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Editorial

Immunotherapy in respiratory allergy

"I have had no hay fever this year, except one attack lasting an hour after walking through a hay field, and no asthma. In former years I had bad hay fever for six weeks and asthma at night."

So said patient 6 of the 20 patients with hay fever who had been treated by hypodermic inoculations of pollen vaccine in the spring of 1911 by Noon[1] and Freeman.[2] Although their study was not placebo controlled they were very conscious of the fact that "Every medical man so desires that his patient's condition shall be improved by his treatment that there is a constant tendency to detect improvement in adventitious fluctuations of health." They were well aware of the novelty of their treatment and felt that their patients were impressed by its "science." Just how scientific Noon and Freeman were can perhaps be judged by our knowledge of this form of treatment 70 years later. They used an extract from Timothy grass pollen because it gave stronger reactions than any other pollen extract when instilled into the conjunctivae of their affected patients. They worked out units for the strength of the pollen vaccine, carefully selected patients for treatment on the basis of conjunctival tests, and monitored the increasing doses of pollen vaccine on the basis of results of provocation. This is a far cry from the haphazard way in which many courses of hyposensitisation with all sorts of allergens are given worldwide today.

In the last two decades the introduction of such drugs as selective β_2-adrenoceptor agonists, sodium cromoglycate, and topically applied corticosteroids has provided an effective and safe method for the treatment of patients with allergy of the respiratory tract without, many would argue, the need for identification of the allergen or attempts at immunotherapy. In Britain sales of hyposensitisation vaccines are on the decline. In other areas of the Western world, however, the position is rather different. In many European countries allergy is a specialty in its own right and in West Germany sales of allergen extracts are on the increase. Leaving aside future developments, we must take seriously the question of whether there is a need for a treatment such as immunotherapy as currently practised. If it is needed, other questions arise: What preparations can be used? Does it really work? Is it safe? And where does it fit into our present treatment for respiratory tract allergy?

Address for reprint requests: Dr Robert J Davies, Academic Department of Respiratory Medicine, St Bartholomew's Hospital, London EC1A 7BE.

WHAT IS IMMUNOTHERAPY?

Immunotherapy can be defined as a form of treatment designed to alter beneficially the immunological reactions concerned in disease processes. In one form or another it is being used increasingly in many different medical specialties. The term is now preferred to desensitisation or hyposensitisation since it is less specific with regard to details of mechanism and effectiveness. In the context of respiratory allergy, immunotherapy currently uses techniques which are considered to modify the interaction of allergen with immunoglobulin E (IgE), which in turn leads to release and formation of chemical mediators from mast or basophil cells or both, leading to the development of allergic rhinitis and asthma. More precise descriptions of this type of treatment vary from the idealistic "administration of slowly increasing quantities of allergen over a period of months which is followed by a degree of tolerance to that allergen as evidenced by the increasing doses that can be given and the decline in the patient's symptoms and medications required"[3] to perhaps the more realistic "exposure of allergic subjects to increasing doses of essentially uncharacterised immunogenic substances at varying intervals for an indeterminate period of time, in an attempt to reduce allergic reactions to these substances."[4] The reader is left to decide which of these descriptions seems nearer to the truth.

IS THERE A PLACE FOR IMMUNOTHERAPY?

Immunotherapy began in the days when pollen was considered to produce a toxin and the ideal—indeed, virtually the only—treatment was to try to produce an antitoxin. Little has changed since the turn of the century with regard to the methods and extracts used, and current views about how immunotherapy might work have swung strongly

back in favour of the development of blocking or neutralising antibodies, which are not too far removed from "antitoxins." Drug treatment designed to prevent mast cell mediator release or block or reverse various aspects of the inflammatory response on the other hand has developed dramatically. Although it is widely accepted that many of the currently available drugs are very effective and are without substantial side effects, some patients do not obtain adequate relief even from topically applied corticosteroids.[5] Other patients, particularly those with both rhinitis and asthma, are inconvenienced by regular insufflation or inhalation (or both) of three or four aerosol sprays or powders up to four times every day. Many patients, particularly those with perennial symptoms, are daunted by the probability that drug treatment may be necessary every day for the foreseeable future, if not for the rest of their lives. Immunotherapy should effect a more fundamental improvement in the patient's disease; but can it, and does it?

