

Systemic reactions occurring during immunotherapy with standardized pollen extracts

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Specific immunotherapy with standardized extracts administered by a rush protocol can induce systemic reactions (SRs). A prospective study was conducted in 454 pollen-allergic patients (aged 5 to 62 years) (1) to compare the incidence of SRs during a 3-day rush or step protocol, (2) to determine if patients presenting pollen asthma or those allergic to multiple pollen species would have an increased incidence of SRs, and (3) to attempt to decrease SRs. According to their sensitivity, patients received standardized extracts of orchard grass, and/or Parietaria, and/or olive, and/or plane-tree pollen with the same allergenically bioequivalent maintenance dose. Patients 10 years of age and older received 2000 BU; patients younger than 10 years, 1000 BU. The occurrence of SRs was carefully monitored. The incidence of SRs per patient ranged from 2.6% to 31.1%. With the rush protocol, there was a significant decrease in SRs after premedication ($p < 0.015$). Severe SRs decreased further when the increase in doses was stopped because large local reactions occurred. Step protocol was only slightly better tolerated than rush protocol. Bronchial adverse reactions occurred more often in patients presenting asthma during previous pollen seasons. No reaction started 45 minutes or later after the last injection. (J ALLERGY CLIN IMMUNOL 1992;89:925-33.)

Key words: Immunotherapy, pollens, rush, systemic reactions, premedication

Specific immunotherapy is an effective treatment under optimal conditions, but its safety is currently being questioned.¹⁻³ It is suggested that potent standardized extracts are more effective but may elicit a greater number of SRs than unstandardized extracts.⁴ Some schedules, such as RIT, may increase the risks,⁵⁻¹⁰ and studies in mite-allergic patients have found that SR per patient during the RIT protocol ranged from 7.3% to 37.6%.^{6,7} Moreover, it has been observed that patients with asthma are particularly prone to develop SR during immunotherapy.^{3,6,7} Finally, SR during RIT can be reduced by use of a more careful protocol and a pretreatment.⁷ Thus, although immunotherapy appears to be basically safe since very few deaths have been reported during the past 20 years, it is advisable to try to decrease SR.

Abbreviations used

BU: Biologic units
RIT: Rush immunotherapy protocol
SR: Systemic reaction

Standardized and lyophilized pollen extracts have been available since 1980, and we administered this extract by RIT performed over 3 days, making it possible to reach the maintenance dose of 2000 BU in subjects older than 10 years of age and 1000 BU in children younger than 10 years of age. The same protocol has been used for the past 7 years and has been demonstrated to significantly alleviate nasal, conjunctival, and bronchial symptoms in children and adults,¹⁰⁻¹² but SRs have often been observed.

A prospective study was performed in 454 patients, suffering from allergies to pollens, rhinoconjunctivitis, and, eventually, asthma, ranging in age from 5 to 62 years, who received the same dose of standardized pollen extracts by means of RIT or step schedules. We examined factors that might be involved in the occurrence of SR. Four different protocols were used in an attempt to decrease the rate and severity of SR.

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TABLE I. Protocol of rush immunotherapy

Day		BU	
1	9 AM	15	Premedication*
	9:30 AM	75	
	10 AM	150	
	11 AM	300	
	2 PM	600	
2	9 AM	1000	Premedication*
	11 AM	1500	
3	9 AM	2000	Premedication*

*Premedication was only administered to patients placed in groups B and C; a dose of 2000 BU corresponds to 10 index of reactivity.

MATERIAL AND METHODS

Patients

Four hundred fifty-four patients, ranging in age from 5 to 62 years, were selected with the following criteria:

1. All patients had symptoms of rhinitis between April and July, and more than half also had symptoms of asthma and/or conjunctivitis during this period of time. The duration of symptoms ranged from 2 to 30 years. Symptoms presented by patients during previous years were assessed by a questionnaire.
2. All patients were tested by means of skin prick tests to common pollens found in the Montpellier atmosphere.¹⁷ All had a positive prick test to a 1/100 (wt/vol) standardized orchard grass-pollen extract (Stallergènes Laboratories, Fresnes, France) and the presence of orchard grass pollen-specific IgE (Phadebas RAST, Pharmacia Diagnostics, Uppsala, Sweden); 47.5% had positive skin tests and RAST to other pollen species, including *Parietaria*, olive, and/or plane-tree pollen.
3. Of the patients tested, 36.1% presented positive skin tests to house dust mites, molds, and cat and dog extracts, but they did not suffer from symptoms of perennial asthma or rhinitis.

