

# Combination of parenteral and oral immunotherapy in grass pollen-allergic children

A double-blind controlled study of clinical and immunological efficacy

N. S. TREDE & R. URBANEK

Universitäts-Kinderklinik Freiburg, W. Germany

Twenty patients with a proven sensitization to grass pollens were treated with parenteral "priming" and subsequently with either oral "booster" ( $n = 10$ ) or placebo ( $n = 10$ ) extension course. The study was carried out in a double-blind manner. Cumulative preseasonal parenteral dosage was 3,100 NU (Noon Units), patients in the oral group subsequently received 123.9 mg of grass pollen extract during the pollen season. No side effects were noted after intake of the oral preparation. No significant differences (95 % confidence interval) were noted comparing results of *in vivo* (skin prick test and conjunctival provocation test) and *in vitro* tests (specific serum IgE- and IgG-antibodies) between the two groups. Analysis of symptom and medication scores as well as subjective assessment of patients revealed no superiority of oral "booster" over placebo. Data obtained in this study does not support the concept of combined parenteral and oral treatment. This is in contrast to work reported previously.

*Key words:* grass pollen hyposensitization; oral extension.

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Since the beginning of this century, immunotherapy has increasingly been used in the management of grass pollen allergy. The efficacy of subcutaneous administration of grass pollen extracts has repeatedly been demonstrated in controlled double-blind studies (9, 12). Because of the inconvenience of this form of treatment, which requires close surveillance by a physician and the pain and swelling at the site of injection, some investigators favour oral immunotherapy (7, 23). Although many clinicians are convinced of the efficacy of such treatment, two placebo controlled studies showed lack of efficacy of oral treatment for grass pollen allergy (3, 17). Recently, a combination of parenteral "priming" and oral "booster" with aeroallergens was shown to provide a strong immunologic stimulus in animals (5).

We performed a double-blind placebo-controlled study to test combined parenteral oral immunotherapy for its clinical and immunologic efficacy in grass pollen-allergic children.

## MATERIAL AND METHODS

### *Patients*

Twenty children and young adults aged 3 to 20 years with a positive history of seasonal rhinoconjunctivitis were admitted to the study. None of the patients had received immunotherapy before. The diagnosis of grass pollen allergy was established through positive skin prick test (SPT) and conjunctival provocation test (CPT). Furthermore, sensitization to grass pollen was

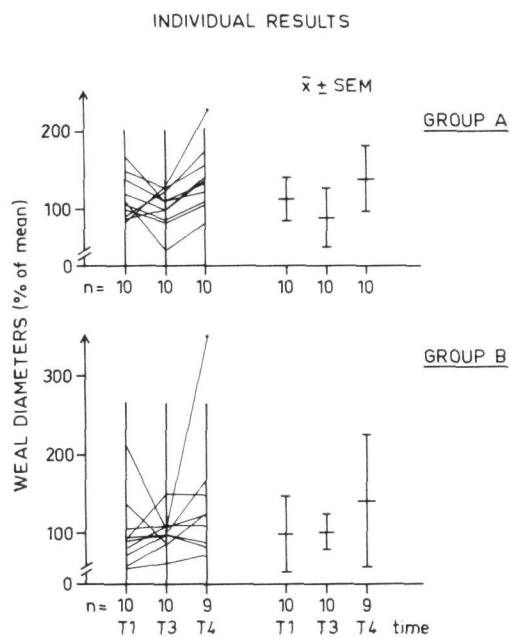


Fig. 1. Skin prick test performed with grass pollen extract (10,000 BU/ml). The values are expressed as a ratio of the allergen and histamine weal (mm) multiplied by 100. The prick test was carried out in duplicate at each investigation term (T1, T3, T4).

proven by determination of specific IgE antibodies (Figs. 1, 2 and 3). After identical parenteral “priming”, patients were randomly allocated to two groups: Group A (2 girls, 8 boys; mean age = 12.6 years) received the active treatment orally in enteric coated capsules, Group B (6 girls, 4 boys; mean age = 13.3