DOES IT WORK?

Immunotherapy had been practised for over 40 years before the first controlled trial of its efficacy was performed by Frankland and Augustin in 1954.[6] Since that time there have been many controlled studies with a wide variety of allergens in both rhinitis and asthma. Overall, favourable results have been obtained more frequently in children, particularly with regard to immunotherapy against the house dust mite (*Dermatophagoides pteronyssinus*), and in the treatment of rhinitis rather than asthma. Review of reports published worldwide shows that conflicting results have frequently been obtained even in controlled studies of immunotherapy with the same allergens. There are many possible explanations for this. Trials of treatment of whatever kind in diseases like asthma and rhinitis, which are characterised by variability of symptoms and signs, are inevitably difficult and require longer-term studies.[7] Careful selection of patients is critical. In one study of asthmatic patients with autumnal (fall) asthma in the United States, only 40 out of 400 were eventually found to have satisfactory histories with positive skin test responses to ragweed.[8] In another study almost one-third of patients referred by chest and allergy specialists as cases of perennial rhinitis due to the house dust mite were found to have negative results in nasal provocation tests with *D pteronyssinus*.[9] On the other hand, in one of the most convincing studies showing benefits from immunotherapy in children with asthma due to allergy to the house dust mite, the participants were selected on the basis of positive responses in bronchial provocation tests.[10] Similarly, in the only placebo-controlled study of immunotherapy with *D pteronyssinus* extracts in adults with perennial rhinitis which has shown both objective and subjective benefit, those taking part were all carefully selected on the basis of positive responses in nasal provocation tests.[11] If nothing else, immunotherapy is a specific form of treatment[12] and it is vital to ensure that those receiving it are indeed suffering from extrinsic rhinitis or extrinsic asthma caused by the particular allergen. The role of provocation testing in the diagnosis of respiratory tract allergy nevertheless remains controversial.

Immunotherapy may be more successful than we think. A reduced response to pollens or mites could be disguised by continuing symptoms due to other allergens, such as mould spores present in the atmosphere, about which we remain so ignorant.

Some of the variability in the results of clinical trials almost certainly results from differences in the allergen preparations themselves, the quantity of the specific allergen present, and the way it has been extracted and prepared. From the very beginnings of immunotherapy in respiratory allergy the belief has been that the higher the doses of allergen that can be administered the better the clinical result. There is some evidence to support this idea, particularly from long-term studies with ragweed extracts in patients with seasonal rhinitis.[13] Further, recent comparative investigations using allergoids (formaldehyde-treated or glutaraldehyde-treated extracts), which allow much higher doses of antigen to be administered, have shown advantages over unaltered allergen in both immunological and clinical responses.[14] Both clinical experience and review of the available literature lead to the conclusion that the most favourable results occur after prolonged treatment. Indeed, in controlled studies of immunotherapy with *D pteronyssinus* in perennial rhinitis advantage in terms of clinical benefit occurred only after one year of treatment.[11 15] Whereas in one co-operative study 15 weekly injections of a moderate dose of house dust extract had little effect,[16] large doses administered over two to three years led to both symptomatic improvement in comparison with controls and a reduction in reactivity on bronchial challenge with the allergen.[17] These points—the variability of the disorder and the effect of multiple hypersensitivities and careful selection of patients on the one hand and the nature, dose, and duration of treatment on the other—must all be considered in the evaluation of the many studies, some apparently favourable and others not, on the effectiveness of immunotherapy in respiratory allergy.

