

Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids

II. Comparison between parameters assessing the efficacy of immunotherapy

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Specific immunotherapy is effective in alleviating symptoms in grass pollen-induced rhinitis, but there are no clear data demonstrating a correlation between symptom-medication scores and objective parameters. Twenty-five patients taking part in a double-blind, placebo-controlled immunotherapy with mixed grass pollen-formalinized allergoids were studied. All patients had the same investigations. Symptom-medication scores were significantly ($p < 0.005$, Mann-Whitney U test) reduced in the treated group by comparison to the placebo-treated patients. Nasal challenges performed with threefold increasing numbers of orchard grass-pollen grains demonstrated that patients treated with allergoid tolerated a significantly ($p < 0.005$, Wilcoxon W test) greater number of grains after treatment, whereas there was no mean difference in the placebo-treated patients. There was a significant ($p < 0.005$, Spearman rank-correlation) correlation between nasal challenges and symptom scores during the season. The skin prick test end point was significantly ($p < 0.001$, Wilcoxon W test) reduced after treatment in the allergoid-treated group and remained unchanged in the placebo-treated group. There was a significant ($p < 0.001$) correlation between the skin prick test end point and symptom scores during the season. Serum grass-pollen IgG titrated by a solid-phase radioimmunoassay with Staphylococcus A protein was significantly ($p < 0.01$, Wilcoxon W test) increased after treatment with allergoid, but there was no significant correlation between IgG titer and symptom scores during the season. Serum grass-pollen IgE increased ($p < 0.04$, Wilcoxon W test) in the treated group but there was no correlation with symptom scores. (J ALLERGY CLIN IMMUNOL 1988;82:439-46.)

Specific immunotherapy has been demonstrated to be effective in grass-pollen allergy.¹⁻³ Its clinical efficacy can be directly assessed by symptom-medication scores during the pollen season^{4,7} or indirectly by means of nasal,^{4, 8-11} conjunctival,¹² or bronchial challenges¹³ or skin tests.^{4, 5, 8, 14} The direct clinical assessment of efficacy by means of symptom scores is subject to the patient's compliance, the subjective assessment of symptoms, and the possible local

Abbreviations used

BU: Biological units
PNU: Protein nitrogen units
SPT: Skin prick test

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variations of the pollen content of the atmosphere. Provocative challenges are often difficult to interpret because they are very different from the natural pollen exposure; however, nasal challenges with pollen grains have recently been proposed and appear to resemble the natural allergen exposure.^{15, 16} Moreover, in ragweed-pollen allergy, it was found with this method that the threshold dose eliciting a positive challenge was significantly increased after specific immunotherapy.¹¹

TABLE I. Protocol of specific immunotherapy

	AM	PNU
Day		
1	9	50
	10	100
	11	200
8	9	300
	10	500
14		700
21		1000
28		1500
35		2000

Four injections of maintenance dose (2000 PNU) before April 1; from April 1 to October 1, bimonthly injection of 1000 PNU.

In grass-pollen allergy, histamine release from blood basophils,¹⁷ the titration of grass-pollen-specific IgG antibodies^{5, 12, 18-21} and IgG subclasses,^{19, 22} or lymphocyte studies²³⁻²⁵ that have evaluated the immune response of the patients and correlations with symptom-medication scores have been attempted without great success.

Mixed pollen-formalinized allergoids²⁶ have been demonstrated to be efficient in significantly protecting pollen-allergic patients from nasal symptoms in ragweed and grass-pollen allergies²⁷⁻³⁰; therefore, this mode of treatment may be suitable for assessing the efficacy of different methods evaluating specific immunotherapy.

A double-blind, placebo-controlled study was carried out analyzing symptom-medication scores and objective parameters (nasal challenges with grass-pollen grains, SPTs, and specific IgG and IGE) to determine their relative effectiveness in evaluating specific immunotherapy.