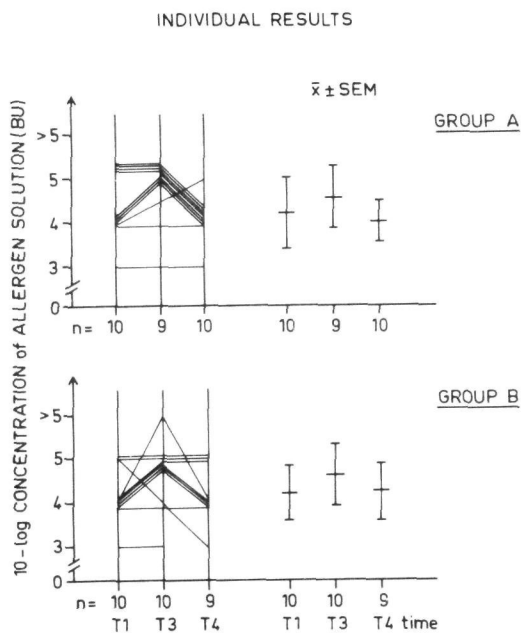


Fig. 2. Conjunctival provocation test performed as an end-point titration and expressed as the allergen concentration (BU/ml) inducing a conjunctival irritation.

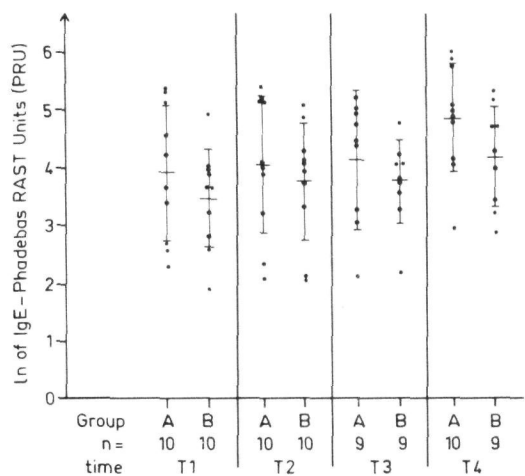


Fig. 3. Specific IgE antibodies (PRU) to grass (*Phleum pratense*). There is no significant change between the investigation terms nor between the serum or placebo-treated patients.

years) received placebo extension course. The study was carried out in a double-blind manner.

Patients and their parents were informed about the aim and the nature of the investigation and parents gave their written consent. One patient (A. K., Group B) was dissatisfied with the treatment result and did not appear at the last visit to the clinic.

Study design

The study was carried out between February 1985 and July 1985 (Table 1). *In vivo* tests (SPT and CPT) were performed at the beginning of the study, before the pollen season (mid April) and at the end of the study. Blood samples for *in vitro* investigation of specific IgE- and IgG-antibodies were taken simultaneously and additionally after the parenteral “priming” (mid March). Diary cards were handed out for the recording of symptoms and medication intake during the season (end of April to beginning of July).

*In vivo* testing was performed employing a lyophilised, standardized grass pollen extract. The solutions used were graded according to biological units (10 BU/ml, 100 BU/ml, 1,000 BU/ml, 10,000 BU/ml and 100,000 BU/ml). Reference solutions were histamine (1 mg/ml) for SPT and 0.9% NaCl-solution for CPT. Skin prick test (SPT) and conjunctival provocation test (CPT) were applied in a titration pro-

Table 1

Schematic representation of the study design

|                                      | February | March                                                                    | April                                                                    | May | June | July |
|--------------------------------------|----------|--------------------------------------------------------------------------|--------------------------------------------------------------------------|-----|------|------|
| Clinical examination                 | T1       | T2                                                                       | T3                                                                       |     |      | T4   |
| Skin prick test                      | x        |                                                                          | x                                                                        |     |      | x    |
| Conjunctival provocation test        | x        |                                                                          | x                                                                        |     |      | x    |
| Blood samples                        | x        | x                                                                        | x                                                                        |     |      | x    |
| Parenteral immunotherapy Groups A, B | xxx      |                                                                          |                                                                          |     |      |      |
| Disruption of capsules               |          | x                                                                        | x                                                                        |     |      |      |
| Capsule intake                       |          | xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx |                                                                          |     |      |      |
| Symptom and medication diary         |          |                                                                          | xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx |     |      |      |

cedure: SPT were performed in duplicate, and the mean of the maximum weal diameters for each concentration was expressed in percent of the weal diameters of the control histamine.

CPT was performed by administering a drop of the reference solution into the conjunctival sac of the left eye. The lowest concentration of the allergen solution was applied in the conjunctival sac of the right eye, and if no reaction occurred within 10 min, the next concentration was applied in the left eye. CPT was regarded as positive if there was conjunctival congestion and itching of the exposed eye.