There can be little doubt from controlled studies performed on both sides of the Atlantic that immunotherapy is more effective than placebo in

the treatment of seasonal rhinitis caused by grass or ragweed pollens,[6 18] though it is rarely curative.[13] Asthma after exposure to pollens is a much less common complaint than rhinitis, though the two may occur together. The evidence that immunotherapy is of value for pollen asthma is less convincing,[19] though there are studies which suggest benefits from grass pollen[6 20] and ragweed[21] injections. More recently studies have concentrated on assessing the value of extracts from fewer grass pollen species and on the "purity" of the preparations. There are 12 widespread grasses in the United Kingdom, differing slightly in the dates when their pollens are shed. Currently the most popular hay grass in England is rye grass *(Lolium perenne)*. We might argue that extracts of this pollen alone should be used for immunotherapy. In a recent controlled study of a small number of children with pollen asthma, however, preseasonal injections of rye grass pollen extract alone proved ineffective when compared with placebo.[22] Longer-term treatment might have been required to show clinical benefit, as seems to be the case for extracts of the house dust mite; and perhaps immunotherapy for seasonal allergy should be continued throughout the year. Both clinical and laboratory studies have shown that there is considerable antigenic similarity between allergens extracted from the common grass pollens[23 24] and it may not matter whether extracts from one or several species are used. Properly controlled clinical trials comparing extracts from a single or a limited number of pollens with extracts from the 12 common grain species are required to settle the point. In principle at least, allergen standardisation and purification should benefit both the reliability and the efficacy of immunotherapy. So far as grass pollens are concerned, however, no advantage could be found in favour of immunotherapy with a purified extract when it was compared with a crude extract of Timothy grass in children with grass pollen allergy.[25]

The commonest allergen causing perennial rhinitis and asthma in northern Europe, though not apparently in the United States, is the house dust mite. The effectiveness of immunotherapy with extracts of *D pteronyssinus* is more open to dispute than such therapy with pollens and controlled trials have given conflicting results. There are, however, at least three studies of childhood asthma in which immunotherapy has been shown to be of clinical value.[10 26 17] As yet it is difficult to be sure whether there is any benefit from immunotherapy with house dust mite extracts in adult asthmatics, though there have been several studies with a favourable result.[27 28] Certainly there is evidence to suggest that such treatment may benefit both children and adults with perennial rhinitis due to allergy to *D pteronyssinus*.[11 15 27] It remains to be determined whether improvement in the preparations of house dust mite extract, perhaps to include larger quantities of the important P_1 allergen, will lead to more consistent and beneficial effects.[29]

Effective immunotherapy would be particularly useful in asthma and rhinitis caused by exposure to animal allergens, whether at work or in the domestic environment. Despite considerable anecdotal support for its benefit, there have been virtually no controlled clinical trials showing that injections of animal allergens have been useful. In one small study, however, the use of cat pelt allergens has been shown to reduce the bronchial sensitivity to this allergen.[30] Possibly a greater knowledge of the allergens from different animal species that are important in the production of disease in man could contribute greatly to the success of immunotherapy.

Bacteria, unlike viruses, probably play a minor part, if any, in the production or precipitation of attacks of asthma. Certainly antibiotic treatment appears to add no therapeutic benefit.[31] Similarly, immunotherapy with bacterial vaccines has been shown in several controlled trials to be of no greater value than placebo.[32 33] The role of many moulds in the production of respiratory tract allergy awaits elucidation, as does the value of immunotherapy with their extracts; and the same applies to food extracts.

HOW DOES IT WORK?

Theoretically, immunotherapy could exert its effect in several ways, but three possibilities appear the most likely. Firstly, allergen injections might lead to a significant decrease in production of specific IgE through interference with the control mechanisms for antibody production. Studies of immunotherapy with ragweed extracts in the United States have shown that the seasonal rise in levels of this specific IgE antibody against ragweed allergen can be prevented by injection treatment,[34] and long term the levels of these antibodies have been shown to decrease.[35] These results have not been found universally. Indeed, no such changes in levels of specific IgE were detected after immunotherapy with Timothy grass allergens.[36] Since decreases at least in circulating levels of specific IgE antibodies are not found in all studies in which clinical benefit occurs, and in any case are only of minor magnitude, it is highly unlikely that immunotherapy as currently practised exerts its effect through suppression of IgE synthesis.[37]

