

Intranasal Immunotherapy with Polymerized Grass Pollen Allergens

A Pilot Study

M. J. SCHUMACHER¹ and M. C. F. PAIN²

¹Clinical Research Unit of the Walter and Eliza Hall Institute of Medical Research,

²Respiratory Unit of the Royal Melbourne Hospital, Australia, and

¹Dept. of Pediatrics, University of Arizona, Tucson, Arizona, U.S.A

The effect of immunotherapy by intranasal insufflation of *Lolium perenne* allergens modified with glutaraldehyde (Glu-Rye) was tested by a stratified placebo-controlled trial in 16 adult subjects with hay fever. The purpose of this pilot study was to determine whether the treatment could reduce nasal sensitivity to allergen in provocation tests. Modification of the allergen was shown to include polymerization and reduced allergenicity, but retained immunogenicity. Nasal sensitivity to unmodified *L. perenne* allergens was measured by threshold-type quantitative nasal challenge and posterior rhinometry for measurement of nasal airway resistance. In challenge tests after a 10-week course of intranasal preseasonal treatment with Glu-Rye, nasal sensitivity was reduced, but this effect was not maintained when testing was repeated 9 months after treatment. Post-seasonal serum levels of IgE antibodies to *L. perenne* were also not significantly affected by the treatment.

Key words: allergy to staphylococci and streptococci; bacterial cell wall; bacterial hypersensitivity; inflammation; intracutaneous test; peptidoglycan.

Accepted for publication 27 October 1981

CLINICAL ASPECTS

Polymerized allergens, being less allergenic and equivalent in immunogenicity to unmodified allergens, can be insufflated into the nose of allergic patients with only minor side effects. The purpose of this placebo-controlled pilot study of nasal immunotherapy, given preseasonally 3 times a week to adults with seasonal allergic rhinitis, was to determine whether nasal treatment with polymerized perennial rye grass pollen allergens could reduce their nasal sensitivity to native rye allergens. Nasal sensitivity, measured by objective methods during quantitative challenge tests, was temporarily suppressed by the treatment. Although this effect could not be corroborated by symptom scoring, the benign nature of the treatment encourages further study, using extended periods of observation to identify efficacy extending beyond transient nonspecific desensitization.

Immunotherapy of hay fever by repeated intranasal applications of allergen extracts is potentially more convenient than conventional allergen injection therapy. Its possible efficacy has been suggested by demonstration of loss of sensitivity to grass pollen allergens in patients repeatedly challenged intranasally with threshold doses of allergens in nasal challenge tests (16). Cook (2) and Mehta & Morrison Smith

(9), using a similar approach, were encouraged by symptomatic improvement of their patients. However, allergic reactions from nasal treatment with unmodified allergens could cause difficulties in the conduct of double-blind trials, and may reduce its general acceptability as an alternative form of immunotherapy. For example, Welsh et al. (17) found that preseasonal intranasal treatment with partially purified but

unmodified short ragweed extract did so at the expense of provoking symptoms during the preceding weeks.

Pollen allergens modified by glutaraldehyde, having reduced allergenicity but retained immunogenicity (10, 13), are potentially more suitable for study of local immunotherapy. In an open trial of intranasal therapy with mixed grass pollen allergens modified by glutaraldehyde and adsorbed to tyrosine, and in a controlled trial of timothy grass allergens modified with glutaraldehyde, nasal treatment was found to be clinically effective (3, 5). However, reports from controlled studies of intranasal treatment with polymerized ragweed allergens have shown conflicting results (7, 11).

This report describes a pilot study of glutaraldehyde-modified perennial rye grass allergen for intranasal immunotherapy of patients with hay fever, tested by a stratified double-blind design. The purpose of the study was to determine whether preseasonal nasal immunotherapy with a polymerized allergen would reduce nasal sensitivity to unmodified allergen in quantitative, objective, nasal challenge tests.

MATERIAL AND METHODS

Subjects

Twenty subjects, aged between 20 and 53 years were studied with their informed written consent. All had hay fever with symptoms during the period September to December, the rye grass pollination season in Melbourne, and none had a history of asthma requiring medications. One subject had received allergen injections 25 years ago. All subjects had weal responses of 20 to 120 mm² to prick tests with *Lolium perenne* extracts at 2 mg dry weight/ml. Two subjects in the treatment group and three subjects given placebo were also sensitive to other allergens including house dust mite (*D. pteronyssinus*).