MATERIAL AND METHODS

Patients

Twenty-five patients (15 men, aged 16 to 44 years, 23.1 ± 11.5 years) volunteered to enter the study after informed consent. They were selected on the following criteria:

1. All patients had severe symptoms of rhinitis during the grass-pollen season, and more than half the patients also had symptoms of asthma and/or conjunctivitis. The duration of symptoms ranged from 4 to 21 years.
2. All patients had a positive SPT to a 1/100 (wt/vol) standardized orchard grass-pollen extract (Stallergenes Laboratories, Fresnes, France) and the presence of orchard grass pollen-specific IgE.

TABLE II. Symptom-medication scores used during the pollen season

No symptom	0
Episodes of sneezing	1
Nasal blockade	1
Rhinorrhea	1
Pruritus of the nose	1
No medication	0
Nasal sprays of disodium cromoglycate	1
Nasal sprays of beclomethasone	2
Terfenadine	3
Oral prednisolone	4

3. None of the patients had received any form of specific immunotherapy to pollen extracts.
4. No patient had a demonstrable tree-pollen allergy.

Extracts

A six-grass pollen allergoid was prepared by mild formalinization according to the method described by Marsh et al.²⁶ with equal weights of pollens from six grass-pollen species (*Dactylis glomerata*, *Festuca elatior*, *Holcus lanatus*, *Lolium perenne*, *Phleum pratense*, and *Poa pratensis*) and previously described in detail.²⁸

Immunotherapy

A double-blind, placebo-controlled immunotherapy was performed according to the protocol indicated in Table I. When a systemic reaction or a large local reaction of >10 cm occurred, the dosage increment was reduced, but all patients were able to reach the maintenance dose. After the maintenance dose had been reached, this was administered four times every 2 weeks. From April 1 to the end of the pollen season, patients received every 2 weeks a dose of 1000 PNU.

Symptom scores

When the patients attended the clinic in April, they were informed of the possible symptoms that could occur and the medication that they should take (Table II).^{5, 28} Patients completed daily forms during the month of June corresponding to the peak of the grass-pollen season.

SPTs

SPTs were performed according to the modified method of Pepys³¹ with a hypodermic needle on the volar surface of the forearm. None of the patients had any factor that might modify the performance of SPTs.³¹ All allergic patients had quantitative SPT with a standard orchard grass-pollen extract (Stallergenes Laboratories) prepared according to a method previously described in detail⁵ and controlled by in vivo and in vitro methods (RAST inhibition, iso elec-

TABLE III. Symptom score used for assessing the positivity of nasal challenges

Sneezing	
3-4	1
>5	2
Rhinorrhea	
Minor anterior rhinorrhea	1
Posterior rhinorrhea	1
Anterior and posterior rhinorrhea	3
Blockage	
Patient can breathe freely	0
Patient can only breathe with difficulty	1
One nostril is blocked	2
Both nostrils are blocked	3
Pruritus	
Nose	1
Palate and/or ear	1
Conjunctivitis	1

tric focusing, and cross immunoelectrophoresis). SPTs were done with threefold dilutions from 8 to 50,000 BU/ml. The end point titer was calculated as the threshold concentration eliciting a wheal similar to that elicited by the 9% codeine phosphate solution as previously described.^{5, 23, 33}

Nasal provocation tests

All patients were tested between 8 AM and 12 PM to avoid possible circadian variations by the same investigator. They were tested from December to January before specific immunotherapy and within 3 weeks (April 1 to 25) after immunotherapy, at a time when no grass pollen is found in the atmosphere. None of the patients was receiving treatment that might affect the performance of nasal challenges. Anti-H₁ histamines, nasal beclomethasone, were discarded 1 week before challenge. Nasal cromoglycate was withdrawn 2 days before testing. Ketotifen and imipramine were stopped 1 month before testing. None of the patients had any form of allergic or infectious rhinitis for the 2 weeks preceding the challenge.

Capsules containing lactose or increasing concentrations of orchard-grass pollens from 50 to 36,450 grains (increasing in threefold increment) were prepared by Stallergenes Laboratories. They were insufflated into the nostrils according to a technique modified from Rosenberg et al.³⁴ with a nasal Spinhaler (Fisons Laboratories, Loughborough, U.K.) and with the patient refraining from breathing during insufflation. For each concentration, the Spinhaler was actuated five times. Each capsule was opened after insufflation to assure that it was completely empty.