For *in vitro* tests specific IgE-antibodies were analysed using the radio allergosorbent test (RAST) as described elsewhere (1). The enzyme-linked immunosorbent test (ELISA) was employed (20) to measure specific IgG-antibodies.

Patients or their parents were instructed to judge daily symptom scores according to an identical pattern (0 to 3 points/day) and to record them in the diary. They were also asked to record daily medication intake.

At the end of treatment the patient's assessment of the effect of treatment was asked in a standardized interview. Options were: "better", "same" or "worse" than last season.

### Treatment

TA Tyrosin-Allergoid Gräserpollen (Beecham Pharmaceuticals) was used for parenteral "priming": Patients of Groups A and B received 200, 800 and 2,000 Noon Units (NU) at weekly intervals Cumulative dose = 3,100 NU.

Group A was continued on capsules containing an enteric-coated mixture of 13 common grass pollens (BRL 42245, Beecham Pharmaceuticals). The characteristics of this preparation have been described elsewhere (22). Treatment was started with low dose therapy (200 µg allergen/capsule/day). Capsules were to be taken half an hour before breakfast with a cup of water. If side effects did not occur after a week, patients were given high dose treatment (1.25 mg/capsule/day) for 14 weeks. Cumulative dose = 123.9 mg.

Group B was continued on placebo capsules (Eudragit L-100, Rohm Pharma GmbH). Placebo and grass pollen capsules were identical as regards to shape and colour, preventing a premature identification by patients.

## Statistics

Student t-test was employed to analyse SPT, specific IgE- and IgG-antibody levels and symptom and medication scores. Wilcoxon test was used to calculate CPT differences between the groups.

## RESULTS

### Side effects

Parenteral injections were well tolerated by all patients. Side effects were restricted to local swelling and pain. A detailed questionnaire about symptoms following intake of capsules

Table 2

Patient diary record: Comparison of symptom scores and medication scores between Groups A and B

| Group     | Symptom score + Medication score |         |
|-----------|----------------------------------|---------|
|           | $\bar{x}$                        | (s)     |
| A (n = 9) | 4.666                            | (1.154) |
| B (n = 9) | 4.883                            | (0.595) |

(covering gastrointestinal, pulmonary, dermatological and circulatory side effects) was filled in by patients. No side effects were recorded during the oral active extension course. One Group B patient (T.W.) complained of headache and sleeplessness several hours after taking capsules.

Analysis of *in vivo* and *in vitro* tests prior to therapy showed that patients of both groups were well matched (data not shown) and distributed (Figs. 1-4).

In vivo tests

In group A a reduction of mucocutaneous sensitivity in the preseasonal period did not reach

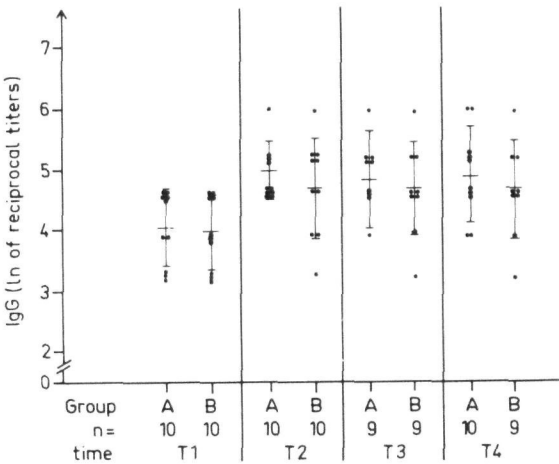


Fig. 4. Specific IgG antibodies to grass (*Phleum pratense*). There is a slight initial increase due to a parenteral induction therapy. No further changes in specific IgG antibodies in serum or placebo oral treatment was demonstrated.

statistical significance. During the season there was significant increase of skin and conjunctival mucosa reaction to the test substances ( $P < 0.01$  each).

In Group B no significant changes were recorded for the SPT, and CPT results were similar to those in Group A (Figs. 1 and 2). No significant changes between the groups were noted.

