

5. Kontoghiorghes GJ, Hoffbrand AV. Orally active α -ketohydroxy pyridine iron chelators intended for clinical use: in vivo studies in rabbits. *Br J Haematol* 1986; **62**: 607-13.
6. Gyparakis M, Porter JB, Hirani S, et al. In vivo evaluation of hydroxypyridone iron chelators in a mouse model. *Acta Haematol* 1987; **78**: 217-21.
7. Kontoghiorghes GJ, Sheppard L. Simple synthesis of the potent iron chelators 1-alkyl-3-hydroxy-2-methylpyrid-4-ones. *Inorg Chim Acta* 1989; **136**: L11-12.
8. Pippard MJ, Callender ST, Finch CA. Ferrioxamine excretion in iron loaded man. *Blood* 1982; **60**: 288-94.
9. Woolf GM, Miller C, Kurian R, Jeejeebhoy KN. Diet for patients with a short bowel: high fat or high carbohydrate? *Gastroenterology* 1983; **84**: 823-28.
10. Hilliard EP, Smith JD. Minimum sample preparation for the determination of 10 elements in pig feces and feeds by atomic absorption spectrophotometry and a spectrophotometric procedure for total phosphorus. *Analyst* 1979; **104**: 312-22.
11. Tesaro A, Leeder S, Benur Y, Klein J, Koren G. A new HPLC method for the measurement of desferrioxamine in body fluids. *Ther Drug Monit* 1989; **11**: 463-70.
12. McCree A, Koren G, Lau P, et al. Cardiac disease free survival in patients with thalassaemia major treated with subcutaneous desferrioxamine: An update of the Toronto cohort. *Blood* 1989; **74**: 311a.
13. Cohen A, Martin M, Schwartz E. Depletion of excessive liver iron stores with desferrioxamine. *Br J Haematol* 1984; **58**: 369-73.
14. Modell B, Lesky E, Flynn DM, Peto R, Weatherall DJ. Survival and desferrioxamine in thalassaemia major. *Br Med J* 1982; **284**: 1081-84.
15. Borgia-Pignatti C, De Stefano P, Zonta L, et al. Growth and sexual maturation in thalassaemia major. *J Pediatr* 1985; **106**: 150-55.
16. Wolfe LC, Olivieri NF, Sallan D, et al. Prevention of cardiac disease by subcutaneous desferrioxamine in patients with thalassaemia major. *N Engl J Med* 1985; **312**: 1600-03.
17. Marcus RE, Davies SC, Bantock HM, Underwood SR, Walton S, Huchins ER. Desferrioxamine to improve cardiac function in iron overloaded patients with thalassaemia major. *Lancet* 1984; **i**: 392-93.
18. Wolfe LC, Nicolosi RJ, Renaud MM, et al. A non-human primate model for the study of oral iron chelators. *Br J Haematol* 1989; **72**: 450-61.
19. Hershko C, Weatherall DJ. Iron chelating therapy. Critical reviews in clinical laboratory sciences, vol 26. Boca Raton, Florida: CRC Press Inc, 1988: 303-45.
20. Porter JB, Gyparakis M, Huchins ER, Hider RC. The relationship between lipophilicity of hydroxypyrid-4-one iron chelators and cellular iron mobilization, using a hepatocyte culture model. *Biochem Soc Trans* 1986; **14**: 1180.
21. Grady RW, Giardina PJ, Hilgartner MW. Total iron balance in response to desferrioxamine. *Blood* 1986; **68**: 40a.
22. Grady RW, Giardina PJ, Hilgartner MW. Intravenous desferrioxamine and total iron balance. *Blood* 1987; **70**: 47a.
23. Porter JB, Hoves KP, Abeyasinghe R, Huchins ER, Hider RC. Animal toxicity of iron chelator L1. *Lancet* 1989; **ii**: 156.
24. Kontoghiorghes GJ, Nasseri-Sina P, Goddard JG, Barr JM, Norrey P, Sheppard L. Safety of oral iron chelator L1. *Lancet* 1989; **ii**: 457-58.
25. Hoffbrand AV, Bartlett AN, Veys PA, O'Connor NTJ, Kontoghiorghes GJ. Agranulocytosis and thrombocytopenia in patient with Blackfan-Ruda anemia during oral chelator trial. *Lancet* 1989; **ii**: 457.
26. Hershko C. Oral chelating drugs: coming but not yet ready for clinical use. *Br Med J* 1988; **296**: 1081.
27. Kontoghiorghes GJ, Hoffbrand AV. Clinical trials with oral iron chelator L1. *Lancet* 1989; **ii**: 1516-17.
28. Editorial. Oral iron chelators. *Lancet* 1989; **ii**: 1016-17.