Animal studies have shown that ongoing IgE production can be prevented by stimulation of suppressor T cells. This can be achieved in experimental animals, and probably in man, by the use of urea-

denatured allergens, which are essentially non-allergenic but still stimulate T cells.[38] Alternative methods of trying to achieve the same goal use polyethylene-glycol-substituted allergens[39] or allergens linked to copolymers of D-glutamic acid D-lysine,[40] but exactly how these preparations exert their effect and whether this will be of any real value in allergic disease in man remains to be determined.[41]

We have known for some years that immunotherapy can decrease histamine release from circulating basophils, at least in some patients.[42] This does not occur in all those who benefit from the injection treatment and we do not know whether the same changes occur in mast cells in the respiratory tract. Overall it seems unlikely that such changes constitute the effective mechanism in immunotherapy.

Recent studies of immunotherapy with bee and wasp venom have reawakened interest in the role of specific immunoglobulin G antibody (IgG), the so-called "blocking" antibody. Passive immunisation with beekeeper's serum has been shown to have some protective effect against subsequent bee stings in allergic patients.[43] Immunotherapy certainly causes a rise in venom-specific IgG in most patients, who are then able to tolerate challenge with deliberate stings.[44] Levels of specific IgG have been shown to rise after immunotherapy with both ragweed and grass pollen. Some but not all studies have shown a correlation between increased blood levels of specific IgG and clinical improvement[13] and reduction in response to direct provocation tests.[36] No changes in specific IgG, however, have been detected in two recent British studies that have shown benefit from immunotherapy with house dust mite extracts.[10 11] During immunotherapy increases in both specific IgG and immunoglobulin A antibodies (IgA) have been shown to occur in secretions from the respiratory tract,[45] which is perhaps the ideal site for the development of "protective" antibodies. So far these changes have not been shown to correlate with clinical improvement.

No unitary hypothesis of how immunotherapy works can yet be proposed. Certainly the immunological changes appear to be complex and probably affect not just one but many aspects of the allergic response, perhaps varying according to the extracts used and the patients treated.

WHAT CAN BE USED?

The ideal preparations for immunotherapy would be those which are easy to administer and require few injections, saving both patients' and doctors' time. They would be stable, well standardised, and safe, and give few unwanted effects; above all, they would be of proved clinical benefit. Pharmaceutical companies have tried to manufacture products which combine all of these properties, but have had to compromise over one aspect or another. There is the further problem, largely unresolved, of which antigenic components should be included in the extract. For example, ragweed contains at least 15 allergens, of which the major one is antigen E, but there is no doubt that the "minor" allergens are of great clinical importance in some patients.[13] Linked to this is the theoretical risk that injection of complete extracts might effectively protect against the major immunising agent but induce a symptomatic level of antibody production against minor components. Considerable care must also be exercised in the choice of chemicals for allergen extraction. For example, pyridine extraction, though acceptable for grass pollen, causes almost complete denaturation of ragweed antigen E.[46] No wonder there is confusion in the ranks of both allergists and pharmacologists. In principle, preparations which have been characterised and standardised would seem best, though we need further information to prove the advantage or otherwise of so-called "purified" extracts.

The desire to maintain potency of allergen extracts and to induce good antibody responses has led to the development of complex treatment regimens, which often require the careful reconstitution of freeze-dried allergens and administration in "cluster" or "rush" regimens that call for repeated injections thoughout the day. In Britain immunotherapy of this type is largely impracticable. It can be used in countries where allergy is a specialty and appropriate clinic and hospital facilities are available, but its benefit over the more "traditional" regimens requires proof.

