

Gene therapy for peanut allergy

The oral delivery of allergen genes in chitosan particles may protect against allergy and anaphylaxis (pages 387–391).



ALL TOPIC DISEASES MEDIATED by immunoglobulin E (IgE) impose a huge burden on westernized societies. One person in five in the United Kingdom will develop hay fever each summer, one child in ten will have eczema and one in seven will suffer from asthma. Food allergies, particularly to peanuts, are the most common cause of anaphylaxis, and approximately 100 people will die in the United States each year from this complication of atopy. The cost of treating asthma alone in the United States has been estimated at \$8,000,000,000 per annum. In these circumstances, any prospect of preventing allergies—the prevalence of which has increased greatly over the past century—should be treated with the greatest respect.

Although the genes for many principal allergens have been cloned, this technical feat has not to date produced any remarkable changes in the traditional treatment of allergy. However, it has allowed some very original approaches to the induction of tolerance, such as the one described in this issue of *Nature Medicine*¹, which may eventually supersede the classical form of therapy—immunotherapy.

Established allergies have been traditionally treated by immunotherapy, which involves the subcutaneous injection of progressively increasing doses of purified allergen. Immunotherapy is only effective in some circumstances, particularly for the treatment of hay fever² and carries a real, albeit remote, risk of anaphylaxis and sudden death. The mechanism of action of immunotherapy is not understood, but it seems to induce an active tolerance that is independent of simple changes in IgE titers or affinity for antigen³.

On page 387 of this issue, Krishnendu Roy and colleagues report a new strategy for the induction of tolerance. The AKR/J mouse, suitably sensitized, serves as a model for peanut allergy and systemic anaphylaxis. Roy and colleagues have taken the gene for the main peanut allergen *Arah2*,

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cloned it into an expression vector and administered it orally to AKR/J mice. The DNA was protected from digestion by being complexed with particles of chitosan, a naturally occurring polysaccharide that is an effective vector for the controlled intestinal delivery of many pharmaceutical agents. Chitosan is adhesive to the intestinal walls and can be endocytosed by cells in the gastrointestinal tract.

As a control, the authors administered the LacZ gene and tested for its expression using β -galactosidase. The resultant brilliant blue of the mouse intestines (Fig. 2 of the paper) is a testament to the extraordinary ability of DNA to make

itself at home wherever it can find the machinery for transcription.

Dosing with the *Arah2* gene and chitosan produced an increase in fecal IgA titres against the allergen, an increase in serum IgG2a titers and a decrease in IgE titers, compared with those of mice given either DNA alone or non-immunized mice. More importantly, the severity of anaphylaxis was blunted considerably when immunized mice subsequently underwent a protocol for sensitization and induction of anaphylaxis by *Arah2*.

Although exciting, the findings are still a long way from the clinic. The authors have not yet studied the efficacy of gene administration to mice that are already sensitized, which is likely to be the case in humans who would require treatment for anaphylaxis to food antigens. Adverse effects, including acute or chronic inflammation of the bowel, are real possibilities to be considered.

Although the notion of ingesting capsules of naked DNA as a treatment for allergy would seem highly improbable to most clinicians, the results of the study of Roy *et al.* also hold the prospect of treatment for a spectrum of allergies.

Events in the first three months of life seem critical in determining the lifetime response to allergens, and it has been hypothesized that manipulation of the immune system by neonatal vaccination may be effective in altering the subsequent risk of allergy⁴. The route of allergen exposure may be as important as the timing in initiating disease. Many children develop transient IgE responses to food allergens, but most of them become tolerant⁵. Tolerance in adults can be induced by the sublingual administration of allergen⁶. Oral tolerance to respiratory allergens might become possible if gene therapy of the kind described by Roy *et al.* becomes a reality.

1. Roy, K., Mao, H.Q., Huang, S.K. & Leong, K.W. Oral gene delivery with chitosan-DNA nanoparticles generate im-

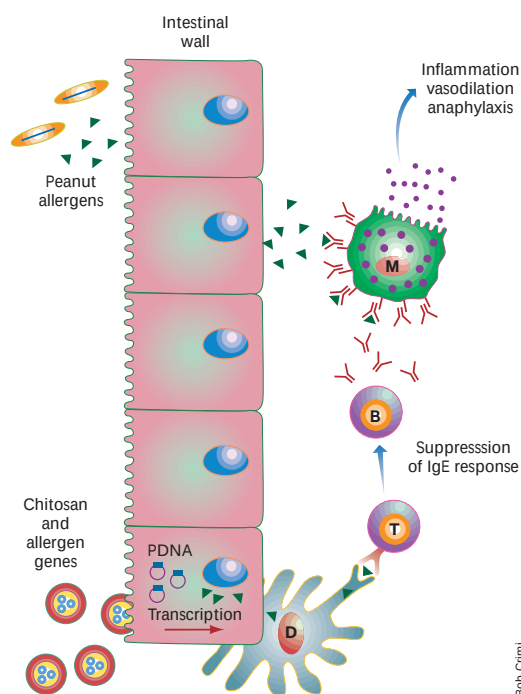


Fig. Top, Food allergens in the intestinal lumen penetrate the mucosa, and can cause cross-linking of IgE bound to receptors on mast cells. Mast cell degranulation leads to local inflammation and systemic anaphylaxis. Bottom, Plasmids containing allergen genes are carried to the mucosa by chitosan particles. Once within the cells, the allergen genes transcribe to create allergen proteins. Allergen is likely to be handled by dendritic cells before presentation in MHC molecules to T-cell receptors. The T cells subsequently suppress B-cell synthesis of IgE, preventing allergen sensitization and mast cell degranulation.