Lactose was insufflated initially, symptoms were recorded for 30 minutes, and the symptom score was calculated (Table II). Then, increasing numbers of grains were deposited on the nasal mucosa every 30 minutes until a symptom score of 5 was obtained according to Table III.

TABLE IV. Comparison of the patient groups

	Placebo-treated	Allergoid-treated
No. of patients	10	15
Age (yr)	22.5 ± 10.7	27.1 ± 9.7
SPT end point	6.7 ± 1.4	6.8 ± 1.2
Nasal challenge (grains)	217 ± 150	430 ± 505
Specific IgE (% binding)	21.3 ± 12.2	23.7 ± 11.6
Rhinitis symptoms		
Duration (yr)	8.5 ± 4.9	9.4 ± 6.1
Severity	3.7 ± 0.5	3.4 ± 0.6

This scoring system was defined according to Østerballe⁴ and Wihl,³⁵ and we subsequently confirmed the validity of this score in a previous study in which it was observed that the release of prostaglandin D₂ in nasal secretions was significantly ($p < 0.005$) associated with a symptom score of 5.³⁶

Mixed grass-pollen serum-specific IgG

Serum-specific IgG was titrated according to the method of Puttonen and Maasch³⁷ with RAST disks prepared with the mixed pollen extract according to the method of Ceska et al.³⁸ IgG coupled on the disk was revealed by *Staphylococcus* protein A radiolabeled with ¹²⁵I. The results were expressed in percent from baseline value.

Mixed grass-pollen serum-specific IgE

Serum-specific IgE was titrated by Phadebas RAST (Pharmacia Diagnostics AB, Uppsala, Sweden) with the same RAST disks as those used for the titration of specific IgG. Results were expressed in percent binding of total added counts.

Design of the study

Before immunotherapy and after four maintenance doses, each patient was investigated by means of nasal challenges, SPTs, and serum-specific IgE and IgG. During the pollen season, patients had follow-up by means of daily symptom-medication scores.

Statistical analysis

Statistical analysis was done with nonparametric tests. The Spearman rank-correlation test was used for regression analysis, the Wilcoxon W test, for paired data, and the Mann-Whitney U test, for unpaired comparisons.

RESULTS

Homogeneity of both groups

Table IV indicates the characteristics of patients placed in the placebo-treated group (10 patients) and

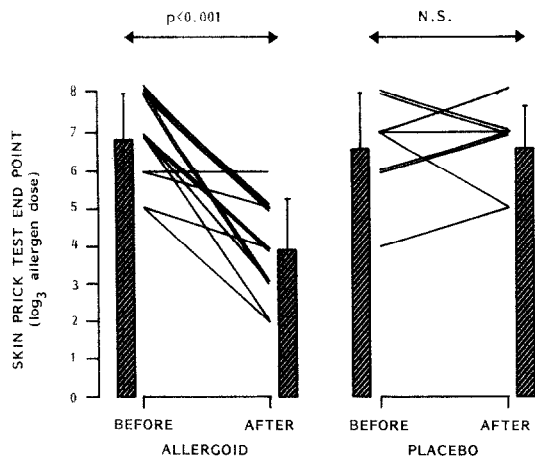


FIG. 3. Evolution of the skin test sensitivity to orchard grass-pollen extracts in patients treated with allergoid or placebo. Statistical analysis by Wilcoxon W test.

DISCUSSION

Our study demonstrated (1) that specific immunotherapy with allergoids is effective in reducing symptoms during the pollen season in grass pollen-allergic patients and (2) that the symptoms are correlated with both nasal challenges with grass-pollen grains and SPTs but not with serum-specific IgG or IgE.