Methods

Extracts. Standardized extracts from orchard grass (*Dactylis glomerata*), olive (*Olea europaea*), plane tree (*Platanus occidentalis*), and *Parietaria officinalis* pollens were prepared according to the proposals of the Allergen Subcommittee of the International Union of Immunological Societies by the Laboratoires des Stallergènes. A complete description of the preparation of the extracts has previously been described.¹⁸ Briefly, extracts were prepared from pure pollens harvested either from cultivated (orchard grass and *Parietaria*) or wild plants during a period of 3 years. Before extraction, pollens were analyzed for purity. Unde-fatted pollens were extracted by ammonium bicarbonate (0.125 mol/L) at pH 8.0. The extracting procedure was performed with continuous stirring at +4° C for 24 hours. The extracting liquid was then passed through a sterilizing grade filter, an aliquot was removed for control testing, and the bulk of the extracting solution was frozen. A control of the potency of an aliquot was done by RAST inhibition, iso-

electric focusing, and crossed immunoelectrophoresis, and compared with the internal standards that were controlled by the same in vitro assays and skin test titration. According to the results of the control procedure, a given amount of the extracting solution was then poured into vials and subsequently lyophilized. A final control of the final product by RAST inhibition and isoelectric focusing was performed. Extracts were labeled in biological units according to the proposals of the Nordic Countries guidelines on Allergen Standardization.¹⁹ The freeze-dried extracts were stored at +4° C. These extracts were used for skin tests and immunotherapy. We also tested the batch-to-batch consistency of these extracts by means of skin tests according to the method of Dreborg.²⁰

Immunotherapy schedules. The maintenance dose was not adapted to every patient according to the sensitivity but was defined as the "optimal dose" inducing the relief of symptoms with the lowest rate of SRs. We studied skin prick test end points before and a week after RIT with various doses and observed that, for a dose of 2000 BU of a standardized orchard-grass extract, the end point was reduced in >80% of patients.¹⁰⁻¹² We also noticed that for this dose, many patients did not present SRs during RIT, whereas for larger doses, the incidence of SRs was enhanced. Then, we confirmed (1) that by nasal challenges with orchard-grass pollens, the dose defined by skin test reduced the threshold dose, inducing a positive challenge, and (2) that during the pollen season, the dose chosen reduced significantly symptom-medication scores.¹⁰⁻¹² We therefore proposed a simple 3-day RIT schedule (Table I). When patients presented an SR, the next injection was reduced by half, and the RIT or step protocols were stopped, the maintenance dose being reached more slowly by weekly injections. Since all extracts used were tested by skin tests, the same allergenically bioequivalent dose was administered for each pollen species.

Initially, immunotherapy was started by an RIT consisting of a rapid increase of allergen doses that made it possible to reach the maintenance dose in 3 days, according to a previously described protocol¹⁰ (Table I, group A). The maintenance dose of each pollen extract was 2000 BU in individuals older than 10 years of age and 1000 BU in children younger than 10 years, and was administered weekly for 6 weeks, then bimonthly until the pollen season. The doses were subsequently reduced by half and administered every 2 weeks.

During 2 years of this treatment, we have observed a large number of SRs and have attempted to decrease them by administering an oral premedication to the patients for 3 days without any modification of the immunotherapy schedule (group B). The premedication consisted in the administration before any injection (9 AM) of methylprednisolone (0.5 mg/kg of body weight), ketotifene (1 mg), and long-acting theophylline (5 mg/kg of body weight), and at 7 PM, ketotifene (1 mg) and long-acting theophylline (5 mg/kg of body weight).

Then, we used the former protocol but stopped the dose increment when there was a large local reaction of >10 cm in diameter at the injection site (group C).

Since the RIT protocol may increase the rate of SRs, we

treated a third group of patients (group D) with a step protocol that made it possible to reach the same maintenance dose within 2 weeks (Table II). In this protocol, premedication was only administered during the first 2 days of treatment.