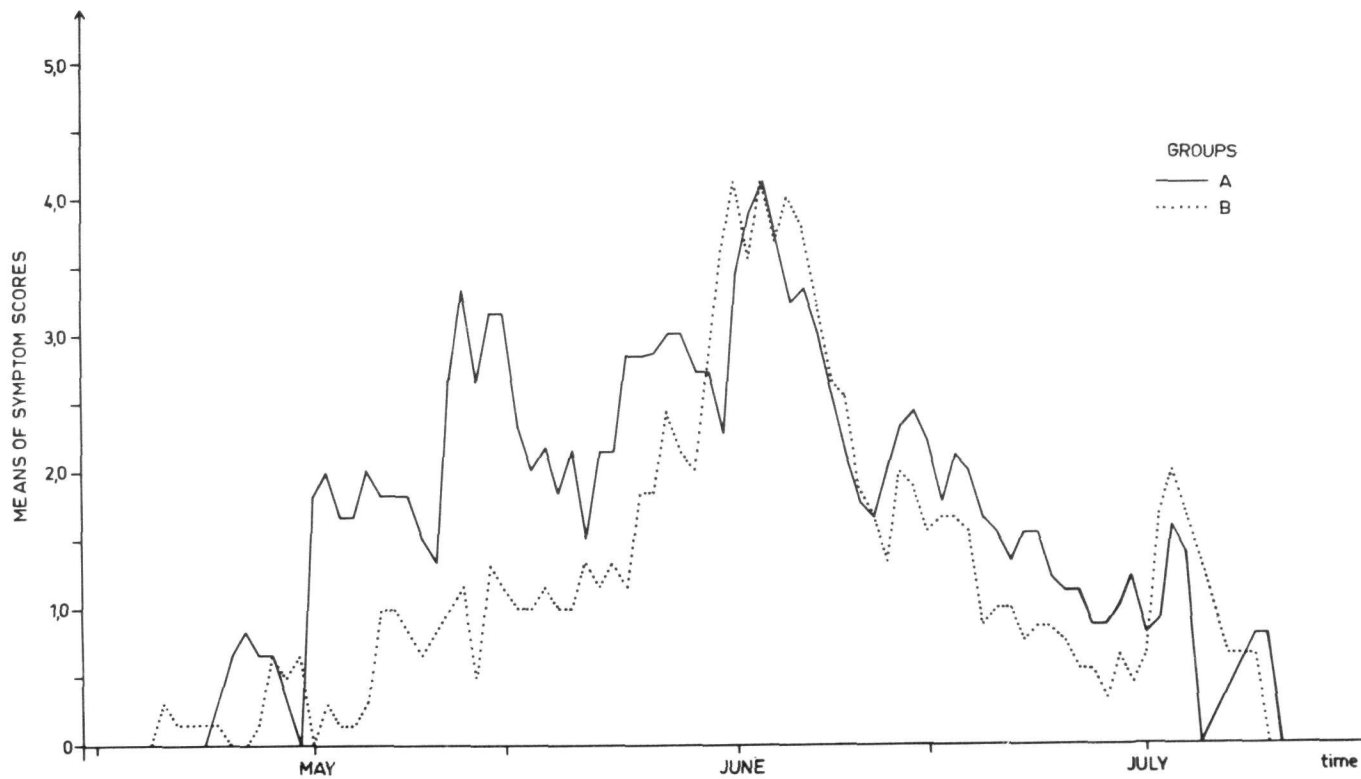


Fig. 5. Daily symptoms scores; there is no significant difference between the serum- or placebo-treated patients (see also Table 2).