All techniques used are simple and have been used by other investigators. Symptom scores were proposed more than 20 years ago in ragweed-pollen allergy^{6,7} and are widely accepted. Nasal challenges with grass-pollen grains appear at present to be one of the most satisfactory because they are correlated with the release of allergic mediators in nasal secretions^{15, 36, 39, 40} and with symptom-medication scores during the pollen season. Moreover, in the present study, it was observed that all but one patient receiving placebo treatment had a similar nasal challenge result on two consecutive occasions. SPTs have been widely studied during specific immunotherapy.^{4, 5, 8, 14} The end point or parallel line bioassays have been equally used and were found to be satisfactory. In the present study, patients treated with placebo had a similar SPT end point before and after immunotherapy, confirming therefore previous studies of our group.^{5, 28, 32} Many different techniques have been proposed to titrate serum-specific IgG. In this study we used a solid-phase radioimmunoassay that was largely used in grass pollen-allergic patients.³⁷ Patients placed in both treated groups had similar levels before immunotherapy, whereas patients treated with placebo did not present major differences after treatment.

Patients treated with placebo presented 25 to 30

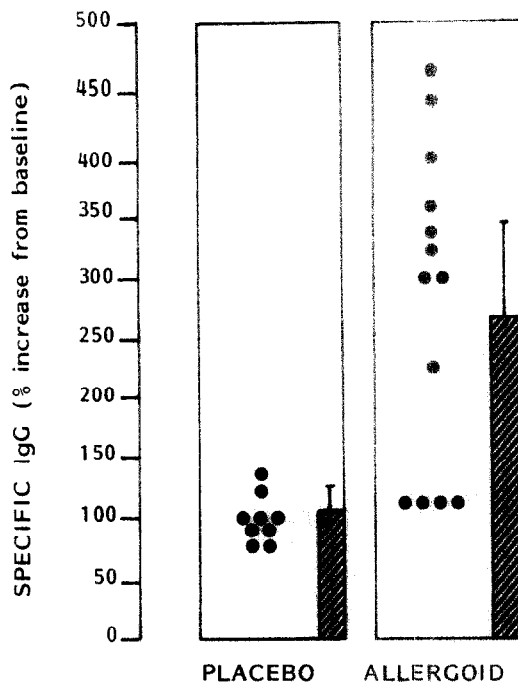


FIG. 4. Evolution of specific IgG. The first titration of IgG (before immunotherapy) was taken as 100%.

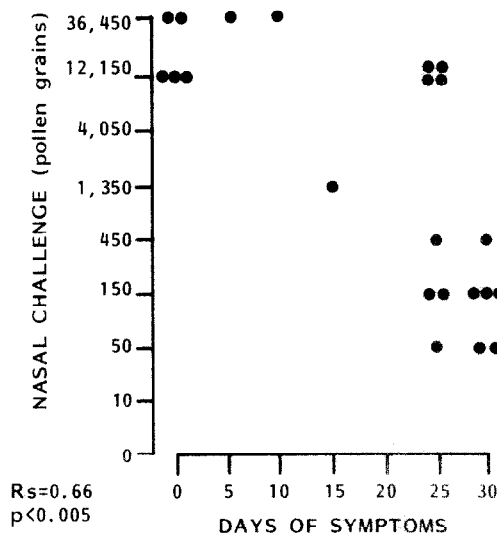


FIG. 5. Correlation between nasal provocation tests and nasal symptoms presented by the patients during the season. Statistical analysis by Spearman rank-correlation test.

days of nasal symptoms during the month of June, suggesting that the treatment was not effective. In contrast, patients treated with allergoids had a significantly better result, confirming therefore previous studies with formalized allergoids.²⁷⁻³⁰