Depot preparations have been used as a means of enabling large amounts of the allergen extract to be administered at infrequent intervals without undue unwanted effects. Alum-precipitated extracts, which appear to allow release of the allergen over a period of about one hour, have been widely used. Treatment is given at weekly intervals for six to 10 weeks and then monthly. More recently suspensions of L-tyrosine or sodium alginate prepared from brown seaweed have been used for the same purpose. The advantage of the use of L-tyrosine is that this material is thought to be fully metabolised at the site of injection,[47] and sodium alginate is considered non-immunogenic—both carriers, it is hoped, avoiding the development of local granuloma. Additional benefits may accrue from adjuvant activity.[47]

Considerable interest has been focused on the use of allergoids, in which chemical treatment with formaldehyde or glutaraldehyde is thought to reduce the ability to cause clinical reactions while

leaving the desired immunological response unaltered. Improved convenience and effectiveness may be additional benefits. The use of a glutaraldehyde-pollen-tyrosine preparation has been shown to reduce to three the least number of injections required for a favourable antibody response[48] and there is a suggestion that injection of allergoids from ragweed may eventually lead to better immunological and clinical responses than the use of unaltered ragweed.[14] The place of allergoids in immunotherapy, though theoretically interesting, has still to be properly established.

The application of allergens directly on to the nasal mucosa has been considered as a method of preseasonal immunotherapy. As expected, aqueous extracts produce severe symptoms. The use of allergoids reduces symptoms and is better than placebo in preventing seasonal rhinitis, but not conjunctivitis, due to grass pollen. Again no correlation has been shown between changes in the antibody content of secretions and clinical benefit.[49]

IS IT SAFE?

Local reactions to injections of allergen extracts and mild exacerbations of rhinitis or asthma or both are reported by 30–50% of patients, but in one study they were no more common after receiving an effective allergen extract than after placebo.[11] Of much greater concern are reports of severe reactions and anaphylaxis leading to death.[50] In an extensive study of nearly 20 000 patients in France the incidence of severe reactions after allergen injections was 0·1%. Asthma, rhinitis, and urticaria were the most common reactions. Anaphylactic shock occurred in only two patients, both of whom were successfully resuscitated with adrenaline.[51] In the United Kingdom over four million injections of one popular alum-precipitated pyridine-extracted allergen extract have been given with no reported deaths,[52] while an incidence of one death for every 750 000 allergen injections has been reported with a similar preparation.[53] The conclusion from the French study was that severe reactions were often related to improper use of the preparations, though this is obviously difficult to prove after the event. Nevertheless, immunotherapy clearly is best administered by doctors experienced in the technique and sensible precautions must be taken. Anecdotal evidence suggests that the frequency of unwanted reactions may be reduced if patients rest for 30 minutes or so before and after the injection and take a potent oral antihistamine before going to the surgery or clinic. It is vital to avoid mistakes in the dosage administered and the site of injection—allergens must never be injected into the blood stream. Such errors seem to be the most likely cause of severe unwanted reactions. The most effective treatment for anaphylaxis is adrenaline (1:1000) administered intramuscularly (or even intravenously) at a dose of 0·5–1·0 ml. Adrenaline must always be readily accessible, preferably already drawn up, when *any* injection of allergen extract is being administered. More minor unwanted reactions can be treated appropriately with β_2-adrenoceptor agonists and antihistamines. Corticosteroids are of no value in the acute anaphylactic response.

Currently there is no evidence to suggest that immunotherapy leads to the development of circulating immune complexes,[54] autoimmune disease, or lymphoproliferative disorders.[55] Occasionally patients appear to have been made worse by allergen injections but it is impossible to tell whether this is a consequence of the injections or the result of "natural" progression of the disease. Immunotherapy appears to have no deleterious effects during pregnancy[56] but is usually postponed during the course of respiratory tract infections or other intercurrent illness on the grounds that the response to treatment might be impaired.

WHEN SHOULD IT BE USED?

Immunotherapy as currently practised should probably not be advised as the first-line treatment for uncomplicated rhinitis or asthma which readily responds to intermittent or daily treatment with topically applied decongestants, bronchodilators, sodium cromoglycate, or corticosteroids, either alone or in combination. Exceptions may be made for patients who would prefer immunotherapy.

