletion or inactivation



Fig. 1. Transgenic rice has benefits and potential limitations in immunotherapy.

(anergy) and protective regulatory cells, which likely arise from interactions with gut-associated lymphoid tissue (GALT) (8). Although oral tolerance has obvious appeal in its potential to suppress inflammatory disorders, its capacity to treat or prevent human disease has not yet been firmly established. There are a number of factors in establishing oral immune tolerance, including the age, genetic background, and species of the host. However, most critical is the identification of relevant antigens and antigen dose and having knowledge of which CD4⁺ T cell subsets are involved in a given disease. Oral administration of the human autoantigen GAD expressed by transgenic tobacco can prevent autoimmune diabetes by specific inhibition of CD4⁺ T helper 1 (Th1) cells (9). In contrast, the success of allergy immunotherapy is critically linked to the opposite response, namely inhibition of CD4⁺ Th2 responses and IgE production. Because suppression of Th1 responses can concurrently allow more Th2 activity, there have been concerns that Th1-directed oral immunotherapy may aggravate allergy and IgE antibody production. Fortunately, in the article by Takagi *et al.* (7), as well as in previous reports, oral administration of allergens clearly inhibits Th2 cells or inhibits Th1 cells without increasing total IgE, reflecting the complexities of oral toler-

ance (9, 10). Takagi *et al.* (7) report the successful use of an orally delivered peptide vaccine in rice that reduces allergen-specific Th2 responses. Although this report demonstrates the potential clinical utility of using genetically modified rice in allergy therapy, it raises interesting questions regarding the capacity of orally delivered plant-expressed antigens to variably regulate Th1 and Th2 cells.

Within the gut, various immunocompetent helper and regulatory T cells encounter nonharmful exogenous food-derived antigens and can distinguish normal intestinal flora from infectious pathogens (11). It therefore seems intuitive to use the gut immune system to attenuate or eliminate peripheral immune responses to antigens that trigger autoimmune disease or allergy. However, specific components of the immune response need to be targeted correctly. In the case of IgE-mediated allergy, the question remains of whether bias in the development of Th2 cell suppression is related to the antigen *per se* or to the form in which it is delivered, or whether this bias is intrinsically related to host factors. Increasing evidence has indicated that early postnatal responses to oral antigens tend to be Th2-biased, consistent with a higher incidence (6–8%) of food allergy in the first 3 years of life. Interestingly, at least in the case of peanut allergy, which is associated with high levels of IgE, peanut antigen does not intrinsically induce Th2 skewing. The type of response depends on the patient's allergic status, and nonallergic children and those who have outgrown their allergy show "normal" Th1 skewing to peanut allergens (12). Given the importance of host factors, the choice of mouse strain in vaccine studies is very important. The use of BALB/c mice used by Takagi *et al.* (7) is well grounded and perhaps even underestimates the Th2 suppression the authors observed using transgenic rice (13). Alternatively divergent results in Th1/Th2 bias in oral tolerance studies may relate to the antigen itself. In studies using germ-free animals, intestinal microbiota can induce a shift toward Th1 immunity to dietary anti-

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gens (14). Inflammatory stimulation including adjuvants at the time of antigen challenge also appears to be very important in triggering Th1/Th2 bias in oral tolerance (15, 16). Therefore, both host and antigen factors will need to be considered in human trials.

