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against Inaba and 60 percent protection against El Tor (currently the most prevalent biotype) in volunteers receiving one oral dose. A live attenuated mutant of El Tor (CVD111) has also been constructed[10]. Phase III trials of a bivalent (CVD103Hg^R/CVD111) live attenuated oral vaccine are underway. A safe and effective cholera vaccine protecting against *V. cholerae* O1 and O139 should be available soon.

An efficacious ETEC vaccine must elicit IgA antibodies in the gut mucosa against the surface appendages of ETEC strains and/or against their heat labile and heat stable (ST) toxins. Vaccine development has been hampered by the diversity of colonization factors and the poor immunogenicity of ST. An oral killed whole-cell ETEC vaccine composed of the five strains that express the most prevalent colonizing factors (together with r-CTB) is immunogenic and deserves to be tested further[9].

A parenteral vaccine consisting of the Vi polysaccharide capsular antigen of *S. typhi* is already commercially available. It is safe and protective in endemic areas, but is an example of the current limitations of pure polysaccharide vaccines: immunogenicity and immune memory are poor in young children. A conjugated vaccine composed of the Vi antigen and a toxoid is set to replace the pure Vi material. The 'old' live attenuated typhoid vaccine consisted of *S. typhi* Ty21a, a strain that was poorly characterized and inconsistently protective. It has been replaced by a new generation of *S. typhi* mutants with deletions in the *aro*C, *aro*D and *htr*A genes, which encode proteins that help bacteria survive inside tissues. Live attenuated oral vaccines of these mutants are immunogenic in humans and the results of phase III trials are eagerly awaited[11]. As two good typhoid vaccines (conjugated and live oral) are in the works, choosing one may be difficult. The major advantage of the Vi conjugate is that it is simple to integrate into the vaccination schedule.

With effective antidiarrheal vaccines on the horizon, new questions arise. Will there be sufficient funds to support clinical trials in the developing and developed world? How and at what stage of the clinical trials should manufacturers be involved? Will regulatory agencies tolerate the diversity of vaccines protecting against several pathogens, but still against a single syndrome? How can vaccination programs be organized so as to reach all children? How can a battery of new antidiarrheal vaccines be effectively introduced into the already busy schedule for child vaccination programs?

No doubt all of these hurdles will eventually be overcome. Let's not forget that slaying the Hydra was just one of the Twelve Labors of Hercules!

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Alchemy for asthma

Techniques that induce allergen tolerance in asthma patients are not yet fully effective or free of side effects.

WILLIAM O.C.M. COOKSON & MIRIAM F. MOFFATT

ASTHMA IS A modern plague, affecting more than ten percent of children in many westernized societies. The prevalence of asthma and other allergic diseases has doubled in the last twenty years. Although much is known about the ubiquitous environmental antigens (known as allergens) that precipitate asthma and allergic disorders, the processes driving the increase in atopic diseases are not fully understood.

Allergens are usually soluble proteins with dimensions that allow penetration into the airways of the nose and lung (see figure). Allergen sources include house dust mites, domestic and farm animals, and grass or tree pollens. Major allergens from a particular source induce an allergic response in many individuals, whereas minor allergens produce disease less frequently. Most major allergens are extremely well characterized, with known protein sequences and even three-dimensional structures. Tiny amounts of allergen precipitate disease, and total yearly exposures to allergen may be measured in nanograms.

Atopic disease is initiated when allergen comes into contact with immunoglobulin E (IgE) bound to the high affinity IgE receptor (FcεRI) on mast cells and other cells of the immune system. Receptor crosslinking releases a mixture of inflammatory mediators and cytokines. Immediate inflammation and associated symptoms resolve and are followed by a second phase, which typically peaks six hours after antigen exposure.

Although the acute response to allergen is mediated by IgE, IgE production and the late phase reaction are modulated by T cells. Helper T cells may be classified into Th1 and Th2 types, according to the pattern of their cytokine production[1]. Although the classification is an oversimplification, in general, Th1 cells produce interferon-γ and enhance cellular immune responses, whereas Th2 cells produce interleukin (IL)-4 and favor humoral responses, IgE production and atopy. The propensity to react to antigen either with a predominant Th1 or a predominant Th2 response appears to be under genetic control. Th2 responses are also encouraged when antigens are presented at mucosal surfaces[2].

Immune tolerance is a distinctive feature of allergy. Many children spontaneously 'grow out' of their asthma. Skin test reactions to allergens decline steadily from the mid-teens, and the total serum IgE concentration at 50 years of age is half that at age 20 (ref.3). The mechanism of the decline is obscure. It is not seen in other immunoglobulin classes.