Allergy treatment with a peptide vaccine

DENIS R. STANWORTH VALERIE M. JONES IAN V. LEWIN
SARITA NAYYAR

An antiserum, obtained by immunising rabbits with a human peptide-protein conjugate, was shown to inhibit histamine release from rat mast cells both in vitro and in vivo. Immunisation of sensitised rats with the same peptide reduced IgE antibody formation and serum histamine concentration, and abolished systemic anaphylactic reactions in response to allergen challenge. This peptide may form the basis of a vaccine in a new approach to the immunotherapy of atopic disease.

Lancet 1990; **336**: 1279-81.

Introduction

Treatment of IgE-mediated allergic responses—eg, asthma and hay-fever, by hyposensitisation—a gradual increase in amount of allergen given to reduce the effects of subsequent exposure to that allergen—has been practised since 1911.¹ Limitations to allergen immunotherapy² include the difficulty in identification of the allergen involved—eg, with food or drug sensitivity. Moreover, the potential of such immunotherapy to cause adverse reactions has restricted its use in the UK.³

Consequently, we have made a peptide vaccine to provide a new form of immunotherapy for patients with IgE-mediated hypersensitivities.

Methods

Peptide antibody preparation

A decapeptide sequence within the CH4 domain of human IgE (Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe-NH₂)₄, which may be the signal to sensitised mast cells after allergen challenge,⁴ was synthesised in an LKB 'Biolynx' automated solid phase

synthesiser with F-moc active ester techniques⁵ and characterised by high-performance liquid chromatography. The peptide was conjugated to keyhole limpet haemocyanin (KLH) or the purified protein derivative (PPD) of tuberculin, with glutaraldehyde.⁶ Rabbits were immunised with the peptide-protein conjugates in Freund's adjuvant for production of polyclonal peptide antibodies.⁵ Peptide antibody concentrations in serum were measured by indirect and inhibition enzyme-linked immunosorbent assay (ELISA).⁶

Mast cell studies

The ability of polyclonal peptide antiserum to inhibit mast cell activation was measured. In-vitro studies involved incubation of samples that contained purified⁷ rat peritoneal mast cells in Hepes-buffered Tyrodes solution (150 μ l with 10⁶ cells for 15 min at 37°C) with equal volumes (100 μ l) of 10⁻⁴ mol/l human ϵ -chain decapeptide solution at variable dilutions of rabbit peptide antiserum (1 in 4, 1 in 8, or 1 in 16). After addition of Hepes-buffered Tyrodes solution and centrifugation (150 g, 5 min), histamine concentration in the supernatant was measured by automated spectrofluorimetry. In-vivo studies measured the ability of rabbit antiserum to inhibit IgE-mediated passive cutaneous anaphylaxis (PCA) reactions in male Wistar rats. Groups of six rats were injected intradermally with 20 μ l of serum (at 1 in 2, 1 in 4, 1 in 8, and 1 in 16 dilutions) obtained from sensitised rats (see below). After 48 h they were challenged intravenously with 5 mg ovalbumin in Evans blue (2% w/v). Rabbit-peptide-antiserum (250 μ l) was given either concurrently with or 2 min before the allergen. In two further groups of laboratory animals, rabbit-peptide-antiserum (100 μ l) or normal rabbit serum (100 μ l) was given 48 h before the allergen challenge. After 2 h, animals were killed with either

ADDRESS: Rheumatology and Allergy Research Unit, Department of Immunology, University of Birmingham, Medical School, Birmingham B15 2TJ, UK (D R Stanworth, DSc, V M Jones, MSc, I V Lewin, HNC, S Nayar, BSc). Correspondence to Dr D R Stanworth.

