

Naked DNA: New shots for allergy?

Injection of an allergen gene into rat muscle prevents specific IgE production and asthmatic reactions (pages 540–544).

The atopic diseases of asthma and allergic rhinitis are amongst the most frequent afflictions of Western society. The medical emergency of anaphylaxis, although less common, is part of the same syndrome. Allergic symptoms result from IgE-mediated responses to foreign proteins or allergens. Common sources of allergen include the house dust mite (HDM), domestic pets and pollens.

Desensitization or immunotherapy by injection of pollen extracts was invented as a treatment for allergic rhinitis in

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allergy, respectively. However, the routine use of an extensive battery of allergens for testing and treatment has been greeted with suspicion by some parts of the medical profession.

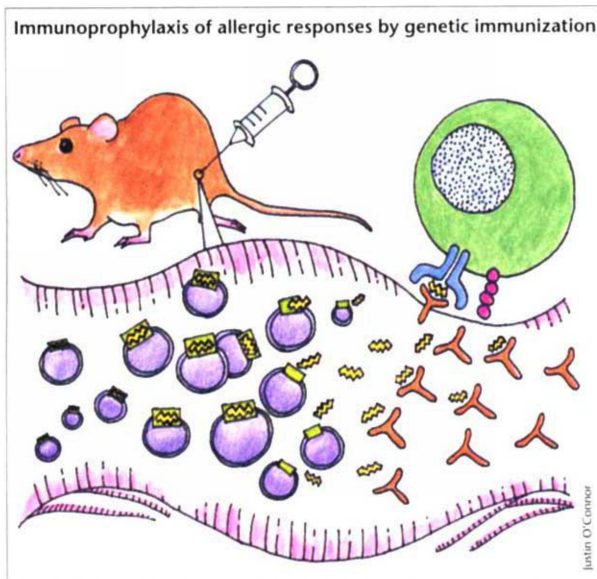
To the cynic, the injection of DNA as a treatment for allergy follows in the tradition of monkey gland transplants and other quackery. However, the results per-

muscle tissues. A transient IgG response to *Der p V* was detected in the serum, but no IgE response was noted. When the animals were subsequently primed and challenged with *Der p V* protein, the resulting IgE response to *Der p V* was greatly reduced, as were airway responses and the release of histamine from cells from bronchoalveolar lavage. The reactions to another antigen, ovalbumin, were not affected, showing that the results of DNA injection were specific for *Der p V* protein.

These findings are strongly underwritten by a literature describing the use of DNA injection for gene therapy and vaccination^{3,4}. Wolff and colleagues were the first to show that plasmid DNA containing reporter genes was expressed when injected into mouse muscle⁵. The observation that cellular and humoral immune responses are raised to the plasmid gene products^{6,7} has led to widespread interest in DNA vaccination for the prophylaxis of a variety of bacterial and viral pathogens. The response to plasmid products is likely to result from their carriage to the cell surface by class I MHC molecules and the subsequent engagement of CD8⁺ effector T cells (see figure). This is in contrast to the usual display of allergens in association with class II HLA molecules on specialized antigen-presenting cells.

Despite advances in the purification and identification of many major allergens and the production of recombinant allergen proteins by genetic engineering, the molecular basis of tolerance in allergy is still not known. Observed increases in specific IgE and IgG do not explain the phenomenon. Immunotherapy causes the cytokine products of helper CD4⁺ T cells to shift from an IL4-dominant to a γ -interferon-dominant profile (that is from T_H2 to T_H1 cell types, so that it has been suggested that CD4⁺ cells control desensitisation⁸. Alternatively, it has been proposed that it is CD8⁺ cells that induce tolerance, through their action on CD4⁺ cells⁹. The results of Hsu *et al.* support the latter hypothesis, as passive transfer of CD8⁺ cells from their vaccinated rats prevented reactions against *Der p V* protein in recipient animals. Although the distribution of cells in the CD8⁺ category is very broad, and a multiplicity of subsets

Plasmids containing the *Der p V* gene are injected into the leg muscle of rats, the gene is transcribed and *Der p V* protein synthesized in muscle cells. *Der p V* protein in the cell cytosol binds to class I MHC molecules and is transported to the cell surface. The MHC/*Der p V* complex is recognized by CD8⁺ T cells which become activated and stimulate production of IgG and suppression of IgE.