Immunotherapy should be considered in the management of patients who find difficulty in using inhaled treatments or who find their application ineffective and those who have symptoms occurring at more than one site. In these circumstances additional treatment with tablets may be required, with an increased risk of unwanted effects, or the patient may be overwhelmed by the frequency of treatment and the number of orifices into which treatment must be placed. Here compliance may be poor. In Britain at present immunotherapy for respiratory tract allergy should be considered only for the treatment of seasonal allergic rhinitis or asthma due to grass pollen or perennial rhinitis or asthma due to *D pteronyssinus*. It is vital to be as certain as possible that allergens from the house dust mite or grass pollen are causing the disease and this may require provocation testing. Immunotherapy should probably not be advised for the treatment of extrinsic asthma or extrinsic rhinitis caused by any other allergen. An irrational exception to this may be made in the case of allergy to animals encountered at work or at home. Removal from exposure may cause consider-

able financial or social distress, and although beneficial effects of immunotherapy with most animal extracts have not been proved it is often considered worth a try.

The particular allergen preparation used must depend on the facilities that are available. The evidence suggests that immunotherapy must be administered long term to obtain maximum benefit, and we ought perhaps to be replacing short courses of preseasonal injections with prolonged immunotherapy at monthly intervals throughout the year.

In theory, the future for the treatment of respiratory allergy should lie with immunotherapy, which may in years to come offer a cure for these common and distressing conditions.

ROBERT J DAVIES
Academic Department of Respiratory Medicine
St Bartholomew's Hospital
London