Nasal challenge tests

Nasal sensitivity to allergen was determined as described previously (14), using quantitative challenges with increasing concentrations of al-

lergen delivered by an electronically controlled metering system. Threshold doses of allergen were determined from the amount required to double the level of nasal airway resistance, measured during a control period, using posterior rhinometry. All of the challenge tests were done with the same batch of allergen, from which a fresh vial was reconstituted each week.

Preparation of allergens

Partly purified *L. perenne* allergen for skin testing and nasal challenge testing was prepared and stored as described previously (14). Briefly, *L. perenne* was extracted, dialysed, and partly purified by Sephadex G-75 gel filtration and DE-52 ion exchange chromatography. Eluted material that was active in prick tests of allergic subjects was pooled, lyophilised, and stored in aliquots under dry nitrogen at -70°C until required. To prepare modified allergen for intranasal treatment, a portion of this material was treated with 100 mM glutaraldehyde for 2 h at pH 7.0 and 20°C, as described by Moran et al. (10). No insoluble material was formed by the glutaraldehyde treatment. The glutaraldehyde-modified rye grass allergen (Glu-Rye) was then dialysed for 3 days against several changes of 0.05 M NH₄HCO₃. A solution containing 320 µg of Glu-Rye and 20 mg of lactose per milliliter was serially diluted in 20 mg/ml lactose, lyophilised, and loaded into three sets of gelatin capsules in 20.0 mg portions, to contain 12.8, 64 and 320 µg of Glu-Rye, respectively. Placebo capsules contained 20 mg of lactose, tinted with caramel to match the three strengths of Glu-Rye capsules.

Characterization of glutaraldehyde-modified allergen

To test the degree of polymerization achieved, samples of Glu-Rye and unmodified rye were reduced with dithiothreitol and analysed by polyacrylamide gel electrophoresis with sodium dodecyl sulphate and urea (6), and high molecular weight standards (Pharmacia AB). The major portion of a Glu-Rye sample would not enter 7.5% acrylamide running gels, indicating molecular weights considerably higher than

500,000 daltons. A minor component of Glu-Rye had a molecular weight similar to the major component of untreated rye allergen, suggesting that Glu-Rye also contained some unpolymerized material. Free amino group determination, performed by a fluorescamine assay method (1) showed 88% substitution of free amino groups by glutaraldehyde. End-point titration of the modified material by prick testing and nasal challenge testing of allergic subjects showed a 50-fold reduction in potency. To check for immunogenicity of the polymerized material, rabbits were immunized with 1 mg of Glu-Rye in complete Freund's adjuvant and boosted with Glu-Rye and adjuvant on days 34 and 98. Control rabbits were immunized with unmodified rye allergens, using the same protocol. Haemagglutinating antibodies (HA) in the rabbit sera were assayed in microtiter trays using tanned sheep erythrocytes coupled with unmodified rye allergens (1 mg/ml) at pH 6.4 (4). Rabbits given Glu-Rye developed HA titres similar to those of rabbits given unmodified rye (Fig. 1).

Experimental design

Three nasal challenge tests with *L. perenne* allergen were given before a 10-week course of nasal treatment with either Glu-Rye or placebo (Table 1). The three challenge tests were then repeated during the following 10 months. After the first three challenge tests, the subjects were

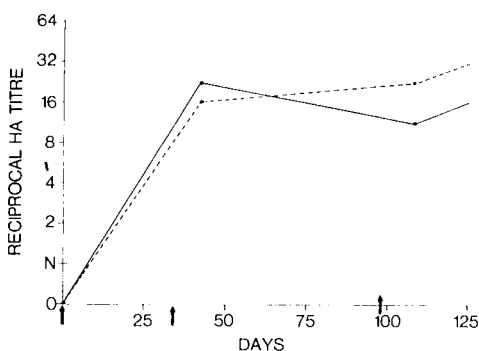


Fig. 1. Titres of haemagglutinating antibodies to *L. perenne* allergen in rabbits immunized with Glu-Rye (—) and with unmodified *L. perenne* allergen (---). Injections given at arrows.