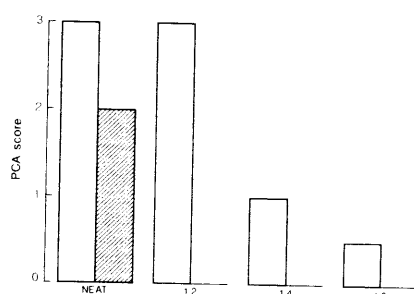


Fig 1—PCA inhibition tests.

PCA score was derived from a combined estimate of the area and intensity of bluing reaction. 1: = Control, normal rabbit serum; 2: = rabbit peptide antiserum.

anaesthetic and their skins were removed and examined from the underside. The intensity of bluing at each site was compared with that recorded at PCA test sites in control rats that had been challenged with either ovalbumin mixed with normal rabbit serum or normal rabbit serum alone.

Sensitisation of rats was completed by subcutaneous injection of 500 µl chicken egg white (200 mg) and *Bordetella pertussis* (10^{11} organisms/2.5 ml).⁸

Immunisation studies

Rats were immunised with human ϵ -chain decapeptide protein carrier (KLH or PPD) conjugate, rat G4 domain mast cell activation peptide (Lys-Tyr-Asn-Gly-Ser-Asn-Gln-Arg-Phe-Phe-Ile-Phe-NH₂) or a non-histamine releasing analogue of this decapeptide (in which the N-terminal lysyl residue was replaced by a glycyl residue) either before or after they had been sensitised. At the end of a 4-week immunisation period (subcutaneous injection of immunogen in complete Freund's adjuvant and later by injection in incomplete adjuvant), "pre-immunised" rats were sensitised a week later (as described above) before being challenged with allergen (ovalbumin) after a further 4 weeks. "Post-immunised" rats were immunised over 4 weeks, 3 weeks after sensitisation, were re-sensitised after 1 week, and finally challenged with ovalbumin 4 weeks later.

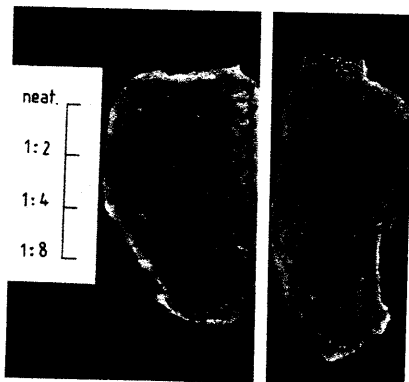


Fig 2—Bluing reactions.

Left, with normal rabbit serum (control); and right, rabbit peptide antiserum.

Peptide antibody and IgE concentrations in immunised rats' sera (tail vein) were measured in animals that received the human conjugate by ELISA. Sensitised rats that had been immunised with peptide were challenged by intravenous injection of near-fatal doses of ovalbumin (5 mg) and compared with non-immunised laboratory animals. Animals were killed 10 min later, and a blood sample obtained by cardiac puncture. Histamine concentrations in pre and post allergen challenge serum samples were measured by automated spectrophotometry. Antibody profiles of serum samples were measured by ELISA.

Results

Mast cell studies

Incubation of human ϵ -chain decapeptide with rabbit-peptide-antiserum reduced peptide induced histamine release from purified rat peritoneal mast cells (78% at 1 in 4, 72% at 1 in 8, and 37% at 1 in 16 dilutions).