London at the beginning of this century¹. The use of immunotherapy with a variety of allergens spread rapidly and for many years (before the advent of effective pharmacological agents) it was the mainstay of treatments for asthma. Modern desensitization regimes usually begin with minute doses of allergen, which are increased over a period of weeks to months. The direct induction of tolerance by "shots" of allergen in this way has been the basis of a substantial and profitable medical subindustry. Treatment regimes have tended to be empirical, and using the diminution of symptoms as an end point of treatment has been problematic in illnesses typified by a fluctuant course. Tolerance can be induced, consistently in some circumstances, to bee and wasp venom and to grass pollen, preventing anaphylaxis and

sented in this issue of *Nature Medicine* by Hsu and colleagues² remind us that IgE-mediated allergy can be a particularly interesting model system for understanding the immune response to exogenous antigen. Hsu *et al.* studied the Brown-Norway rat, an animal that shows many features of human atopic disease. After priming by intraperitoneal injection of allergen or ovalbumin, these rodents exhibit exuberant IgE production, eosinophilia, and early and late asthmatic airway responses.

The authors describe experiments in which a plasmid expression vector, containing the gene for the minor HDM allergen *Der p V*, was injected into the leg muscle of rats. In animals injected with the *Der p V* gene, the authors observed that expression of *Der p V* protein persisted for at least six months in the

are likely to be found within this group, these results may be a good starting point from which to proceed with unraveling the mechanisms of tolerance.

From the clinical point of view, the injection of DNA is not without potential difficulties. First, plasmid DNA may be integrated into the host genome, with the possibility of disruption of normal genes and malignant transformation. The reassuring evidence so far is that integration does not take place, even after long periods³. Second, it is not clear what the outcome will be if *Der p V* plasmids are injected into animals already sensitized to the allergen. The capacity to induce anaphylaxis or other untoward reactions needs to be investigated in further animal studies. Third, although immunization has been the chief objective of DNA vaccination, in the study of Hsu *et al.* the immune response is one of tolerance. This paradox has yet to be explained.

Finally, orthodox theories of immune surveillance predict that myocytes expressing a new protein would be recognized as foreign cells and attacked. The absence of myositis in animal tissues expressing foreign genes is most intriguing and suggests that antigen presentation and recognition are not alone sufficient to induce immune attack.

The results of Hsu and colleagues will not lead immediately to injections of DNA as routine treatments for asthma and allergic rhinitis. Their data do, however, suggest a number of new and very interesting approaches to allergic desensitization, and pose questions of substantial interest to immunologists.

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Shaking down new epilepsy genes

The neural function of cystatin B, recently implicated in EPM1, is unclear but may lead to new insights into the mechanisms of epilepsy.

Positional cloning has been relatively slow to make an impact on the study of human epilepsy. However, a large step forward has been taken with the recent publication in *Science* of a paper by Pennachio *et al.*¹ describing the gene responsible for progressive myoclonic epilepsy of the Unverricht-Lundborg type (EPM1). The appeal of positional cloning is of course that it does not require knowledge of gene function. Consequently one escapes the limits of what current fashion implies is important, and is forced to confront what Mother Nature has designated important. In the case of epilepsy, there is the hope that identification of the responsible genes will enable development of novel drug treatments for the 20–30% of epileptics who are refractory to current therapies. However, although some sources suggest that up to half of all cases of

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epilepsy have an important genetic component, this disease is heterogeneous, with many “nongenetic” causes includ-

ing head trauma, infection, tumors, drugs and alcohol. Therefore, genetic analysis requires epileptic diagnoses that can be distinguished from those due to “nongenetic” causes that may also occur in the same pedigrees. Thus, linkage analysis frequently centers on relatively rare epilepsies that have complex, even bizarre, features that typically require far more than three words of description (see table). One hopes (and already there is some positive evidence) that some of the genes involved in these unusual types of epilepsy will also be important in the more common forms of this disease. Consequently the identification by Pennachio and colleagues of mutations in the cystatin B gene that are responsible for EPM1 is an exciting breakthrough. Whether it will ultimately broaden our understanding of other epilepsies remains to be determined.

Human epilepsy genes

Disorder	Location	Gene
Idiopathic generalized epilepsies		
Juvenile myoclonic epilepsy (EJM1) ¹³	6p	?
Benign familial neonatal convulsions (BFNC1) ¹³	20q	CHRNA4 (?)
Benign familial neonatal convulsions (BFNC2) ¹³	8q	?
Idiopathic generalized epilepsy (IGE) ¹⁴	8q	?
Idiopathic partial epilepsies		
Partial epilepsy with auditory symptoms (EPT) ¹³	10q	?
Nocturnal frontal lobe epilepsy (ADNFLE) ¹⁵	20q	CHRNA4
Single-gene disorders with symptomatic epilepsy as a major feature		
Unverricht-Lundborg disease (EPM1) ¹	21q22.3	cystatin B
Myoclonic epilepsy with ragged red fibers (MERRF) ¹³	mitochondrial	tRNA(Lys)
Northern epilepsy syndrome (EPMR) ¹⁶	8p	?
Ceroid lipofuscinosis, juvenile type (CLN3) ¹⁷	16p	CLN3
Myoclonic epilepsy of Lafora (MELF) ¹⁸	6q23	?
Periventricular heterotopia (PH) ¹⁹	Xq28	?

Table modified from Ryan¹³.