Table 1

Schedule for nasal challenge testing, bleeding and intranasal treatment with modified allergen

Time	Event
October to December, 1977	Rye grass pollen season
October & November, 1977	Nasal challenge test (spring)
January & February, 1978	Nasal challenge test and RAST (summer)
May & June, 1978	Nasal challenge test (early winter)
July to September, 1978	Treatment period
October to December, 1978	Rye grass pollen season
October & November, 1978	Nasal challenge test
January & February, 1979	Nasal challenge test and RAST
May & June, 1979	Nasal challenge test

ranked in 10 pairs according to the level of their nasal sensitivity, and then one member of each pair was randomly assigned to the Glu-Rye or placebo group by an independent statistician. Glu-Rye or placebo capsules were then dispensed into containers for each subject by the statistician. Two subjects, including one who developed an intercurrent viral infection and sinusitis, withdrew from the trial during treatment. Therefore the subjects with whom they were matched were also excluded from subsequent analysis, leaving eight treated with Glu-Rye, and eight with placebo. The assignment code was withheld until the sixth nasal challenge test was completed.

Treatment protocol

During a 10-week period in late winter and early spring (July–Sept., 1978), each subject was given either Glu-Rye or placebo with a Rynacrom® intranasal insufflator. Three times a week, the contents of one capsule were insufflated into the nose, evenly divided between both nostrils, 45 min after two sprays of 2% w/v disodium cromoglycate into each nostril to minimize symptoms that could be attributed to allergens. The Glu-Rye capsules given initially contained 12.8 µg of allergen, and doses were increased during the second and third week, as tolerated. Five of the subjects given allergen could tolerate the highest strength (320 µg) during the third week. After maintenance doses

Table 2

Nasal sensitivity to unmodified *L. perenne* allergen before and after nasal treatment with Glu-Rye*

Subject	Total dose of Glu-Rye (mg)	Threshold doses of <i>L. perenne</i> allergen (μ g) in nasal challenges					
		1	2	3	4	5	6
		Spring 1977	Summer 1978	Winter 1978	Spring 1978	Summer 1979	Winter 1979
JW	3.75	5.91	147	29.4	ND	29.4	29.4
JQ	5.36	5.91	5.91	5.91	5.91	ND	5.91
MW _o	2.80	5.91	29.4	1.16	5.91	5.91	5.92
RT	5.11	3.0	5.91	5.91	ND	29.4	1.16
JS	2.93	1.16	5.91	1.16	1.16	5.91	1.16
MW _a	3.62	3.0	1.16	1.16	1.16	5.91	1.16
PA	2.87	1.16	5.91	1.16	5.91	5.91	1.16
RF	3.00	1.16	0.26	1.16	ND	0.26	0.26

* Glu-Rye = glutaraldehyde-modified *L. perenne* allergen given in late winter, 1978, after challenge # 3.

ND: not done.

Table 3

Nasal sensitivity to *L. perenne* before and after nasal treatment with placebo*

Subject	Threshold doses of <i>L. perenne</i> allergen (μ g) in nasal challenges					
	1	2	3	4	5	6
	Spring 1977	Summer 1978	Winter 1978	Spring 1978	Summer 1979	Winter 1979
PT	29.4	29.4	29.4	1.16	5.91	147
AC	29.4	5.91	5.91	29.4	5.91	1.16
SL	3.0	29.4	5.91	ND	5.91	29.4
RD	5.91	29.4	5.91	5.91	5.91	29.4
CJ	29.4	1.16	0.26	1.16	0.26	5.91
DH	3.0	5.91	1.16	0.26	5.91	5.91
MN	0.3	0.26	29.4	5.91	29.4	29.4
MT	1.16	1.16	0.26	1.16	1.16	1.16

* See footnote to Table 2.

ND: not done.

were achieved, it was necessary to reduce the treatment strength for two subjects (R.T. and P.A.) because of sneezing and rhinorrhoea after each treatment. The average total dose of Glu-Rye given over 10 weeks was 3.68 mg of protein (range 2.80 to 5.63 mg). The Glu-Rye group subjects used slightly fewer maximum strength capsules than did the placebo group, but this was not statistically significant.