Only patients placed in group A were hospitalized for the first night of the RIT. Other patients stayed in the clinic throughout the day of RIT but returned home 2 hours after the last injection.

Study design

Patients received either an orchard grass-pollen extract when they were allergic only to grass pollens or several pollen extracts if they had more than one sensitivity. However, we treated the patients only with orchard grass and *Parietaria*, plane tree, or olive-pollen extracts, and more complex mixtures were not used since the other pollen species are likely to have only a minimal effect on patients from our area and were not, at the time of the initiation of the study, in a standardized form. All allergens were mixed in the same vial. Patients allergic to cypress pollen were not included in the study.

During the course of RIT or step protocol, all patients were carefully monitored before and between each injection. Moreover, they were followed for up to 2 hours after the last injection. In particular, patients were always examined carefully before each injection, observing for possible wheezing. When an SR occurred, pulse rate and blood pressure were checked.

SRs were classified as asthma, urticaria, or anaphylaxis. Severe anaphylaxis was characterized by symptoms including tachycardia, hypotension, generalized urticaria and/or angioedema, and possibly wheezing. Reactions, including both urticaria and wheezing without hypotension, were classified as minor anaphylaxis. If a patient presented more than one SR on separate injections, the patient was only counted as one. Minor SRs, such as generalized urticaria, were treated with antihistamines and sometimes systemic corticosteroids. Asthma was treated with 200 to 400 µg of salbutamol administered by metered-dose inhalers, and if these were required, by theophylline infusion, oxygen and subcutaneous terbutaline. Finally, when rapidly extensive generalized urticaria or symptoms of severe anaphylaxis were observed, subcutaneous and/or intramuscular epinephrine was administered, and oxygen was sometimes administered.²¹

Late-phase reactions were defined as the recurrence of asthma and/or urticaria 4 to 12 hours after the last injection.

The patients were examined for local reactions before each injection and only those indurations >15 cm and erythema >50 cm were classified as large local reactions.²²

Statistical analysis was calculated by means of Student's *t* test or chi-square analysis.

RESULTS

Patients included in the four groups were similar in terms of mean age, skin test end point, percentage of asthma or rhinitis, and allergenic sensitivities (Table III).

TABLE II. Protocol of step immunotherapy

Day	BU		
1	9 AM	15	Premedication
	9:30 AM	75	
	10 AM	150	
	11 AM	300	
2	9 AM	600	Premedication
5		1000	
8		1500	
11		2000	

The maintenance dose was only reached according to the protocol in patients who did not present any SR. It was reached more slowly in patients that presented an SR, and the number of injections was related to (1) the dose inducing the first SR, (2) the severity of the SR, and (3) the possible occurrence of other SRs. The mean maximal dose reached during the RIT protocol was similar in groups A and B and significantly ($p < 0.0001$, Student's *t* test) reduced in group C during the 2-day step schedule. However, all patients in the four groups reached the top dose of 1000 and 2000 BU in children and adults, respectively.

The incidence of SR per patient ranged from 31.1% in group A to 2.6% in group D. Asthma ranged from 9.7% to 0%, urticaria from 16.3% to 2.6%, and anaphylaxis from 16.3% to 0% (Table III). Most anaphylactic reactions were classified as severe and required adrenaline. No patient had to be admitted to an intensive care unit. In group D, all SRs occurred during the first 2 days of the protocol. The severity of SR decreased from group A to group D, since the necessity for administration of adrenaline had been mainly occasioned by patients in groups A and B and since there was a significantly decreased percentage of patients requiring β_2 -agonists from group A to group D. Very few patients had more than one SR; therefore, the rate of reaction per injection ranged from 5.5% to 0.5%. SRs were observed for doses ranging from 100 to 2000 BU. Patients treated with several pollen species did not present a significantly larger number of SRs.

The premedication by corticosteroids, antiallergic drugs, and bronchodilators reduced the risk of SR by twofold (differences between groups A and B, $p < 0.015$, chi-square analysis). The step protocol (group D) reduced slightly the incidence of SR. Less than 1% of patients developed an anaphylactic reaction in groups C and D; thus, for this parameter, it was not possible to assess the difference between RIT and the step schedule.