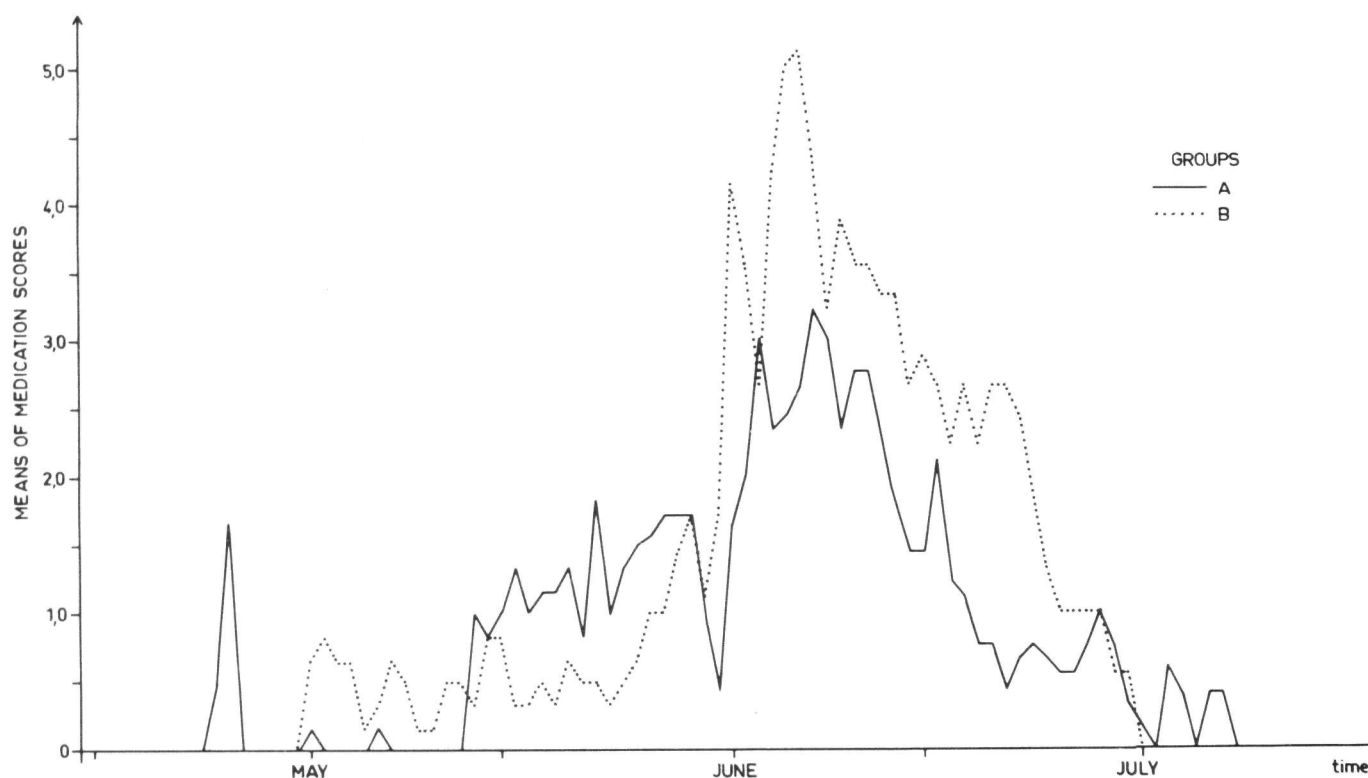


Fig. 6. Daily medication scores; there is no significant difference between the serum- or placebo-treated patients (see also Table 2).

#### *In vitro tests*

In the preseasonal period a non-significant increase of specific IgE-antibodies was noted in both groups. Increases were more marked during the season but did not reach statistical significance (Group A:  $P > 0.1$ , Group B:  $P > 0.5$ ) (Fig. 3). During preseasonal injection therapy, a significant increase in specific IgG-antibodies was noted in both groups (Group A:  $P < 0.01$ , Group B:  $P < 0.05$ ). Thereafter increases and decreases of specific IgG antibodies were balanced in both groups (Fig. 4). No significant *in vitro* tests differences occurred between the groups.

As regards the quantitative relationship between IgG and IgE antibodies (IgG/IgE ratio), a significant increase was noted only in Group A during parenteral "priming" ( $P < 0.05$ ). In this group the IgG/IgE ratio decreased significantly during the season ( $P < 0.005$ ). In Group B no significant changes were recorded.

There was no significant difference between Groups A and B as regards the IgG/IgE ratio.

#### *Symptom- and medication-scores*

Group A patients experienced slightly more symptoms but made less use of symptomatic treatment to control their symptoms than Group B patients (Figs. 5 and 6). Statistically significant results between the groups were not noted (Table 2). Symptom scores of eyes, nose and pulmonary systems on an individual basis were not significantly different.

#### *Subjective assessment*

Five of 10 Group A patients and three of the nine Group B patients felt improved compared to the preceding season. Three Group A patients and two Group B patients had experienced more symptoms than the year before.

If one considers clinical and immunological results of the eight improved patients (5 in Group A and 3 in Group B) and the five patients with deteriorated symptoms, no striking differences were observed. Improved patients seem to respond more markedly to pre-

seasonal injection therapy with a reduction, and to coseasonal pollen exposition with an increase, of mucocutaneous sensitivity. At the same time specific IgE-as well as IgG-antibodies were consistently higher than in the patients who reported worse symptoms (data not shown).