Radioallergosorbent tests (RAST)

Sera collected in January and February of 1978 and 1979 were stored at -70°C until assayed for IgE antibodies to *L. perenne* by Phadebas[®] RAST. Results were calculated as percentages of radiolabelled anti-IgE bound.

Symptom scoring

The severity of sneezing, nasal obstruction, nasal discharge and eye irritation was scored and recorded by the subjects daily during the pollen season after intranasal treatment as follows. Scores for each symptom persisting for less than 2 h: mild = 1, moderate = 2, severe = 3; symptoms persisting for more than 2 h: mild = 2, moderate = 4, severe = 6. Scores for all symptoms were added to give total daily and weekly scores.

Statistical methods

Wilcoxon signed rank sum tests for matched pairs were used to compare logarithms of nasal sensitivities and IgE antibody levels before and after treatment. Significance of differences between symptom scores of the Glu-Rye and placebo treatment groups was determined by the Mann-Whitney U test. Both of these nonparametric ranking tests are applicable to small samples and are independent of distribution (15).

RESULTS

Nasal sensitivity to allergen

As seen in Table 2, nasal sensitivity to native rye allergens in subjects treated intranasally with Glu-Rye decreased significantly when challenge tests in winter 1978 (before treatment) and summer 1979 (4 months after treatment) were compared ($P < 0.05$, one-tailed). In contrast, subjects given placebo showed no change in nasal sensitivity during this period (Table 3). However, reduction in sensitivity of subjects treated with Glu-Rye was not maintained, because their sensitivities in challenge tests returned to pretreatment levels within 9 months of the treatment period.

Nasal symptom scores

The eight subjects given Glu-Rye and seven of the eight subjects given placebo completed symptom score forms. During the pollen season

Table 4

Symptom scores during the rye grass pollen season (October 8 to December 31, 1978) following treatment with Glu-Rye or placebo

Placebo treatment		Glu-Rye treatment	
Subject	Average weekly score	Subject	Average weekly score
PT	50.6	JW	41.1
AC	2.6	JQ	35.5
RD	32.4	MW _o	61.4
CJ	41.8	RT	80.5
DH	23.9	JS	17.6
MN	30.9	MW _a	84.7
MT	45.6	PA	38.8
		RF	54.0

Table 5

L. perenne-specific IgE antibodies in summer-time sera before and after nasal treatment

Percent binding*			Percent binding*		
Subject	before treatment		Subject	before treatment	
	after treatment			after treatment	
PT	39.4	45.0	JW	3.1	6.3
AC	14.9	9.4	JQ	46.1	47.9
SL	28.1	38.3	MW _o	42.9	44.1
RD	31.3	33.0	RT	28.8	32.5
CJ	39.5	38.6	JS	33.1	34.3
DH	8.2	9.1	MW _a	7.4	8.4
MN	38.8	40.5	PA	40.5	40.9
MT	19.2	22.5	RF	43.8	44.7

* Percent ¹²⁵I-labelled anti-IgE bound to paper discs.

that followed the course of nasal treatment, the averages of the weekly symptom scores of subjects given Glu-Rye (range 17.6–84.7) were higher than scores of subjects given placebo (range 2.6–50.6), but the differences were not significant at the 5% level (Table 4). When the eight subjects given Glu-Rye were asked to identify their treatment, five were correct. However, three of eight subjects given placebo also believed that they had been given Glu-Rye. During the 2-h period after most of the treatment doses, sneezing and rhinorrhoea occurred in

five subjects given Glu-Rye, of whom three correctly identified their treatment. Only occasional mild symptoms followed placebo treatment.

Serum IgE antibody levels

IgE antibody levels specific for *L. perenne* in paired sera taken in the summers of 1978 and 1979 (12 months apart), before and after treatment, increased slightly in both the group treated with Glu-Rye and the group treated with placebo (Table 5). However, the changes were statistically significant only for the placebo group ($P = 0.01$).

DISCUSSION

This study differs from the published investigations of nasal immunotherapy in that its principal objective was to determine whether this form of therapy could induce a long-lasting suppression of nasal sensitivity to allergen, as measured objectively by quantitative nasal challenge. Perhaps unique to this study was the objective measurement of nasal sensitivity using a quantitative allergen delivery system and measurement of nasal airway resistance, performed repeatedly for 10 months before treatment and for 9 months after treatment. This was done to determine whether any effects of nasal immunotherapy were limited to transient desensitization, as seen in "rush" treatment (8).