Peptide antigens, such as those used by Takagi *et al.* (7), have advantages and potential disadvantages in immunotherapy. Immunodominant peptides can be used for their exquisite specificity in targeting immune responses. Also, because short linear peptide sequences generally lack the ability to crosslink antigen-specific IgE, a particular advantage of T cell epitope peptides in desensitization is the avoidance of IgE-mediated activation. Thus, inhibition of Th2 responses can occur without anaphylaxis. Early promise with peptide therapy in experimental models of allergy has led to evaluation of synthetic peptides for immunotherapy in clinical trials (17). However, because peptide vaccines must contain at least one major histocompatibility complex (MHC) binding motif to be successfully presented to the immune system, the polymorphic nature of human MHC may impede the success of peptide therapy (18). Takagi *et al.* (7) demonstrate in rice that the production of mouse T cell immunodominant peptide (Cry jI and Cry jII) allergens of Japanese cedar (*Cryptomeria japonica*) pollen is abundant when fused to the soybean seed storage protein glycinin. Daily oral delivery of transgenic rice seeds containing $\approx 70 \mu\text{g}$ of the fusion protein for 4 weeks inhibited the development of allergen-specific serum IgE and IgG antibodies in BALB/c mice; reduced the allergen-specific proliferative responses of CD4⁺ T cells; decreased the secretion of allergen-specific Th2 cytokines such as IL-4, IL-5, and IL-13; and decreased the level of serum histamine in mice systemically primed with intact cedar pollen antigen. Takagi *et al.* (7) were also able to show clinical benefit in a mouse model of upper-airway pollen allergy using orally administered transgenic rice seeds.

These results strongly support the clinical feasibility of allergen-derived peptides expressed in rice being used in treating airway allergy. Alternatively, the use of intact allergens rather than peptides in plants has shown benefit; oral consumption of transgenic lupin seeds expressing sunflower seed albumin can attenuate sunflower seed hypersensitivity and experimental asthma (19). Although the potential benefit of transgenic plants is obvious, the complexities of MHC as well as T and B cell agretopes may remain formidable hurdles in developing peptide vaccines in plants for allergic diseases, particularly in those in which little is known about the relevant allergens.

The benefits of a plant-based oral vaccine for allergy include affordability, absence of complicated storage or delivery systems, safety from human pathogens, and possibly greater efficacy than with other systems. The use of rice seed systems as a carrier has advantages beyond transgenic plant leaf or root expression. Seeds, as a major storage organ, are designed to be natural reservoirs of products required for seedling growth, including substantial amounts of protein. Rice is easy to store and transport, and recombinant proteins expressed in seeds are highly stable at room temperature. One of the major limitations of the expression of recombinant antigens in transgenic plants for oral tolerance remains the achievement of high levels of expression. To be clinically feasible, expression levels must be sufficiently high that protein purification with its associated high costs are not required. Currently, the level of expression achieved for the majority of transgenic proteins of medical interest has been in the range of 0.01–0.40% of total soluble proteins (20). Achieving high levels of expression of recombinant peptide antigens in transgenic plants is challenging, because small peptides cannot form stable secondary structures and thus are more vulnerable to proteolytic degradation in plant cells. Takagi *et al.* (7) address this challenge by expressing the two

mouse dominant T cell epitope peptides of Cry jI and Cry jII allergens as a fusion protein with the soybean seed storage protein glycinin under the control of the robust rice seed storage protein glutelin promoter *GluB-1*. Using this strategy, accumulation reached 0.5% of the total seed protein, which is likely sufficient for clinical testing in humans, given the edible nature of rice. Because properties of antigen can influence Th1/Th2 responses, it is intriguing to consider the potential influence of the allergen fusion to the soybean storage protein glycinin A1aB1b in the enhanced Th2 suppression noted (7).

The work presented here by Takagi *et al.* (7) demonstrates that it is feasible to develop an effective peptide-based oral vaccine for allergy treatment using a cereal food crop for both expression and delivery. These results extend previous work using transgenic plants and human autoantigens, and so plants are emerging as an important new therapeutic tool for both allergy and autoimmunity. It will be several years before plant-based vaccines for allergy likely become available, because hurdles need to be overcome. Variation in expression yield between individual seeds will hamper the control of consistent dosing, and extensive processing of rice may alter or reduce antigenicity. The selection of targets for oral immune tolerance will require extensive knowledge of relevant trigger antigens and the Th1/Th2 balance in any specific disease. Despite safeguards, concerns will be voiced regarding the potential escape of transgenes from genetically altered edible plants. Nonetheless, the prospects for the therapeutic use of transgenic plants in immune-related diseases will remain bright if clinical studies confirm efficacy and transgenic plants address practical issues of cost and production. Finally, although preventing allergic diseases in infants and children is a powerful incentive for further studies, transgenic plants for medical applications will require greater public appreciation of potential benefits before widespread acceptance occurs.

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