Nasal challenges demonstrated that the threshold

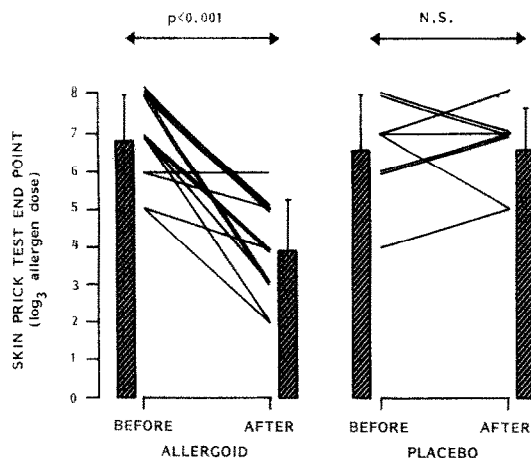


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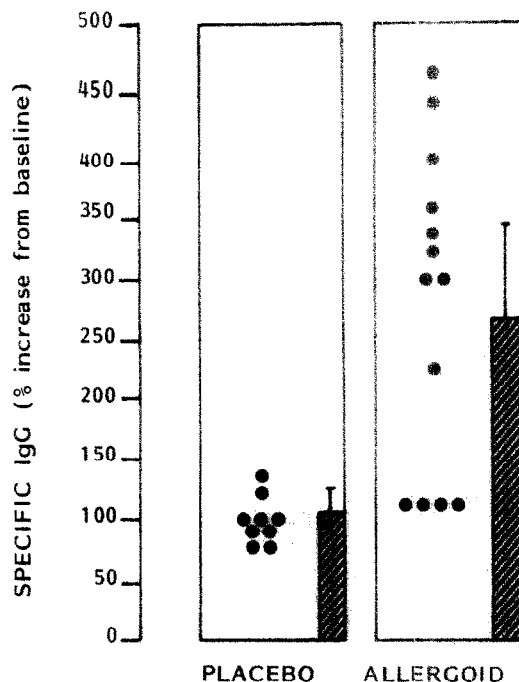


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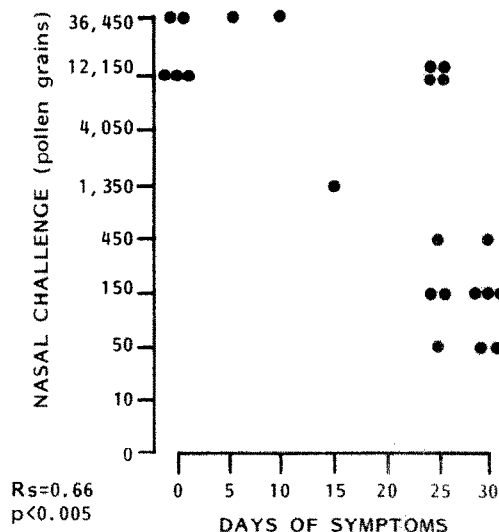


FIG. 5. Correlation between nasal provocation tests and nasal symptoms presented by the patients during the season. Statistical analysis by Spearman rank-correlation test.

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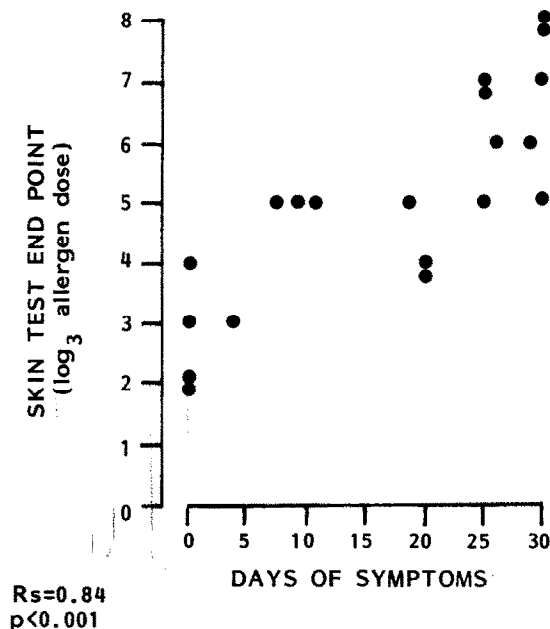


FIG. 6. Correlation between the SPT end point and nasal symptoms presented by the patients during the season. Statistical analysis by Spearman rank-correlation test.

number of grains eliciting symptoms was significantly increased in allergoid-treated patients, and this result accords with previous data collected either with grass-pollen extracts^{9, 10} or with ragweed-pollen grains.¹¹ Our results are very close to the latter study. The correlation between nasal challenges and symptoms was significant, although four patients tolerating a high number of pollen grains during the challenge presented many days of symptoms during the season. Nasal challenges with pollen grains may therefore be used (1) to assess the efficacy of specific immunotherapy during trials, (2) to be eventually a predictor of response of immunotherapy before the pollen season starts, and (3) to compare the benefits of immunologic and pharmacologic treatments.