Using this approach, it was shown that there was evidence of decreased nasal sensitivity to allergen when pretreatment challenge tests were compared with tests done after the grass pollen season that immediately followed the preseasonal treatment. These results agree with the findings of Johansson et al. (5) who found that patients treated with a glutaraldehyde-modified timothy grass extract were less sensitive to allergen immediately after treatment. However, continued testing of our subjects showed that desensitization to rye allergen was only a temporary phenomenon. Nickelsen et al. (11), who treated subjects intranasally with glutaraldehyde-polymerized short ragweed ex-

tract, were not able to show any significant change in nasal sensitivity to unmodified allergen, even in tests done immediately after treatment.

Although the nasal treatment did lead to some reduction in nasal sensitivity after the grass pollen season, this was not reflected in a significant difference between the seasonal symptom scores of the treated subjects and the scores of the placebo group. This finding agrees with results of Matthews et al. (7) and conflicts with findings of symptomatic improvement in three other studies (5, 11, 17). It is acknowledged that symptom scoring as employed in our trial has only limited validity, because the patients were not stratified according to their pretreatment symptom scores. Stratification according to the patients' levels of nasal sensitivity to allergen was required by the primary objective of studying effects of treatment on challenge test results.

A further problem in basing controlled nasal immunotherapy trials on results of symptom scores, aside from the imprecision of quantifying subjective sensations, lies in the possibility that the reported studies may not have been truly double-blind. In at least two reports (5, 12) it was conceded that all of the treated subjects had allergic symptoms following each nasal treatment: in our study we could not prevent some treatment reactions by concomitant use of nasal cromoglycate. Nickelsen et al. found that unmodified allergen, given intranasally and preseasonally, resulted in more severe adverse reactions than reactions from polymerized allergens, but was more effective than polymerized allergen in reducing seasonal symptoms. This is consistent with findings of Welsh et al. (17) who showed that reduced seasonal symptoms followed preseasonal occurrence of symptoms caused by treatment, suggesting that nasal immunotherapy may work by a mechanism dependent on depletion of mast cell mediators. If this hypothesis is correct, we may have rendered our treatment clinically ineffective by combining it with nasal cromoglycate therapy.

Another possible explanation of lack of clinical efficacy in our study may be an insufficient

total dose given to each subject, which was between 2 and 6 mg of protein. Johansson et al. (5) gave approximately 6 times this amount, but the extract used in that study was from a different species of grass, which, unlike our preparation, was not chromatographically purified, and allergenic potencies of the two preparations were not available for comparison. Comparisons between doses of grass allergens and doses of ragweed allergens used in apparently successful U.S. studies (11, 17) are even more speculative. Inadequate polymerization of material used in the present study was not a likely explanation, because polymerization of the major proportion of the material was demonstrated in analytical studies and immunogenicity was retained in the face of reduced allergenicity, as described for glutaraldehyde-modified timothy pollen extract (10).

Post-seasonal IgE antibodies to *L. perenne* in serum were not significantly changed by nasal treatment with Glu-Rye. This agrees with other studies (5, 12) in which polymerized allergen given intranasally did not affect the post-seasonal rise of serum IgE antibody. IgG and secretory IgA antibodies were not studied in nasal secretions in this investigation, but it appears that these antibodies do not correlate well with clinical response to nasal immunotherapy (12). It is suggested that in future studies of nasal immunotherapy, carefully performed nasal challenge tests should be done for extended periods before and after nasal treatment, to be certain that loss of sensitivity is maintained. It may also be necessary to consider use of histamine and methacholine in placebo preparations, to ensure that a symptom scoring study is properly controlled by adequate blinding.

ACKNOWLEDGEMENTS

The authors are indebted to Ann Dunstone and Carmen Wagner for technical assistance, Jonathon Buckley for statistical analysis, and to Fisons Pty. Ltd. for gifts of Rynacrom® (disodium cromoglycate) nasal sprays and Rynacrom® insufflators. The work was supported by grants from the National Health and Medical Research Council of Australia,

and the Bencard Division of Beecham Research Laboratories, Australia.