This inhibitory activity in vitro was also shown in in-vivo PCA tests. A significant reduction in PCA reactivity (as assessed by both measurement of area of bluing, and an estimate of colour intensity) was observed when rabbit-peptide-antiserum was given 2 min before or at the same time as allergen (ovalbumin). In addition, there was a reduction in PCA activity when peptide-antiserum was given with sensitised allergic rat serum as shown by subsequent allergen challenge (figs 1 and 2).

Immunisation studies

In 5 of 6 rats immunised with a human peptide vaccine before sensitisation to egg white, there were no increases in serum histamine concentrations and no evidence of anaphylactic responses after 1 mg ovalbumin injection. In the sixth animal no peptide antibody was produced and the serum histamine concentration increased. In contrast, 2 non-immunised control rats died from anaphylactic shock and 2 showed evidence of anaphylaxis—eg, dyspnoea and cyanosis, after intravenous allergen challenge.

Effects of the human peptide vaccine on histamine concentrations are shown in fig 3. Pre-immunisation (fig 3a) caused a reduction in serum histamine from 330 to 70 ng/ml; immunisation after sensitisation (fig 3b) reduced histamine concentration from 2110 to 200 ng/ml ($p \leq 0.05$, Mann Whitney). Pre and post immunised rats showed significant IgG antibody responses to the peptide vaccine (fig 4a). However, IgE antibody concentration to ovalbumin fell after immunisation in both laboratory animal groups (fig 4b).

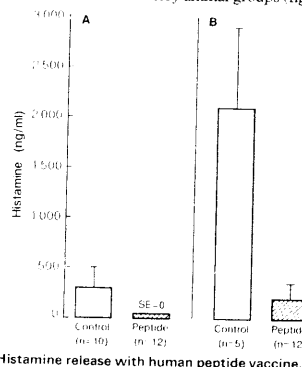


Fig 3—Histamine release with human peptide vaccine.

(a) Pre-immunised, and (b) post-immunised laboratory animals.

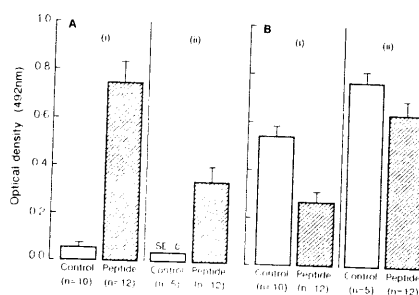


Fig 4—Immunoglobulin responses with human peptide vaccine. (a) IgG (i) pre-immunised and (ii) post-immunised (b) IgE (i) pre-immunised (p < 0.025) and (ii) post-immunised (p < 0.01).

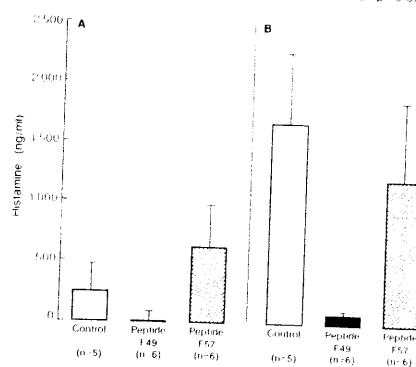


Fig 5—Histamine release with rat peptide vaccines. (a) Pre-immunised, and (b) post-immunised laboratory animals.

A vaccine generated from a rat immunogenic peptide-PPD conjugate (F49) led to a fall in serum histamine concentration after ovalbumin challenge in pre-immunised rats from 300 to 0 ng/ml (fig 5a) and in post-immunised rats from 950 to 50 ng/ml (fig 5b, $p < 0.01$). A rat peptide analogue (F57) had no significant effect on allergen-induced histamine release.

Discussion

The reduction in circulating histamine concentrations and the protection of sensitised rats from the consequences of subsequent systemic allergen challenge by pre-immunisation with a human ϵ -chain decapeptide, suggests a new and effective form of immunotherapy.