Most reactions started within 15 minutes after the last injection, and no reaction started 45 minutes or

TABLE III. Characteristics of patients and global results

Groups	A	B	C	D	p A/B	p A/C	p B/C	p C/D
No. patients	74	102	200	78				
Age (yr)	24.6 ± 12.5	23.6 ± 12.6	29.6 ± 13.6	23.8 ± 13.1	NS	NS	NS	NS
Skin test end point	6.2 ± 2.3	6.0 ± 1.9	6.4 ± 2.5	5.8 ± 1.9	NS	NS	NS	NS
Asthma (%)	39.2	44.1	34.8	34.6	NS	NS	NS	NS
Rhinitis (%)	100	100	100	100	NS	NS	NS	NS
Protocol	RIT	RIT	RIT	Step				
Maximal dose (BU)	2000	2000	2000	1000 BU* 2000 BU†				
IT with multiple allergens (%)	41.9	49.0	42.5	39.5				
Premedication	No	Yes	Yes	Yes				
Preventive measures	No	No	Yes	Yes				
Mean dose reached	1608 ± 577	1488 ± 531	1130 ± 261	660 ± 293*	NS	<0.0001	<0.0001	—
SR during the increase in doses								
SR per patient (%)	31.3	14.7	7.5	2.6	<0.015	<0.005	NS	NS
SR per injection (%)	5.5	3.1	2.3	2	<0.04	<0.005	NS	<0.05
Asthma per patient	9.7	5.8	2.5	0	NS	<0.03	<0.05	<0.05
Urticaria per patient	16.3	5.8	0.3	2.6	NS	NS	NS	NS
Anaphylaxis per patient (%)	16.3	5.8	0.3	0	<0.05	<0.005	<0.005	<0.005
Adrenaline per patient (%)	14.9	5.8	0.8	0	<0.05	<0.005	<0.005	NS
β ₂ -agonist per patient (%)	9.5	5.8	0.8	0	NS	<0.005	<0.005	NS
SR during maintenance dose								
Per patient (%)	8.1	5.9	6.0	2.6				

*Dose reached after the first 2 days of the protocol.

†Dose reached at the end of the step protocol.

later after the last injection. Only three mild SRs in the form of urticaria started between 30 and 40 minutes after the last injection. No patient had a late onset SR. The quicker the onset of the reaction, the more severe (Fig. 1).

In groups A and B in which the largest number of reactors was observed, we examined the difference in SR rate according to the symptoms presented by patients during previous pollen seasons and the sensi-

tization of patients (Fig. 2). No difference was demonstrated between patients treated with grass pollens or several pollen species, but patients who had asthma during previous pollen seasons had significantly more asthma during the course of RIT than patients with rhinitis only. Patients with asthma during previous pollen seasons had a similar sensitization pattern toward perennial allergens than patients without bronchial symptoms.