REFERENCES

1. Böhlen, P., Stein, S., Dairman, W. & Udenfriend, S.: Fluorometric assay of proteins in the nanogram range. *Arch. Biochem. Biophys.* 155, 213–220, 1973.
2. Cook, N.: Pre-seasonal local nasal desensitization in hay fever. *J. Laryngol. Otol.* 88, 1169–1174, 1974.
3. Deuschl, H. & Johansson, S. G. O.: Hyposensitization of patients with allergic rhinitis by intranasal administration of chemically modified grass pollen allergen. A pilot study. *Acta Allergol.* 32, 248–262, 1977.
4. Herbert, W. J.: Passive haemagglutination with special reference to the tanned cell technique. In Weir, D. M. (ed.): *Handbook of experimental immunology*, pp. 20.1–20.20. Blackwell, Oxford, 1978.
5. Johansson, S. G. O., Deuschl, H. & Zetterström, O.: Use of glutaraldehyde-modified Timothy grass pollen extract in nasal hypsensitization treatment of hay fever. *Int. Arch. Allergy Appl. Immunol.* 60, 447–460, 1979.
6. King, J. & Laemmli, U. K.: Polypeptides of the tail fibres of bacteriophage T4. *J. Mol. Biol.* 62, 465–477, 1971.
7. Mathews, K. P., Bayne, N. K. & Banas, J. M.: A controlled study of intranasal immunotherapy with polymerized ragweed antigen. *J. Allergy Clin. Immunol.* 65, 191, 1980 (Abstract).
8. May, C. D., Schumacher, M. J. & Williams, C. D.: Significance of concordant fluctuation and loss of leukocyte sensitivity to two allergens during injection therapy with one. Nonspecific desensitization. *J. Allergy Clin. Immunol.* 50, 99–108, 1972.
9. Mehta, S. B. & Morrison Smith, J.: Nasal hypsensitization and hay fever. *Clin. Allergy* 5, 279–284, 1975.
10. Moran, D. M., Wheeler, A. W., Overell, B. G. & Woronicki, S. R.: Chemical modification of crude Timothy grass pollen extract. III. The effect of glutaraldehyde-induced aggregation on antigenic and immunogenic properties. *Int. Arch. Allergy Appl. Immunol.* 54, 315–321, 1977.
11. Nickelsen, J. A., Goldstein, S., Mueller, U., Wypich, J., Reisman, R. E. & Arbesman, C. E.: Local nasal immunotherapy for ragweed allergic rhinitis. I. Clinical response. *J. Allergy Clin. Immunol.* 63, 33–40, 1981.
12. Nickelsen, J. A., Goldstein, S., Mueller, U., Wypich, J., Reisman, R. E. & Arbesman, C. E.: Local nasal immunotherapy for ragweed allergic rhinitis. II. Immunologic response. *J. Allergy Clin. Immunol.* 68, 41–45, 1981.
13. Patterson, R., Suszko, I. M., Pruzansky, J. J. & Zeiss, C. R.: Polymerized ragweed antigen E. II. *In vivo* elimination studies and reactivity with IgE antibody systems. *J. Immunol.* 110, 1413–1418, 1973.
14. Schumacher, M. J. & Pain, M. C. F.: Nasal challenge testing in grass pollen hay fever. *J. Allergy Clin. Immunol.* 64, 202–208, 1979.
15. Siegel, S.: *Nonparametric statistics: for the behavioral*

sciences. McGraw-Hill Kogakusha Ltd., Tokyo, 1956.

16. Taylor, G. & Shivalkar, P. R.: Local nasal desensitization in allergic rhinitis. *Clin. Allergy* 2, 125–136, 1972.
17. Welsh, P. W., Zimmerman, E. M., Yunginger, J. W., Kern, E. B. & Gleich, G. J.: Preseasonal intranasal immunotherapy with nebulized short ragweed extract. *J. Allergy Clin. Immunol.* 67, 237–242, 1981.

Address:

M. J. Schumacher, M.B., F.R.A.C.P.
Dept. of Pediatrics
University of Arizona Health Sciences Center
Tucson, Arizona 85724
U.S.A.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.