References

[1] Noon L. Prophylaxis inoculation against hay fever. *Lancet* 1911;i:1572–3.
[2] Freeman J. Further observations on the treatment of hay fever by hypodermic inoculations of pollen vaccines. *Lancet* 1911;ii:814–7.
[3] Patterson R, Lieberman P, Irons JS, *et al.* Immunotherapy. In: Middleton E, Reed CE, Ellis EF, eds. *Allergy: principles and practice.* Philadelphia: CV Mosby Company, 1978:877–98.
[4] Warner JO. Hyposensitization in asthma: a review. *J Roy Soc Med* 1981;**74**:60–5.
[5] Cockcroft DW, Hargreave FE, Dolovich J. Intranasal beclomethasone dipropionate in rhinitis. In: Mygind N, Clark TJH, eds. *Topical steroid treatment for asthma and rhinitis.* London: Ballière Tindall, 1980:143–54.
[6] Frankland AW, Augustin R. Prophylaxis of summer hay fever and asthma: controlled trial comparing crude grass pollen extracts with isolated main protein component. *Lancet* 1954;i:1055–7.
[7] Davies RJ, Moodley I. Anti-allergic compounds. *Pharmacology and Therapeutics* 1982;**17**:279–97.
[8] Bruce CA, Norman PS, Rosenthal RR, Lichtenstein LM. The role of ragweed pollen in autumnal asthma. *J Allergy Clin Immunol* 1977;**59**:449–59.
[9] Davies RJ, Penketh ARL, Phillips MJ. The value of nasal provocation tests using the technique of anterior rhinomanometry in the diagnosis of perennial rhinitis caused by allergy to the house dust mite. *Allergologia et Immunophathologia* 1980;**80**:390.
[10] Warner J, Price JF, Soothill JF, Hey EN. Controlled trial of hyposensitisation to *Dermatophagoides pteronyssinus* in children with asthma. *Lancet* 1978;ii:912–5.
[11] Davies RJ, Blainey AD. Evaluation and treatment of house dust mite allergy in perennial rhinitis. In: Kerr JW, ed. *Proceedings of the XI International Congress of Allergology and Clinical Immunology.* Basingstoke: MacMillan, 1983.
[12] Norman PS, Lichtenstein LM. The clinical and immunologic specificity of immunotherapy. *J Allergy Clin Immunol* 1978;**61**:370–7.
[13] Norman PS. An overview of immunotherapy: implications for the future. *J Allergy Clin Immunol* 1980;**65**:87–96.
[14] Norman PS, Lichtenstein LM, Katgey-Sobotka A, Marsh DG. Controlled evaluation of allergoid in the immunotherapy of ragweed hay fever. *J Allergy Clin Immunol* 1982;**70**:248–60.
[15] Gabriel M, Ng HK, Allan WGL, Hill LE, Nunn AJ. A study of prolonged hyposensitisation with *D pteronyssinus* extract in allergic rhinitis. *Clin Allergy* 1977;**7**:325–36.
[16] Forgacs P, Swan AV. Treatment of house dust allergy. *Br Med J* 1968;iii:774–7.
[17] Aas K. Hyposensitization in house dust allergy asthma. *Acta Paed Scand* 1971;**60**:264–8.
[18] Lowell FC, Franklin WA. A "double blind" study of the effectiveness and specificity of injection therapy in ragweed hay fever. *N Engl J Med* 1965;**273**:675–9.
[19] Lichtenstein LM. An evaluation of the role of immunotherapy in asthma. *Am Rev Respir Dis* 1978;**117**:191–7.
[20] McAllen MK, Heaf PJD, McInroy P. Depot grass pollen injections in asthma. Effect of repeated treatment on clinical response and measured bronchial sensitivity. *Br Med J* 1967;i:22–5.
[21] Johnstone DE. Study of the role of antigen dosage in the treatment of pollenosis and pollen asthma. *Am J Dis Child* 1957;**94**:1–5.
[22] Hill DJ, Hosking CS, Shelton MJ, Turner MW. Failure of hyposensitisation in treatment of children with grass pollen asthma. *Br Med J* 1982;**284**:306–9.
[23] Milner FH, Tees EC. Specific sensitivity to individual grass pollens in some hay fever patients. *Clin Allergy* 1972;**2**:83–90.
[24] Lowenstein H. Immunological partial identity and in-vitro inhibitory effect of two major Timothy pollen allergens to whole pollen extract of four grasses. *Int Arch Allergy Appl Immunol* 1978;**57**:379–83.
[25] Berg T, Nordvall SL, Lanner A. Clinical studies of a purified Timothy pollen extract. Desensitisation therapy with a purified Timothy pollen preparation compared to a crude Timothy pollen extract. I—Results of tests in-vivo. *Int Arch Allergy Appl Immunol* 1980;**63**:266–74.
[26] Taylor B, Sander SS, Norman AP. A double blind controlled trial of house mite fortified dust vaccine in childhood asthma. *Clin Allergy* 1974;**4**:35–40.
[27] D'Souza MF, Pepys J, Wells ID, *et al.* Hyposensitisation with *Dermatophagoides pteronyssinus* in house dust allergy: a controlled study of clinical and immunological effects. *Clin Allergy* 1973;**3**:177–93.
[28] Smith AP. Hyposensitisation with *Dermatophagoides pteronyssinus* antigen: trial in asthma induced by house dust. *Br Med J* 1971;iv:204–6.
[29] Chapman MD, Platts-Mills TAE. Purification and characterisation of the major allergen from *Dermatophagoides pteronyssinus*—antigen P_1. *J Immunol* 1980;**125**:587–92.
[30] Taylor WW, Ohman JL, Lowell FC. Immunotherapy in cat induced asthma. Double blind trial with evaluation of bronchial responses and histamine. *J Allergy Clin Immunol* 1978;**61**:283–7.
[31] Graham VAL, Milton AF, Knowles GK, Davies RJ. The

role of bacterial infection in asthma: a double blind study of a broad spectrum antibiotic. *Lancet* 1982;i:418–20.

[32] Frankland AW, Hughes WH, Gorrell RH. Autogenous bacterial vaccines in the treatment of asthma. *Br Med J* 1955;ii:941–4.

[33] Barr SE, Brown H, Fuch M, *et al.* A double blind study of the effects of bacterial vaccine on infective asthma. *J Allergy* 1965;**36**:47–61.

[34] Gleich FJ, Jacob GL, Yunginger JW, Henderson LL. Measurement of the absolute levels of IgE antibodies in patients with ragweed hay fever: effect of immunotherapy on seasonal changes and relationship to IgG antibodies. *J Allergy Clin Immunol* 1977;**60**:188–98.

[35] Connell JT, Sherman WB. Changes in skin sensitising antibody titer after injections of aqueous pollen extract. *J Allergy* 1969;**43**:22–3.