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Receptor seeks ligand: On the way to cloning the molecular receptors for sweet and bitter taste

The cloning of two new taste receptors represents a scrumptious advance for this field of research, but determining their flavor still leaves one feeling slightly hungry

CHOCOLATE ... FRENCH CUISINE ... Chinese food ... much of our life quality depends on the proper function of taste receptors. Their large hedonic value has long kindled scientific curiosity. More practical considerations have contributed to this interest: the design of artificial sweeteners and bitter blockers based on taste receptor structure is a long-standing dream of those engaged in food manufacture. The annual global production of artificial sweeteners alone exceeds 100 million tons.

For at least the past 7 years the race has been on to clone taste receptors. Now, Mark A. Hoon and colleagues have reported success¹. They describe two new clones of G-protein-coupled receptors (GPCRs) that are specific for taste tissue—a great achievement. Furthermore, they have interpreted the topographic expression pattern of one of the clones to suggest involvement in sweet taste and using the same method have linked the other clone to bitter taste. But in these functional assignments I believe they have more than a sporting chance of being wrong.

It was often suspected that sweet receptors and some of the bitter receptors would be GPCRs because their downstream targets are adenylate cyclase, phosphodiesterase or/and phospholipase C. The discovery of a taste-specific G protein, α -gustducin, in 1992, and the loss of taste function caused by its

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deletion², strengthened this belief (reviewed in 3). Therefore, Hoon and colleagues searched for taste-tissue-specific proteins of the heptahelical, GPCR type.

After isolating rat and mice taste buds and taste cells, Hoon's team generated cDNA libraries from taste buds in various

vomer nasal tissue. Rat, mouse and human clones were fully sequenced. The predicted membrane topology showed a long extracellular N terminal that presumably constitutes the ligand binding site. Homology with the Ca^{2+} receptor CaSR of the parathyroid, with metabotropic glutamate receptors and with the vomeronasal receptors V2R allowed the TRs to be grouped into one superfamily with these receptors.

TR1 and 2 were localized to the apical pole of taste cells using immunocytochemistry, which is precisely where they should be found if they are taste-sensory and not 'housekeeping' receptors. Moreover, TR1 and 2 were found only in a subset of receptor cells. Surprisingly, this set was not identical to the subset containing α -gustducin, reinforcing the belief that these are truly novel taste receptors. In addition, *in situ* hybridization showed TR1 and 2 to be concentrated in different sensory areas of the mouth. In conclusion, using an impressive arsenal of molecular methods, Hoon and colleagues

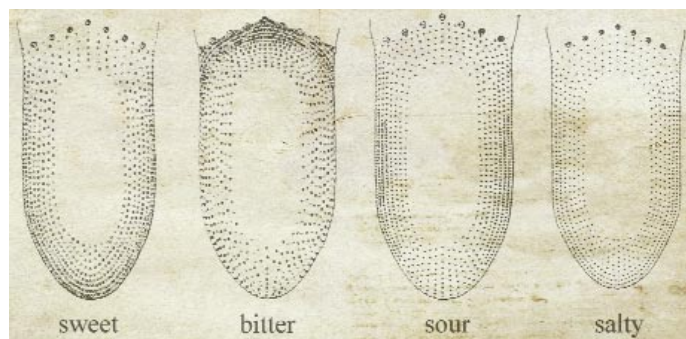


Fig. Chemotopic representation of the human tongue according to Hänig, the first source on tongue maps¹⁰. Taste sensitivity (inverse detection threshold) is represented by the density of symbols. For each of the four modalities shown, sensitivity extends across anterior, lateral and posterior (vallate) parts of the tongue—highest for sucrose-sweet at the tip, sour for HCl at the sides and bitter at the back for quinine. But the differences in sensitivity within each quality are moderate and also controversial. In addition, taste buds are found on the palate (not included here). The popular textbook versions, most of which convey a different impression, probably arose from these diagrams, (now 98 years old) by 'graphical evolution'. The chemotopic diagram of a rat tongue would look quite different from the human diagram but in the rat, too, each area is sensitive to more than one taste quality.

areas of the tongue, as well as from single taste cells isolated from the buds. The advanced techniques of random partial sequencing and subtractive screening were combined, focusing on taste tissue-specific molecules. Only two clones, TR1 and TR2, were found in taste tissue (and testis) that were absent in lingual epithelium, brain and the olfactory and

leagues have discovered two clones of GPCRs that seem to be taste specific and are likely candidates for taste receptors.

Other attempts have been made to characterize and/or clone taste receptors have been made. Two putative sweet receptor proteins have been purified but not yet sequenced⁴. An arginine taste receptor has also been purified.