#### *Comparison of individual test results*

Three of the 20 patients in this study (1 from Group A and 2 from Group B) did not show any change over their initial IgG antibody levels throughout the observation period. IgE levels increased markedly only during the pollen season, but no IgE booster following immunotherapy was noted.

Conversely, eight patients (5 from Group A and 3 from Group B) had increased specific IgG-antibody levels by 2–3 reciprocal titer steps. In these patients a steady increase in IgE-antibody levels was noted throughout the observation period (data not shown).

## DISCUSSION

Oral immunotherapy was originally introduced as early as 1900 (4). Since that time it has been widely used to improve hay fever symptoms during the pollen season. Though its relative safe and easy administration is obviously advantageous, a treatment effect has convincingly been demonstrated only in animal studies. The beneficial effect of this form of treatment is probably based on the induction of systemic tolerance, which might be achieved by an activation of suppressor T cells (11). Further, a generalized mucosal immune response following oral administration of antigens has been demonstrated (2). Increased secretion of IgA-antibodies in organs belonging to the “mucosal associated tissue system” (MALT) is thought to be the consequence of migration of B cell immunoblasts from intestinal Peyer’s patches to other mucosal tissues and secretory glands (19). In contrast to these results, parenteral “priming” with grass pollen prior to the oral administration of the antigen seems to provide a potent

immune stimulus with boosting of serum specific antibodies in mice (5, 22). Similarly, a much higher degree of clinical protection was reported after sequential parenteral oral administration of cholera toxoid to dogs compared to subcutaneous or oral immunization alone (14). This is the rationale for the use of a combined parenteral/oral immunotherapy in this study.

The grass pollen extract used for parenteral “priming” has been shown to be of low immunogenicity and reduced clinical efficacy if administered alone without the inclusion of an extension course of subcutaneous therapy (8). This characteristic of the preparation was advantageous for our investigation, in that it prevented masking of possible changes in *in vivo* and *in vitro* parameters induced by subsequent oral treatment. We chose this preparation because a similar extract had been used in preliminary animal studies, the oral preparation contained the same set of pollen allergens, and its treatment schedule is convenient (three injections only at weekly intervals).

For objective assessment of treatment results, *in vivo* and *in vitro* tests were conducted throughout the investigation. SPT and CPT are safe, easy to perform and yield reproducible results. Both are good indicators of changes in the sensitivity of clinically involved organs. Whereas the sensitizing role of specific serum IgE-antibodies seems to be well established in allergy, there is still some controversy as to the role of specific serum IgG-antibodies. Though their protective effect in hymenoptera sting allergy has been demonstrated (21), a positive correlation between serum IgG-levels and clinical protection has not convincingly been shown in grass pollen allergy. Regardless of their possible role in modifying clinical symptoms, we measured both classes of antibodies in order to determine any systemic antibody changes brought about by antigen absorption from subcutaneous and intestinal tissues during immunotherapy.

Clinical protection during the grass pollen season as measured by SPT and CPT was absent. IgE-antibodies were boosted in both groups only during the pollen season to a marked, albeit nonsignificant, degree. IgG-antibodies increased significantly only dur-

ing parenteral immunotherapy and remained stable during the oral "booster" phase of treatment.

As regards individual test results, the three patients who showed no change in their IgG levels and whose IgE levels rose only during the pollen season, might have been immunological "non-responders". The antibody response of the eight patients showing a 4-8-fold increase of IgG antibodies and a steady increase of IgE-levels, satisfied "immunological responders" criteria. "Responder" patients tended to assess treatment results more favourably (4 of 8 "better") than "non-responders" (2 of 3 "worse"). Statistical analysis of test results has obviously to be ruled out because of the small number of patients in each subgroup. Larger studies exploring this observation to a further degree might be of some interest.

Possibly the 95% confidence interval is too high as standard for the evaluation of a single-year of immunotherapy. However, not even trends of differences between the two groups could be discerned. Thus, our results are neither in favour of a beneficial effect of oral "boosting" compared with placebo extension course, nor do they indicate an absorption of the oral preparation from the gut. Another sign pointing to inefficient absorption is the complete absence of local or systemic side effects following the oral administration of the antigen. Local side effects have been a consistent trait in two successful oral birch pollen studies in children (10) as well as in adults (18), and, occasionally, even a systemic reaction was noted following ingestion of capsules. Both were double-blind placebo-controlled studies showing a significant superiority of oral immunotherapy over placebo, though very high dose levels of birch pollen were used.