SPT results were significantly reduced after specific immunotherapy, as has been demonstrated in many studies with grass pollen^{4, 5, 14, 28}; however, in most of these studies, there was no correlation between symptom scores and SPTs. In the present study, we observed a significant correlation between SPT reactivity and symptoms during the pollen season. In the study by Østerballe,⁴ it was indicated that the threshold concentration eliciting a positive SPT was correlated with symptoms. These data suggest that SPTs may be used as a predictor of response of the patients to specific immunotherapy, although mast cells present in the skin and in the surface layers of the nasal mucosa appear to be different.^{41, 42}

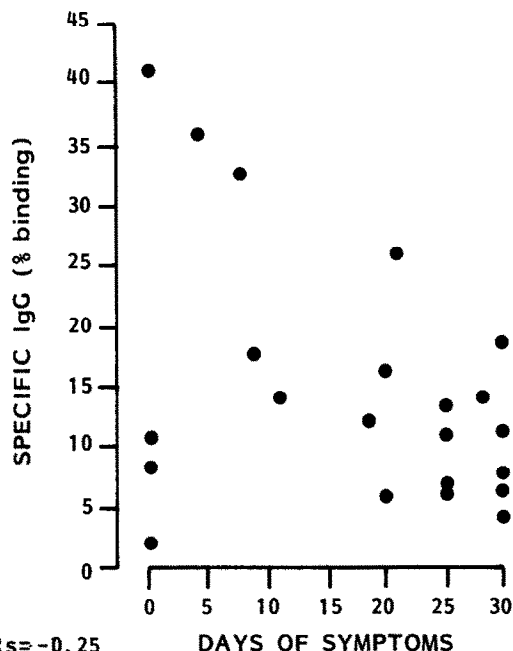


FIG. 7. Correlation between specific IgG after immunotherapy and nasal symptoms presented by the patients during the season. Statistical analysis by Spearman rank-correlation test.

Although serum-specific IgG was increased after specific immunotherapy, there was no correlation with either nasal challenge or symptoms during the pollen season. This finding accords with other studies performed with grass-pollen immunotherapy^{18-21, 30} with different methods. However, with other techniques of titration of IgG, it may eventually be possible to observe significant correlations, but this has yet to be demonstrated. It is however possible that patients treated with grass pollens react differently from patients treated with either ragweed pollens or Hymenoptera venoms. It does not therefore appear from this study and other studies that IgG may be a predictor of response for immunotherapy with grass-pollen allergens.

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REFERENCES

1. Norman PS. Immunotherapy. *Prog Allergy* 1981;32:38-47.
2. Patterson R, Lieberman P, Irons JS, Pruzansky JJ, Metzger WJ, Zeiss CR. Immunotherapy. In: Middleton E Jr, Reed CE, Ellis EF, eds. *Allergy: principles and practice*. 2nd ed. St. Louis: CV Mosby, 1983:1119-40.
3. Rocklin RE. Clinical and immunologic aspects of allergen-specific immunotherapy in patients with seasonal allergic rhinitis and/or allergic asthma. *J ALLERGY CLIN IMMUNOL* 1983;72:323-34.