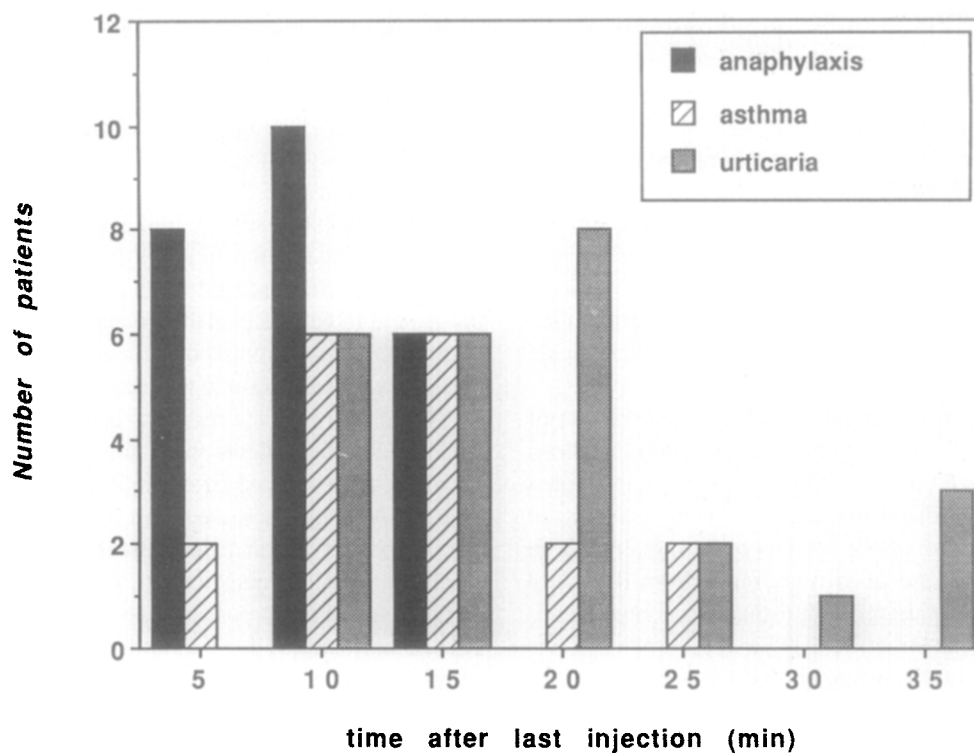


FIG. 1. Time of onset of reactions according to their severity.

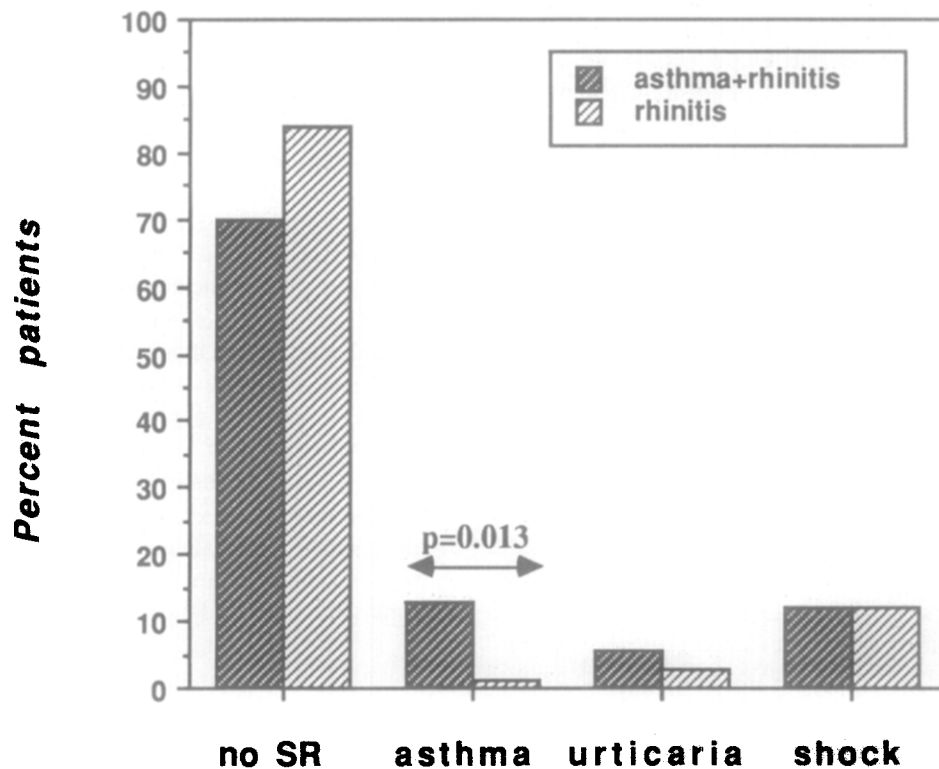


FIG. 2. SRs occurring in 176 patients receiving RIT with grass pollen or several pollen extracts placed in groups A and B according to symptoms presented during previous pollen seasons; statistical analysis by paired Student's *t* test.

DISCUSSION

Standardized pollen extracts were found to induce a large number of SRs when a 3-day RIT protocol without any premedication was applied. However, by use of a premedication, the rate and severity of SR were reduced. With the same maintenance dose, a step protocol is only slightly safer than RIT. Patients treated with several pollen species presented a slightly, but not significantly, larger number of SRs. Patients who suffered from pollen asthma in previous pollen seasons had a significantly larger number of asthmatic episodes during RIT.