The laboratory data concerning the grass pollen extract seems to ensure its protection against degradation from gastric juices and rapid dissolution in the intestine. Preclinical *in vitro* studies indicated a slow degradation of preparation in a simulated duodenal milieu (22). Because of bile juices and the numerous pancreatic and mucosal enzymes, the exact influences of the intestinal milieu on antigenic

sites are difficult to evaluate *in vitro*. The increased incidence of nutritional allergy in patients with exocrine pancreatic insufficiency seems to point to a possible role of pancreatic enzymes in the degradation of antigenic sites (13). Differences in pancreatic enzyme constellation between guinea pigs and humans might explain why the treatment was effective in the former and not in the latter.

Potential structural differences caused by glutaraldehyde modification of the parenteral preparation may be the reason for lack of "priming" potential and hence of inefficient oral absorption.

One might also consider insufficient dosage of the oral preparation as a possible reason for lack of absorption. However, earlier double-blind controlled oral grass pollen studies showed that increasing the administered doses of unprotected extract did not lead to any clinical improvement. In one adult study even a total dosage as high as 8,915,000 PNU did not result in a clinical effect (17). If the antigen was absorbed, this should at least lead to a change, albeit modest, in serum antibody titers. However, in the case of protected birch pollen high doses were effective.

The work of Pierce et al. (14) in dogs parenterally immunized to cholera toxoid indicates an intestinal inactivation of the bacteria by serum IgG-antibody which is thought to leak into the gut lumen. If the same is true in parenteral grass pollen immunotherapy, one could imagine specific IgG-antibody formed during parenteral "priming" to prevent absorption of the oral extract. Furthermore, the induction of a secretory immune response following parenteral immunotherapy, as shown in nasal washings (15), could lead to the formation of specific IgA-antibodies in the intestinal mucosa via a generalized activation of the MALT system. These antibodies could equally inactivate the antigen in the gut lumen.

There is, however, strong evidence against this proposition. The results of Pierce show clearly that, in spite of inactivating *Vibrio cholerae* in the gut lumen, IgG-antibodies do not interfere with intestinal absorption of the antigen, as subsequent oral "boosting" led to a

prolonged clinical protection. In addition, Platts-Mills (16) demonstrated that local production of IgA-antibody following parenteral grass pollen injections is strictly limited to the nasal mucosa. Therefore premature antibody-mediated inactivation of orally administered grass pollen extract as a reason for inefficient absorption is unlikely.

A recent investigation (6), using a similar protocol as the one employed in this study, indicated a significant difference between nasal and eye symptoms in adults with oral extension course and placebo. If compared with our study, these results seem surprising in that a shorter oral extension course led to a positive clinical outcome in adults, where mucosal immunity is less favourable to allergen ingress than in younger patients.

As discussed above, intestinal degradation of the grass-pollen extract best explains the lack of clinical efficacy of oral immunotherapy with this pollen species. However, two studies showed clinical and immunological efficacy of oral immunotherapy with birch pollen (10, 18). Preliminary investigations indicate that a greater stability of birch pollen antigenic sites in intestinal fluids may be responsible for the observed differences (18).

### Conclusion

Combined parenteral/oral immunotherapy with grass pollen extracts used in this study was shown to lack clinical and immunological efficacy. Poor absorption of this oral extract is the possible reason, as indicated by lacking immune response. Further investigations on intestinal antigen uptake and degradation are necessary, in view of other successful trials by oral protected birch pollen extract, and of the previously reported positive results with a combined parenteral protected oral extract.