4. Østerballe Ø. Immunotherapy in hay fever with two major allergens 19, 25 and partially purified extract of timothy grass pollen. A controlled double-blind study. In vivo variables, season I. *Allergy* 1980;35:473-89.
5. Bousquet J, Guérin B, Dotte A, Dhivert H, Djoukhadar F, Hewitt B, Michel FB. Comparison of rush immunotherapy with a standardized grass-pollen extract and classical immunotherapy with a pyridine extracted alum adjuved extract. *Clin Allergy* 1985;15:179-94.
6. Franklin W, Lowell FC. Comparison of two dosages of ragweed extract in the treatment of pollenosis. *JAMA* 1967;201:915-7.
7. Norman PS, Winkenwerder WL, Lichtenstein LM. Immunotherapy of hay fever with ragweed antigen E: comparison with whole pollen extract and placebo. *J ALLERGY* 1968;42:93-108.
8. Berg T, Nordvall SI, Lanner A. Clinical studies of a purified timothy-pollen extract: desensitization 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.
9. Irander K, Olofsson J, Aschan G. Hyposensitization in allergic seasonal rhinitis as evaluated by rhinomanometry. *Acta Otolaryngol [Suppl]* (Stockh) 1979;360:24-30.
10. Brown HM, McIntyre D. Immunotherapy for grass pollenosis with Allpyral allergen extracts: improved results by objective control of treatment requirement. *Acta Allergol* 1977;32:321-32.
11. Creticos PS, Adkinson NF Jr, Kagey-Sobotka A, et al. Nasal challenge with ragweed pollen in hay fever patients: effect of immunotherapy. *J Clin Invest* 1985;76:2247-53.
12. Frostad AS, Grimmer Ø, Sandvik L, Aas K. Hyposensitization comparing a purified (refined) allergen preparation and a crude aqueous extract from timothy pollen. *Allergy* 1980;35:81-5.
13. Mac Allen MK. Hyposensitization in grass-pollen hay fever: a double-blind trial of alum-precipitated pollen extract and depot emulsion pollen extract: compared with placebo injections. *Acta Allergol* 1969;24:421-31.
14. Kjellman N-IM, Lanner A. Hyposensitization in childhood hay fever: a comparison of refined and whole timothy extracts. *Allergy* 1980;35:323-34.
15. Naclerio RM, Meier HL, Kagey-Sobotka A, Adkinson NF Jr, Meyers DA, Norman PS, Lichtenstein LM. Mediator release after nasal spray airway challenge with allergen. *Am Rev Respir Dis* 1983;128:597-602.
16. Bousquet J, Lebel B, Dhivert H, Bataille Y, Martinot B, Michel FB. Nasal challenge with pollen grains, skin tests, specific IgE in patients with grass-pollen allergy. *Clin Allergy* 1987;17:529-36.
17. Østerballe O, Lichtenstein LM, Norm S, et al. Immunotherapy with grass pollen major allergens: immunological results from a prospective 3-year double-blind study. *Allergy* 1982;37:491-501.
18. Deuschl H, Johansson SGO, Fagerberg E. IgE, IgG, and IgA antibodies in serum and nasal secretion during parenteral hyposensitization. *Clin Allergy* 1977;7:315-24.
19. Djurup R. The subclass nature and clinical significance of the IgG antibody response in patients undergoing allergen-specific immunotherapy. *Allergy* 1985;40:469-86.
20. Østerballe Ø, Løwenstein H, Prah P, Skov P, Weeke B. Immunotherapy in hay fever with two major allergens 19, 25 and partially purified extracts of timothy pollen. A controlled double-blind study. In vitro variables, season I. *Allergy* 1981;36:183-99.
21. Nordvall SL, Berg T, Johansson SGO, Lanner A. Clinical studies of a purified timothy-pollen extract: desensitization 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* 1978;67:132-8.
22. van der Giessen M, Homan WL, van Kernbeek G, Aalberse RC, Dieges PH. Subclass typing of IgG antibodies formed by grass pollen-allergic patients during immunotherapy. *Int Arch Allergy Appl Immunol* 1976;50:625-40.