The reductions in anaphylactic responsiveness to allergen challenge, achieved with this heterologous peptide in sensitised rats, may not necessarily take place if allergic human beings are immunised with this same human peptide. However, this possibility is unlikely because pre or post immunisation of sensitised rats with a rat C ϵ 4 dodecapeptide, which was structurally homologous to the human peptide, gave comparable results. It is possible that the autoantigenic epitope, of which the synthetic rat dodecapeptide is representative, is sequestered within the

rat IgE molecule and not recognised as self. This is suggested by our failure to show reactivity of the peptide immunised rats' sera with rat or human IgE by ELISA.

The major peptide antibody response was IgG and not IgE. Our finding of a reduced IgE response to allergen in laboratory animals is consistent with earlier evidence that shows that the human ϵ -chain decapeptide behaves like interleukin-4,⁹ which upregulates expression of low-affinity IgE Fc ϵ RII receptors on B lymphocytes, and enhances IgE production. The peptide antibody made in response to immunisation of rats with peptide-protein conjugate may neutralise the effect of free ϵ -chain peptide on IgE synthesis.

The application of these results to immunotherapy of atopic patients may be important because of inhibition of mast cell activation and reduced synthesis of IgE by B lymphocytes. The definition of optimum immunisation conditions for long-term protection, the applicability of these results to other allergens, and the possibility of passive immunisation with murine monoclonal antibodies are being investigated. Clinical trials will be needed to examine the value of this new immunotherapeutic technique.

REFERENCES

1. Noon L. Prophylactic inoculation against hay fever. *Lancet* 1911; i: 1572-73.
2. World Health Organisation and International Union of Immunological Societies working group report: current status of allergen immunotherapy. *Lancet* 1989; i: 259-61.
3. Committee on Safety of Drugs update. Desensitising vaccines. *Br Med J* 1989; 299: 948.
4. Stanworth DR. The role of non-antigen receptors in mast cell signalling processes. *Mol Immunol* 1984; 21: 118-120.
5. Sheppard RC. Modern methods of solid phase peptide synthesis. *Science Tools* 1986; 33: 9-10.
6. Burt DS, Hastings GZ, Stanworth DR. Use of synthetic peptides in the production and characterisation of antibodies directed against predetermined specificities in rat immunoglobulin E. *Mol Immunol* 1986; 23: 181-91.
7. Cooper PH, Stanworth DR. Isolation of rat peritoneal mast cells in high yield and purity. In: Prescott DM, ed. *Methods in Cell Biology*. New York: Academic Press, 1976: 365-78.
8. Jasani B, Stanworth DR. An investigation of the optimal conditions for the in-vivo production of immunologically sensitised rat mast cells. *J Immunol Methods* 1979; 30: 55-68.
9. Stanworth DR, Ghaderi AA. The role of high and low affinity IgE receptors in cell signalling processes. *Mol Immunol* in press.

From The Lancet

Outpatient departments in hospitals

The outpatient departments of hospitals form a rich mine, which is very imperfectly utilized and very insufficiently appreciated by medical students. Attendance on the physicians and surgeons who have charge of the inpatients is compulsory on students; it is that which is required by the examining boards. Attendance on the outpatient rooms is given or withheld commonly at the will of the student. Hence it is comparatively rare to find them at the elbow of the medical officer in charge of this department, where they could see and learn so much that is of the highest importance; while they may be found in troops at the heels of the inpatient attendant, where often, from their number, they can hardly see or learn anything. Students who attend at hospitals, however, to qualify for the profession in the true and best sense of that much-abused word, should by no means neglect the outpatients. The multitude and variety of their slighter ailments or earlier stages of disorder afford the most valuable means of instruction: these are the phases of disease which make up the mass of ordinary practice. The severer forms and later stages of disease are proportionately rare.

(Oct 7, 1865)