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### REFERENCES

1. Ceska, M., Eriksson, R. & Varga, J. M.: Radioimmunosorbent assay of allergens. *J. Allergy Clin. Immunol.* 49, 1, 1972.
2. Challacombe, S. J. & Tomasi, T. B. Jr.: Systemic tolerance and secretory immunity after oral immunization. *J. Exp. Med.* 152, 1459-1472, 1980.
3. Cooper, P. J.: Oral hyposensitization in seasonal asthma and rhinitis in children (abstract). *Clin. Allergy* 14, 541-550, 1984.
4. Curtis, H. H.: The immunizing cure of hay fever. *Med. News (N.Y.)* 77, 16, 1900.
5. Henderson, D. C. & Moran, D. M.: Antibody responses of mice to intragastric and parenterally administered aeroallergens. *Int. Arch. Allergy Appl. Immunol.* 79, 66-71, 1986.
6. Horak, F. & Wheeler, A. W.: Oral hyposensitization with enteric-coated allergens as extension therapy following a basic subcutaneous course of injections. *Int. Arch. Allergy Appl. Immunol.* 84, 74-78, 1987.
7. Jorde, W.: Die orale Desensibilisierung, theoretische Überlegungen und praktisches Vorgehen. *Ther. Gegenw.* 117, 823, 1978.
8. Kuhn, W., Urbancek, R., Forster, J., Scheyer, H., Wais, U.: Vorteile einer Hyposensibilisierung mit höherdosiertem Allergoid bei Pollenallergie. *Klein. Padiatr.* 195, 411-414, 1983.
9. Lowell, F. C. & Franklin, W.: A double-blind study of the effectiveness and specificity of injection therapy in ragweed hay fever. *N. Engl. J. Med.* 273, 675-679, 1965.
10. Möller, C., Dreborg, S., Lanner, A. & Björkstén, B.: Oral immunotherapy of children with rhinoconjunctivitis due to birch pollen allergy. A double blind study. *Allergy* 41, 271-279, 1986.
11. Ngan, J. & Kind, L. S.: Suppressor T cells for IgE and IgG in Peyer's patches of mice made tolerant by oral administration of ovalbumin. *J. Immunol.* 120, 861-865, 1978.
12. Österballe, O., Löwenstein, H., Norn, S., et al.: Immunotherapy with grass pollen major allergens. *Allergy* 37, 491-501, 1982.
13. Palme, G.: Orale Hyposensibilisierung - Resorption und Persorption aus der Sicht des pädiatrischen Gastroenterologen. 1. Tagung der Regionalgruppe Berlin der Ärztlichen Arbeitsgruppe für Angewandte Allergologie e.V., Mai 1980.
14. Pierce, N. F., Sack, R. B. & Sircar, B. K.: Immunity to experimental cholera. III. Enhanced duration of protection after sequential parenteral-oral administration of toxoids to dogs. *J. Infect. Dis.* 135, 888-895, 1977.
15. Platts-Mills, T. A. E., von Maur, R. K., Ishizaka, K., Norman, P. S. & Lichtenstein, L. M.: IgA and IgG anti-ragweed antibodies in nasal secretions. *J. Clin. Invest.* 57, 1041-1050, 1976.
16. Platts-Mills, T. A. E.: Local production of IgG, IgA and IgE antibodies in grass pollen hay fever. *J. Immunol.* 122, 2218-2225, 1979.

17. Taudorf, E., Laursen, L. C., Djurup, R., et al.: Oral administration of grass pollen to hay fever patients. *Allergy* *40*, 321-335, 1985.
18. Taudorf, E., Laursen, L. C., Lanner, A., et al.: Oral immunotherapy in birch pollen hay fever. *J. Allergy Clin. Immunol.* *80*, 153-161, 1987.
19. Tomasi, T. B. Jr., Larsen, L., Challacombe, S. & McNabb, P.: Mucosal immunity: The origin and migration patterns of cells in the secretory system. *J. Allergy Clin. Immunol.* *65*, 12-19, 1980.
20. Urbanek, R., Forster, J., Karitzky, D. & Ziupa, J.: The prognostic significance of specific IgG antibody in insect sting allergy. *Eur. J. Pediatr.* *136*, 31-34, 1981.
21. Urbanek, R., Forster, J. & Kuhn, W.: Insektenstichallergie-gelöste und ungelöste Probleme. *Dtsch. Med. Wochenschr.* *107*, 506-509, 1982.
22. Wheeler, A. W., Hendersson, D. C., Youlten, L. J. F., et al.: Immunogenicity in guinea-pigs and tolerance in grass pollen sensitive volunteers of enteric-coated grass pollen allergens. *Int. Arch. Allergy Appl. Immunol.* *83*, 354-358, 1987.
23. Wortman, F.: Oral hyposensitization of children with pollenosis or house-dust asthma. *Allergol. Immunopathol.* *15*, 5-26, 1977.

Address:

Prof. Dr. R. Urbanek  
 Universitäts-Kinderklinik  
 Mathildestraße 1  
 D-7800 Freiburg  
 West Germany

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