23. Romagnani S, Biliotti G, Ricci M. Depressed lymphocyte responses after hyposensitization. *Clin Exp Immunol* 1976;19:83-92.
24. Möller C, Juto P, Dreborg S, Björkstén B. Blood lymphocyte proliferation response to pollen extract as a monitor of immunotherapy. *Allergy* 1984;39:291-6.
25. Nagaya M, Lee SK, Reddy PM, Pascual H, Jerome D, Sadia J. Lymphocyte response to grass pollen antigens: a correlation with radioallergosorbent test and effect of immunotherapy. *Ann Allergy* 1977;39:246-52.
26. Marsh DG, Norman PS, Roebber M, Lichtenstein LM. Studies on allergoids from naturally occurring allergens. III. Preparation of ragweed pollen allergoids by aldehyde modification in two steps. *J ALLERGY CLIN IMMUNOL* 1981;68:449-59.
27. Norman PS, Lichtenstein LM, Kagey-Sobotka A, Marsh DG. Controlled evaluation of allergoid in the immunotherapy of ragweed hay fever. *J ALLERGY CLIN IMMUNOL* 1982;70:248-590.
28. Bousquet J, Hejjaoui A, Skassa-Brociek W, Guérin B, Maasch HJ, Dhivert H, Michel FB. Double-blind placebo-controlled immunotherapy with mixed grass-pollen allergoids. I. Rush immunotherapy with allergoids and standardized orchard grass-pollen. *J ALLERGY CLIN IMMUNOL* 1987;80:591-8.
29. Meriney DK, Kothari H, Chinoy P, Grieco MH. The clinical and immunologic efficacy of immunotherapy with modified ragweed extract (allergoid) for ragweed hay fever. *Ann Allergy* 1986;56:34-38.
30. Bousquet J, Frank E, Soussana M, Hejjaoui A, Maasch H, Michel FB. Double-blind placebo-controlled immunotherapy with high molecular weight formalinized allergoid in grass-pollen allergy. *Int Arch Allergy Appl Immunol* 1987;82:550-2.
31. Pepys J. Skin testing. *Br J Hosp Med* 1976;14:412-20.
32. Bousquet J, Calvayrac P, Guérin B, Hewitt B, Hejjaoui A, Dhivert H, Michel FB. Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. I. In vivo and in vitro parameters after a short course of treatment. *J ALLERGY CLIN IMMUNOL* 1985;76:734-44.
33. Bousquet J, Djoukhadar F, Hewitt B, Guérin B, Michel FB. Shelf-life of a pollen and a mite standardized extract. *Clin Allergy* 1985;15:29-36.
34. Rosenberg GL, Rosenthal RR, Norman PS. Inhalation challenge with ragweed pollen in ragweed-sensitive asthmatics. *J ALLERGY CLIN IMMUNOL* 1983;71:302-10.
35. Wihl JA. Methods for assessing nasal reactivity. *Eur J Respir Dis* 1983;64(suppl 128):175-9.
36. Michel FB, Bousquet J, Lebel B, et al. Correlation between symptoms and release of mediators in secretions during nasal challenge with pollen grains. *J ALLERGY CLIN IMMUNOL* 1987;79:247.
37. Puttonen F, Maasch HJ. Measurement of "blocking antibodies" to pollen antigens by radiolabelled protein A from *Staphylococcus aureus*. *Int Arch Allergy Appl Immunol* 1981;64:157-63.

38. Ceska M, Eriksson P, Varga JM. Radioimmunosorbent assay for allergens. *J ALLERGY* 1972;49:1-8.
39. Creticos PS, Peters SP, Adkinson NF Jr, Naclerio RM, Hayes EC, Norman PS, Lichtenstein LM. Peptide leukotriene release after antigen challenge in patients sensitive to ragweed. *N Engl J Med* 1984;310:1626-30.
40. Shaw RJ, Fitzharris P, Cromwell O, Wardlaw AJ, Kay AB. Allergen-induced release of sulphidopeptide leukotrienes (SRS-A) and LTB_4 in allergic patients. *Allergy* 1985;40:1-6.
41. Otsuka H, Denburg J, Dolovitch M, et al. Heterogeneity of metachromatic cells in human nose: significance of mucosal mast cells. *J ALLERGY CLIN IMMUNOL* 1985;76:695-702.
42. Lawrence ID, Warner JA, Bascom R, Kagey-Sobotka A, Lichtenstein LM. Heterogeneity of mast cells. *J ALLERGY CLIN IMMUNOL* 1987;79:179.