The combination of inappropriate immune responses, well-known antigens and immune tolerance is a promising mixture from which to forge treatment by vac-

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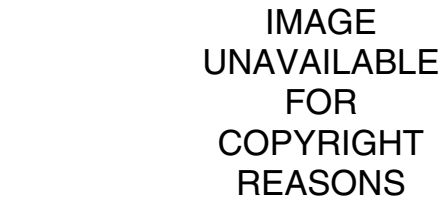
cination. Vaccination may be directed against the specific allergens to which an atopic individual responds, or more ambitiously against the general underlying atopic state.

Specific allergy has been treated by immunotherapy since the beginning of the century[4]. In this process, increasing doses of purified allergen are given subcutaneously over a period of months. The starting dose is below that which will precipitate symptoms. Therapy is often carried out repeatedly over several years.

Immunotherapy is potentially dangerous, with side effects that include systemic anaphylaxis and sudden death. Controlled clinical trials of its efficacy were lacking until recently[5]. It works best when disease is caused by a single antigen source such as pollen or bee venom. Immunotherapy against house dust mite allergens, the principal precipitant of asthma, does not seem effective. Clinical treatment has often been costly to the patient and conducted without clear endpoints. Finally and frustratingly, despite the immense popularity of immunotherapy, its mechanism of action is completely unknown. The abatement of mast cell degranulation can take place despite persistence of allergen-specific IgE, a process that cannot be convincingly explained by current immunological models. The effort to deal with these dangers and inconsistent responses has led to several ingenious new approaches to immunotherapy.



Allergens induce disease in sensitized individuals by the crosslinking of IgE bound to high-affinity receptors on mast cells. Mast cell degranulation initiates an inflammatory cascade, airway edema and allergic symptoms. Th2 cells upregulate IgE production, whereas Th1 cells downregulate it.

Many allergen genes have been cloned, but a therapeutic role for recombinant allergens has not been established. This is partly because of the problems of faithful folding and glycosylation of recombinant proteins. In addition, it has been recognized that allergens exist in polymorphic isoforms, and that individuals may react specifically to certain isoforms. Apocryphal stories of patients who were allergic to their neighbor's birch tree and not their own have therefore at last received scientific support. Therapy may consequently be necessary with mixtures of isoforms, as occurs with allergen preparation from natural sources.

Attempts have been made to induce T cell tolerance directly, bypassing IgE recognition of antigen and anaphylactoid side effects. In one model, therapy depends on administration of peptide fragments of allergen[6]. Peptides are selected to contain T cell epitopes that react with the T cell receptor, while failing to engage IgE bound on mast cells. Despite the elegance of the theory, peptides unexpectedly produce a similar range of side effects as the whole antigens[7]. This finding emphasizes that T cells are effectors as well as controllers of allergic inflammation.

Another strategy for immune bypass involves the injection of naked allergen DNA into muscle. Production of the allergen by muscle cells blocks subsequent allergic sensitization, perhaps because the tissue does not express class II HLA proteins[8]. At the moment this approach has only been studied in mice.

Conversely, IgE may be met head-on, by encouraging polyclonal IgE to block FcεRI receptors on mast cells, and to prevent the binding of allergen-specific IgE. Deliberate worm infestation (to elicit polyclonal IgE production) seems unlikely to be as socially acceptable as the allergist's shots, but controlled stimulation of the production of IgE with low affinity for allergen remains a therapeutic possibility.

Other approaches to vaccination depend on good evidence that the first three months of life are critical in priming the lifelong immune response to allergens[9]. It is conjectured that environmental agents acting at this time initiate Th2 responses and subsequent allergic disease.

Vaccinations with mycobacteria have been advocated to pull the entire neonatal immune system towards a Th1 response[10]. However, universal inoculation with attenuated mycobacteria (BCG) still results in a population divided clearly into those who respond primarily with cellular immunity and those who respond primarily with immunoglobulins and develop atopy[10]. More promising perhaps may be strategies to present specific antigens by a particular route or with particular adjuvants to induce Th1 rather than Th2 responses to that allergen[2]. Such studies are still in their infancy.

Despite much research activity, a silver bullet for atopic disease still does not exist. Of the various new approaches, those aimed at prevention of allergy in the first few months after birth appeal most, because antigen administration at that time carries no risk of anaphylaxis, and protection may be permanent. It is likely that progress would be faster if the mechanisms underlying allergic tolerance could be identified.

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