[36] Nordvall SL, Berg T, Johansson SGO, Lanner A. Clinical studies of a purified Timothy pollen extract: desensitisation therapy with a purified Timothy pollen preparation compared to a crude Timothy pollen extract. II—Results of the tests in-vitro and their relation to symptoms and tests in-vivo. *Int Arch Allergy Appl Immunol* 1982;**67**:132–8.

[37] Lichtenstein LM, Ishizaka K, Norman PS, Sobotka AK, Hill BM. IgE measurements in ragweed hay fever. Relationship to clinical severity and results of immunotherapy. *J Clin Invest* 1973;**52**:472–82.

[38] Ishizaka K, Okudeira H, King TP. Immunogenic properties of modified antigen E. II—Ability of urea denatured antigen and a polypeptide chain to prime cells specific for antigen E. *J Immunol* 1975;**114**:110–5.

[39] Lee WY, Sehon AH. Abrogation of reaginic antibodies with modified allergens. *Nature* 1977;**267**:618.

[40] Chiorazzi N, Eshbar Z, Katz D. Induction of immunological tolerance to the major antigenic determinant of penicillin: a therapeutic approach to penicillin allergy. *Proc Natl Acad Sci USA* 1976;**73**:209.

[41] Bernstein IL. Tolerogenic vaccines in human allergy. *J Allergy Clin Immunol* 1980;**65**:165.

[42] Pruzansky JJ, Patterson R. Histamine release from leukocytes of hypersensitive individuals. II—Reduced sensitivity of leukocytes after injection therapy. *J Allergy* 1967;**39**:44–50.

[43] Lessof MH, Sobotka AK, Lichtenstein LM. Protection against anaphylaxis in hymenoptera-sensitive patients by passive immunisation. *J Allergy Clin Immunol* 1976;**57**:246.

[44] Lichtenstein LM, Valentin MD, Sobotka AK. Insect allergy: the state of the art. *J Allergy Clin Immunol* 1979;**64**:5–12.

[45] Platts-Mills TAE, van Maur RK, Ishizaka K, Norman PS, Lichtenstein LM. IgA and IgG anti-ragweed antibodies in nasal secretions: quantitative measurements of antibodies and correlation with inhibition of histamine release. *J Clin Invest* 1976;**57**:1041–50.

[46] Lichtenstein LM, Norman PS. Letter to the editor. *J Allergy* 1968;**41**:237–9.

[47] Miller ACML, Tees EC. A metabolisable adjuvant: clinical trial of grass pollen-tyrosine adsorbate. *Clin Allergy* 1974;**4**:49–55.

[48] Johansson SGO, Miller ACML, Mullan N, *et al.* Glutaraldehyde-pollen-tyrosine: clinical and immunological studies. *Clin Allergy* 1974;**4**:255–63.

[49] Georgitis JW, Reisman RE, Clayton WF, *et al.* Local nasal immunotherapy for grass-allergic rhinitis. *J Allergy Clin Immunol* 1983;**71**:71–6.

[50] Rands DA. Anaphylactic reaction to desensitisation for allergic rhinitis and asthma. *Br Med J* 1980;**281**:854.

[51] Vervloet D, Khairallah E, Arnaud A, Charpin J. A prospective national study of the safety of immunotherapy. *Clin Allergy* 1980;**10**:59–64.

[52] Ancill R, Wainscott G. Anaphylactic reaction to desensitisation. *Br Med J* 1980;**281**:1429.

[53] Frankland AW. Anaphylactic reaction to desensitisation. *Br Med J* 1980;**281**:1429.

[54] Kemler BJ, Franklin WD, Alpert E, Bloch KJ. Failure to detect circulating immune complexes in allergic patients on injection therapy. *Clinical Allergy* 1979;**9**:473–8.

[55] Levinson AI, Summers RJ, Lawley TJ, Evans R, Frank MM. Evaluation of the adverse effects of long-term hyposensitisation. *J Allergy Clin Immunol* 1978;**62**:109–14.

[56] Metzger WJ, Turner E, Patterson R. The safety of immunotherapy during pregnancy. *J Allergy Clin Immunol* 1978;**61**:268–72.