Results from the literature indicate that the rate of SR ranges from 1 to 17 reactions per 1000 injections and is observed in 1% to 50% of the patients treated with unstandardized extracts.²²⁻²⁷ With standardized extracts, the rate of SR appears to be higher, and an additional increase in SR may result from RIT. In a large series of patients with asthma receiving a standardized mite extract by RIT or step protocols, we had previously demonstrated that the number and severity of SRs could be reduced with premedication and by stopping the protocol when very large local reactions occurred.⁷ The present study was devised to confirm our findings with a different type of allergen extract. Moreover, we wanted to determine whether many patients receiving mite allergens presented SRs because of the potency of the extract or because they had severe asthma and, eventually, because the allergen was still present in the environment. In the present study, none of the patients had severe asthma, and pollens were not present in the environment during the RIT or step procedures. The conclusions of the present study indicate that the potency of the allergen extract (mite and pollen studies), as well as the severity of asthma (mite study), are of great importance in determining the rate of SRs during immunotherapy.

In the first group of 74 patients in which RIT was administered without any precaution or premedication, SR occurred frequently (31.1% of patients and 9.6% of injections). This rate is unacceptable but should be compared with other studies. RIT with either pollens,⁹ animal extracts,^{15, 16} mites,⁶⁻⁹ or molds^{13, 14} elicits at least a similar rate of SR. Thus, the safety of RIT should be questioned. In grass-pollen allergy, the rate of SR per patient, with potent aqueous extracts administered with a classic protocol, ranged from 6% to 44%.^{28, 29} In ragweed-pollen allergy, with potent aqueous extracts administered with a classic protocol, two studies reported no SR,^{30, 31} but in other studies, the rate of SR per patient ranged from 23% to 77%.³²⁻³⁸ Thus, comparing our findings with the literature and the differences observed in the present study between RIT in group C and step immunother-

apy (group D), it appears that the RIT is only partly correlated with the high rate of SR found in the first group of patients. In the present study it is possible that the hospitalization increased the rate of SR because of its psychologic impact, but we did not find such an increase of SR when we compared venom-allergic patients receiving immunotherapy with and without hospitalization (unpublished observation).

The rate of SR observed in the first group of patients was found to be unacceptable in number and severity, and we therefore attempted to reduce the occurrence. We gave premedication to patients to reduce the onset of SR and to avoid late reactions occurring during the night, since the patients were not hospitalized. This premedication reduced by one half the number of SRs and significantly decreased their severity. Moreover, not one patient had an asthma attack at night, possibly since we used a premedication with corticosteroids. In this study, it is not possible to determine the relative role of corticosteroids or ketotifene in the reduction of SRs, since we did not perform a double-blind study with single drugs alone. Corticosteroids require some hours to be effective³⁹ and are unlikely to prevent reactions occurring during the first four injections, but they may be effective in preventing late-phase reactions. Ketotifene has both anti-H₁-receptor antagonist and antiallergic properties occurring rapidly after its administration, whereas the antiasthmatic efficacy requires some days to become maximal.⁴⁰ However, although this treatment did not completely abolish SR, we obtained an acceptable rate of SR of a milder severity, suggesting that RIT should be performed with care. The importance of a premedication in reducing the number and severity of SR was already reported by Jarisch et al.⁴¹ in patients allergic to various allergen species and by our group in mite RIT.⁷ Jarisch et al.⁴¹ used a premedication with antihistamines and reduced by half the maximal dose. They noticed that severe SRs, observed in 0.2% of injections, were completely abolished with their new protocol. In the mite study,⁷ we observed results similar to results reported in this study, indicating that a premedication is able to decrease the rate and severity of SR during RIT. Premedication is, however, not advised in the United States, and the cocktail used might have masked minor SRs, which are treatable and which necessitate allergen modification in dosage. The potential of a serious life-threatening SR breaking through the premedication is real. However, these data of the present study do not support this hypothesis because premedication by itself decreased significantly the rate of SR from 31.1% to 14.7% (groups A and B) and the rate of severe SR, from 16.3% to 5.8% in the same groups.

These considerations may help to significantly reduce SR during immunotherapy with standardized extracts. An optimal dose should be defined and administered, eliciting a significant relief of symptoms with the lowest rate of SR possible. Moreover, a premedication may help to decrease SR further. Both RIT or step procedures may be used, although the first regimen might be associated with a higher rate of SR. Furthermore, care should be taken with regard to large local reactions, as noticed in the present study. Using these recommendations of an optimally designed dose with premedication and taking care of large local reactions, we were able to treat efficiently a group of *Alternaria*-allergic patients without inducing any SR.⁴²

Other suggestions have been proposed to increase the safety of immunotherapy. Adsorption on aluminium is widely used in Europe, but the rate of SR may still be high⁴³ and even present in >50% of patients.⁴⁴ Pyridine-extracted, alum-adsorbed extracts are safer but not totally devoid of SRs,^{45, 46} and their efficacy appears to be significantly lower than standardized extracts.¹⁰ Polymerized extracts are effective, but when they are not fractionated, SRs may occur.^{47, 48} By comparison with aqueous extracts, some studies have found a decreased rate of SR,^{29, 48} but other investigators have observed a similar or higher rate.^{11, 12, 28, 35} High-molecular-weight polymerized preparations appear to be safer than aqueous preparations or unfractionated polymerized extracts, both in grass,^{12, 49, 50} ragweed,^{32, 51, 52} or tree-pollen allergy,⁵³ severe SR being mainly absent.

During the RIT or step protocol, all SRs occurred within 45 minutes after the last injection. Most severe SRs started within 15 minutes after the last injection, and only three mild SRs occurred after 30 minutes. Although these observations do not completely agree with data recently published,²⁴ this study confirms a recent position paper⁵⁴ indicating that patients should be kept under supervision for 20 minutes when allergen doses are increased, at least with potent standardized extracts, and 30 minutes for high-risk patients.

Finally, it has been suggested in many articles and studies that subjects with asthma are more prone to develop bronchial adverse reactions during immunotherapy.^{2-4, 6, 7} This study confirms such observations because patients, presenting asthma during previous pollen seasons, had significantly more bronchial adverse reactions during RIT. However, although these findings agree with the lower tolerance of patients with asthma toward immunotherapy, these findings must be interpreted with some care: (1) asthma, during previous pollen seasons, was characterized by a ques-

tionnaire and not by a follow-up of the patients using symptom-medication scores and peak flows, and (2) a proportion of these patients was also allergic to perennial allergens, and although they did not claim the existence of perennial symptoms nor had they had an abnormal pulmonary function before RIT, they might have had an increased bronchial hyperactivity.

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54. The waiting period after allergen skin testing and immunotherapy. Position paper.

Vascular permeability and airway narrowing during late asthmatic response in dogs treated with Metopirone

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Recently, we have developed an animal model of late asthmatic response (LAR) by treating naturally sensitized dogs to *Ascaris suum* antigen with the cortisol-synthesizing inhibitor, Metopirone. By using this animal model, we examined the contribution of edema in the airway wall to the development of LAR. To study whether airway microvascular leakage is increased in association with LAR, we performed antigen challenge in dogs treated with Metopirone. We measured the amount of extravasated Evans blue (EB) dye from the esophagus, trachea, and large and small bronchi 8 hours after the antigen challenge in dogs demonstrating immediate asthmatic response alone (IAR) and in dogs demonstrating both IAR and LAR. Airway responses to *A. suum* antigen were assessed by changes in respiratory resistance measured with the force oscillation technique at 3 Hz. EB dye extravasation did not increase significantly from that of control in any tissues in IAR ($p > 0.10$), but in LAR, it increased significantly from that of control ($p < 0.01$) and IAR ($p < 0.05$) in large and small bronchi. Histologic assessment of vascular permeability revealed that Monastral blue-labeled leaking vessels were only in sections from LAR, and leaking vessels were limited to small vessels (10 to 25 μm) in the trachea, large (diameter, >5 mm) and small bronchi (2 to 4 mm in diameter), and bronchiole. The permeability index defined as the ratio of area of small vessels labeled with Monastral blue to that of the total small vessels in the walls was highest in the small bronchi. LAR significantly increased submucosal thickness of the small bronchi ($p < 0.05$) compared with that in IAR. Both EB dye extravasation and permeability index in large and small bronchi also significantly increased during IAR within 3 minutes after the antigen challenge ($p < 0.05$), but IAR did not alter the submucosal thickness of the small bronchi. These results imply that the increase in vascular permeability and submucosal thickness, especially in small bronchi, may be an important factor in the pathogenesis of LAR. (*J ALLERGY CLIN IMMUNOL* 1992;89:933-43.)

Key words: Asthma, bronchial provocation tests, airway obstruction, bronchi, edema, adrenalectomy, steroid

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